SYNTHESIS AND EVALUATION OF VALUE-ADDED PRODUCTS FROM GLYCEROL

YUSRABBIL AMIYATI YUSOF

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YUSRABBIL AMIYATI YUSOF

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ABSTRACT

Reactions to produce glyceryl ethers from glycerol were carried out using one-pot and two-step methods. In one-pot synthesis, reaction of glycerol with *tert*-butanol in the presence of acidic catalyst had resulted in 80 % conversion of glycerol to glyceryl ethers (*mono*-*tert*-butoxypropanediol and *di*-*tert*-butoxypropanediol) wherein *mono*-*tert*-butoxypropanediol is the major component. On the other hand, glyceryl ethers (*mono*-methoxypropanediol, *mono*-ethoxypropanediol and *mono*-*tert*-butoxy propanediol) were successfully obtained in the range of 10 % to 45 % yield from the two-step reaction when the ring opening was conducted in basic reaction condition. Meanwhile, glyceryl ethers namely *mono*-methoxypropanediol, *mono*-ethoxypropanediol, *mono*-propoxypropanediol, *mono*-butoxypropanediol, *mono*-isopropoxypropanediol, *mono*-isobutoxypropanediol and *mono*-*tert*-butoxypropanediol were successfully synthesized in the range of 15 % to 60 % yield from the ring opening in acidic reaction medium. The *in vitro* dermal irritection assay showed that glyceryl ethers produced (*mono*-methoxypropanediol, *mono*-ethoxypropanediol, *mono*-propoxypropanediol, *mono*-butoxypropanediol, *mono*-isobutoxypropanediol and *mono*-*tert*-butoxypropanediol) are classified as non-irritant to the skin as the Human Irritancy Equivalent (HIE) score was below 0.9. In addition, *mono*-*tert*-butoxypropanediol was found to be practically non-toxic to aquatic system as the LC_{50} of the compound was more than 100 mg/L. All synthesized compounds exhibited antibacterial properties against gram positive and gram negative bacteria with 7.5 ± 0.7 mm to 16.0 ± 1.4 mm zone of inhibition. The scaling up process of *mono*-*tert*-butoxypropanediol by one-pot reaction (direct etherification method) was conducted for its application evaluations. The etherification was carried out at 1:4 mole ratio of glycerol/*tert*-butanol in the presence of 5 % acidic catalyst for 5 hours at the temperature of 80 °C to 85 °C. A product consists of more than 60 % *mono*-*tert*-butoxypropanediol with less than 5 % glycerol was successfully obtained in sufficient volume from the scaling up process. The study showed that glyceryl ether (*mono*-*tert*-butoxypropanediol) has potential applications in macroemulsion, microemulsion and transparent soap making. Macroemulsions with glyceryl ether showed lower viscosity values (0.48 - 0.51 Pa.s) than macroemulsions with glycerol (0.54 - 0.60 Pa.s). Furthermore, the macroemulsions containing glyceryl ether also exhibited moisturizing property in which the variation in hydration was 27 - 59 % compared to control macroemulsion (variation in hydration = 19 - 34 %). Glyceryl ether is suitable to be used in cosmetic products which require reduced viscosity but retain its skin hydration property. In addition, glyceryl ether is also suitable to be used as a co-surfactant in microemulsion formation. Furthermore, a natural yellow transparent soap with desired transparency value (0.87 ± 0.01) and soap hardness was successfully made by the incorporation of glyceryl ether in the absence of colouring agent. The take-home message from this research is that glyceryl ethers can be synthesized from glycerol *via* one-pot and two-step reactions, and the findings from the application evaluations of glyceryl ether look promising.

ABSTRAK

Tindak balas untuk menghasilkan eter gliseril daripada gliserol telah dilakukan melalui kaedah 'one-pot' dan dua langkah. Dalam sintesis 'one-pot', tindak balas antara gliserol dan *tert*-butanol dengan kehadiran mangkin asid telah memberikan penukaran 80 % gliserol kepada eter gliseril (*mono-tert*-butoksipropanadiol dan *di-tert*-butoksipropanadiol) yang mana *mono-tert*-butoksipropanadiol adalah komponen utama. Sebaliknya, eter gliseril (*mono*-metoksipropanadiol, *mono*-etoksipropanadiol dan *mono-tert*-butoksipropanadiol) telah berjaya diperolehi dalam julat hasil antara 10 % ke 45 % daripada tindak balas dua langkah yang mana pembukaan gelang dilakukan dalam keadaan tindak balas beralkali. Manakala, eter gliseril iaitu *mono*-metoksipropanadiol, *mono*-etoksipropanadiol, *mono*-propoksipropanadiol, *mono*-butoksipropanadiol, *mono*-isopropoksipropanadiol, *mono*-isobutoksipropanadiol dan *mono-tert*-butoksipropanadiol telah berjaya disintesis dalam julat hasil antara 15 % ke 60 % daripada pembukaan gelang dalam keadaan tindak balas berasid. Ujian iritasi dermal *in vitro* menunjukkan eter gliseril yang dihasilkan (*mono*-metoksipropanadiol, *mono*-etoksipropanadiol, *mono*-propoksipropanadiol, *mono*-butoksipropanadiol, *mono*-isobutoksipropanadiol dan *mono-tert*-butoksipropanadiol) dikelaskan sebagai bahan yang tidak iritasi kepada kulit yang mana skor 'Human Irritancy Equivalent' (HIE) adalah di bawah 0.9. Malah, *mono-tert*-butoksipropanadiol tidak toksik kepada sistem akuatik yang mana LC_{50} untuk sebatian tersebut adalah lebih daripada 100 mg/L. Semua sebatian yang dihasilkan mempamerkan ciri antibakteria terhadap bakteria gram positif dan gram negatif dengan zon perencatan 7.5 ± 0.7 mm ke 16.0 ± 1.4. Proses untuk menghasilkan *mono-tert*-butoksipropanadiol pada skala besar melalui kaedah 'one-pot' (pengeteran langsung) telah dilakukan untuk penilaian aplikasinya. Proses pengeteran telah dilakukan pada nisbah mol 1:4 gliserol/*tert*-butanol dengan kehadiran 5 % mangkin asid selama 5 jam pada suhu antara 80 °C ke 85 °C. Produk yang mengandungi lebih 60 % *mono-tert*-butoksipropanadiol dengan kandungan gliserol kurang daripada 5 % telah berjaya diperolehi dalam amaun yang mencukupi daripada proses tersebut. Kajian menunjukkan eter gliseril (*mono-tert*-butoksipropanadiol) berpotensi digunakan dalam makroemulsi, mikroemulsi dan pembuatan sabun lutsinar. Makroemulsi yang mengandungi eter gliseril mempunyai kelikatan yang rendah (0.48 - 0.51 Pa.s) berbanding makroemulsi yang mengandungi gliserol (0.54 - 0.60 Pa.s). Makroemulsi yang mengandungi eter gliseril tersebut mempamerkan ciri kelembapan dengan 27 - 59 % variasi penghidratan berbanding makroemulsi kawalan (variasi penghidratan = 19 - 34 %). Eter gliseril sesuai untuk digunakan dalam produk kosmetik yang memerlukan kelikatan yang rendah tetapi pada masa yang sama mengekalkan ciri penghidratan pada kulit. Eter gliseril juga sesuai untuk digunakan sebagai ko-surfaktan dalam pembentukan mikroemulsi. Di samping itu, sabun lutsinar kuning dengan nilai lutsinar (0.87 ± 0.01) dan kekerasan sabun yang dikehendaki telah berjaya dihasilkan dengan penambahan eter gliseril tanpa kehadiran agen pewarna. Mesej yang diperolehi daripada penyelidikan ini ialah eter gliseril boleh dihasilkan melalui tindak balas 'one-pot' dan dua langkah, dan penemuan daripada kajian aplikasi eter gliseril amat memberansangkan.

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Last but not the least, I owe my deepest gratitude to the one above all of us, Allah the Almighty, for answering my prayers for giving me the strength to finish and complete this thesis.

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LIST OF SYMBOLS AND ABBREVIATIONS

d	doublet
DCM	Dichloromethane
dd	doublet of doublet
DMF	*N*,*N*-dimethylformamide
DMSO	Dimethylsulfoxide
FID	Flame Ionization Detector
FTIR	Fourier Transform Infrared
GC	Gas Chromatography
GCMS	Gas Chromatography Mass Spectroscopy
HCl	Hydrochloric acid
HIE	Human Irritancy Equivalent
hr	hour
IR	Infrared
g	gram
L	Liter
m	multiplet
m	*meta*
mbar	millibar
MCT	Medium Chain Triglycerides
min	minute
mg	milligram
mL	milliliter
MPOB	Malaysian Palm Oil Board
N	Normality

NMR	Nuclear Magnetic Resonance
OECD	Organization for Economic Cooperation and Development
p	*para*
PbO	Plumbum oxide
POME	Palm Methyl ester
ppm	part per million
p-TsOH	*para*-toluenesulfonic acid
RT	Retention Time
rt	room temperature
s	singlet
SD	Standard Deviation
t	triplet
tert-	tertiary
TLC	Thin Layer Chromatography
Δ	heat
>	more than
<	less than
ν	stretching
µL	microliter

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 General Introduction

Glycerol is the simplest triol (Figure 1.1), having the chemical formula of $C_3H_8O_3$ with more correctly name 1,2,3-propanetriol (Jungermann & Sonntag, 1991). The molecular mass of glycerol is 92.09 $g.mol^{-1}$. It is composed of three carbon atoms, five hydrogen atoms and three hydroxyl or –OH groups. The hydroxyl groups are known as the functional alcohol groups, placing glycerol in the alcohol group of chemical compounds.

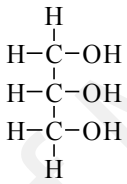


Figure 1.1 : Chemical structure of glycerol

Glycerol is a clear, water-white, sweet, viscous, odourless, colourless and hygroscopic liquid at ordinary room temperature above its melting point (Heming, 1999; Knothe *et al.*, 2005). It is stable under most conditions, non-toxic, easily digested and environmentally safe (Jungermann & Sonntag, 1991). Glycerol has three hydroxyl groups that are responsible for its solubility in water and its hygroscopic nature. Some of the physicochemical properties of glycerol are listed in Table 1.1.

Table 1.1 : Physicochemical properties of glycerol at 20 °C
(Pagliaro and Rossi, 2010)

Property	Value
Molecular mass	92.09382 g/mol
Density	1.261 g/cm^3
Viscosity	1.5 Pa.s
Melting point	18.2 °C
Boiling point	290.0 °C
Flash point	160 °C (closed cup)
Surface tension	64.00 mN/m

Glycerol was first discovered by K.W. Scheele (1742-1786), the Swedish chemist in 1779. It was discovered accidentally while he was heating a mixture of litharge (lead oxide) and olive oil (Knothe *et al.*, 2005; Jungermann & Sonntag, 1991).

The term glycerine, glycerin or glycerol refers to the purity of the product. "Glycerol" applies only to the pure chemical compound 1,2,3-propanetriol while "glycerine" (or "glycerin") applies to the purified commercial products normally containing more than 95 % of glycerol (Knothe *et al.*, 2005).

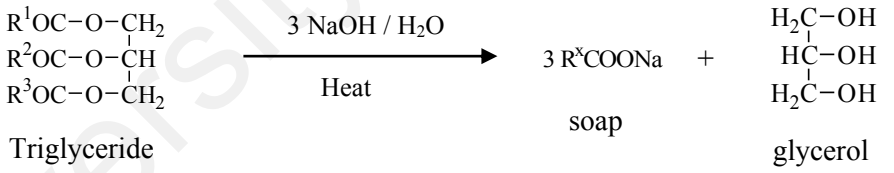
According to the Soap and Detergent Association (1990), there are several grades of glycerine as tabulated in Table 1.2. They differ somewhat in their glycerol content and other characteristics such as colour, odour and trace impurities.

Table 1.2 : Glycerine grades (Soap and Detergent Association, 1990)

Grade	Description
USP GLYCERIN(E)	It is a high purity of glycerine with taste and odour characteristics desirable for pharmaceutical and food purposes. It is clear and almost colourless product. The designation USP (U.S. Pharmacopeia) signifies that the glycerine meets or exceeds the standard established in U.S. Pharmacopeia (USP XXII, 1990). It has official legal status in the United States. Commercially, USP glycerine is commonly available at 96 %, 99 % and 99.5 % anhydrous glycerol content. Concentrations of more than 99.5 % are also available. The equivalence of USP in European Countries is the PH.Eur.
CP GLYCERINE	It is chemically pure glycerine and generally understood to be of the same quality as USP glycerine.
FOOD GRADE GLYCERINE	It meets the requirements outlined in the monograph Glycerin contained in the Food Chemicals Codex prepared by the Committee on Food Protection of the National Research Council in the United States. The requirements are similar to the USP standards.
HIGH GRAVITY GLYCERINE	The designation used in the United States for a commercial grade of glycerine that is clear, almost colourless and conforms to Federal Specification 0-G-491C. It also conforms to Standard Specification for High-Gravity Glycerin, D1257, issued by the American Society for Testing and Materials (ASTM). The glycerol content must not less than 98.7 % based on specific gravity of 1.2587 minimum. It is commonly supplied at not less than 99.0 % glycerol content.
DYNAMITE GLYCERINE	In the United States, it meets all the High Gravity grade specifications except colour but the colour of the glycerine cannot be darker than the Federal Colour Standard.
SAPONIFICATION (88 %) CRUDE AND SOAP LYE (80 %) CRUDE	Generic terms used in the United States to designate grades of crude glycerine recovered from triglycerides where the percentages refer to the glycerol content of the crudes.

Glycerine is found widely in nature as a component of thousands of natural substances (Jungermann & Sonntag, 1991). Naturally occurring glycerol is in the form of glyceride. It occurs in combined form in all animal and vegetable fats and oils. It is usually present as triglycerides (also called triacylglycerols). Triglycerides are *tri*-esters of fatty acids with glycerol where typically the fatty acids are different. That means different fatty acids can be attached to one glycerol backbone. Glycerol also occurs naturally in all animal and vegetable cells in the form of lipids such as lecithin and cephalins (Knothe *et al.*, 2005).

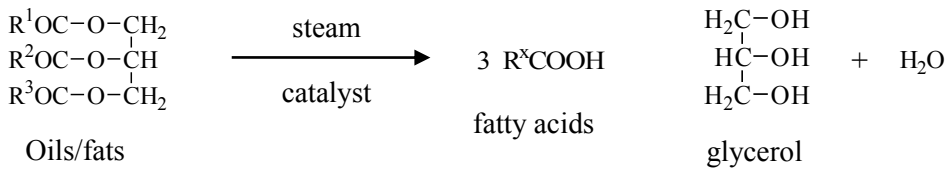
Glycerol is obtained from glycerides through three sources namely soap manufacturing, fatty acid production and fatty ester production. The first source of natural glycerol is the soap manufacturing where the fat is boiled with a caustic soda (sodium hydroxide) solution as shown in Scheme 1.1 (Spitz, 1990; Jungermann & Sonntag, 1991).



Scheme 1.1: Soap manufacturing from fat, producing glycerol as co-product. R^x is a mixture of different alkyl chains; x = 1,2 and 3

The soap and glycerol are formed when fats react with caustic soda and the presence of salt helps to separate the soap from glycerol where the glycerol will go into lower layer which is referred to as spent lye. The glycerol obtained from the soap making is recovered from the spent lye by removing the excess alkali and undergoes several treatments to meet the standard commercial grades.

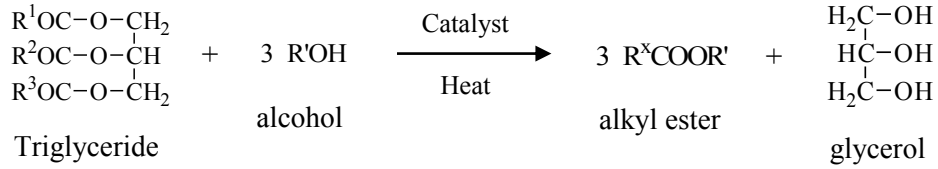
The second source of glycerol is the fatty acid production where the fat or oil is split into fatty acids and glycerol as shown in Scheme 1.2 (Basiron *et al.*, 2000; Spitz, 1990).



Scheme 1.2 : Fatty acid production from fats or oils, producing glycerol as co-product. R^x is a mixture of different alkyl chains; x = 1,2 and 3

The fatty acids from splitting process are used to make soap, reduced to the corresponding fatty alcohol or marketed as fatty acids. The crude glycerol or as being called sweet water obtained from splitting process should be processed promptly after splitting to avoid degradation and loss of glycerol by fermentation.

The last but not least, the source of natural glycerol is the transesterification of oils or fats with alcohol to produce fatty esters as shown in Scheme 1.3 (Knothe *et al.*, 2005; Spitz, 1990). The resulting fatty esters are separated from glycerol by washing with water. The crude glycerol with a few percent of salt content is obtained after acidification process with hydrochloric acid and removal of residual methanol. Historically, the methyl esters have been principally reduced to the corresponding fatty alcohols, but with the advent of biodiesel, methyl esters are used in the fuel industry besides the detergent industry.



Scheme 1.3: Fatty esters production from oils or fats, producing glycerol as co-product. R^x is a mixture of different alkyl chains; x = 1, 2 and 3. The alcohol for producing biodiesel is usually methanol (R' = CH_3)

Glycerol can also be produced *via* synthesis route where it can be produced either from microbial synthesis (fermentation) or chemical synthesis. According to Wang *et al.* (2001), the production of glycerol by yeast fermentation has been known since the investigations of Pasteur in 1858. The microbial production of glycerol has been known for 150 years and it was produced commercially during World War I. However, the production by microbial synthesis subsequently declined since it was unable to compete with chemical synthesis due to the low glycerol yields and the difficulty to purify the glycerol obtained. However, significant improvements have been made in recent years by using osmotolerant yeasts to produce glycerol on a commercial scale in China.

As mentioned earlier, glycerol can be manufactured by chemical synthesis where it can be produced from petroleum feedstock namely propylene. Chlorination of propylene at 510 °C to produce allyl chloride with some co-products is one of the processes where the allyl chloride produced is further treated with hypochlorous acid at 38 °C to produce glycerin dichlorohydrin ($CH_2ClCHClCH_2OH$). The glycerin dichlorohydrin then can be hydrolyzed to glycerin by caustic soda. The production path of glycerol from propylene can be summarized in Scheme 1.4 (Speight, 2002).

$$CH_3CH{=}CH_2 + Cl_2 \rightarrow CH_2ClCH{=}CH_2 + HCl$$

$$CH_2ClCH{=}CH_2 + HOCl \rightarrow CH_2ClCHClCH_2OH$$

$$CH_2ClCHClCH_2OH + 2\,NaOH \rightarrow CH_2OHCHOHCH_2OH + 2\,NaCl$$

Scheme 1.4 : Production path of glycerol from propylene

In addition, Saletan *et al.* (1977) patented the production of glycerol by the hydrolysis reaction of chlorohydrins reactant mixtures containing epichlorohydrin and glycerol *mono*- and *di*-chlorohydrins in the presence of alkali metal carbonate. The temperature of the reaction was about 50 °C to 70 °C and it was done from about 20 hrs to about 150 hrs in a two phase agitated reaction system.

Glycerol has been produced commercially by synthesis from propylene since 1949 (Knothe *et al.*, 2005). The production of synthetic glycerol peaked in the 1960s and 1970s, when it accounted for 50 % to 60 % of the market. However, as the availability of natural glycerine increased, most synthetic producers closed their plants as the process no longer economical. According to Ariyanchira *et al.,* there was a reduction in volume of glycerol from synthetic route from 1999 to 2004. People now prefer natural glycerol to synthetic glycerol because of the many hazardous chemicals involved in the synthesis route. The decrease in glycerol volume from soap manufacturing could also due to alternative soaps manufacturing routes which produce soaps without the formation of glycerol as a by-product. The volume of glycerol from fatty acid and fatty alcohol productions was not much different from 1999 to 2004. But the amount of glycerol from biodiesel production showed a significant increase from 7 % in 1999 to 18 % in 2004 (Ariyanchira *et al*., 2005). In fact, it is projected that the amount of glycerol from biodiesel will increase in the future as shown in Figure 1.2 (as cited in Ciriminna *et al.*, 2014).

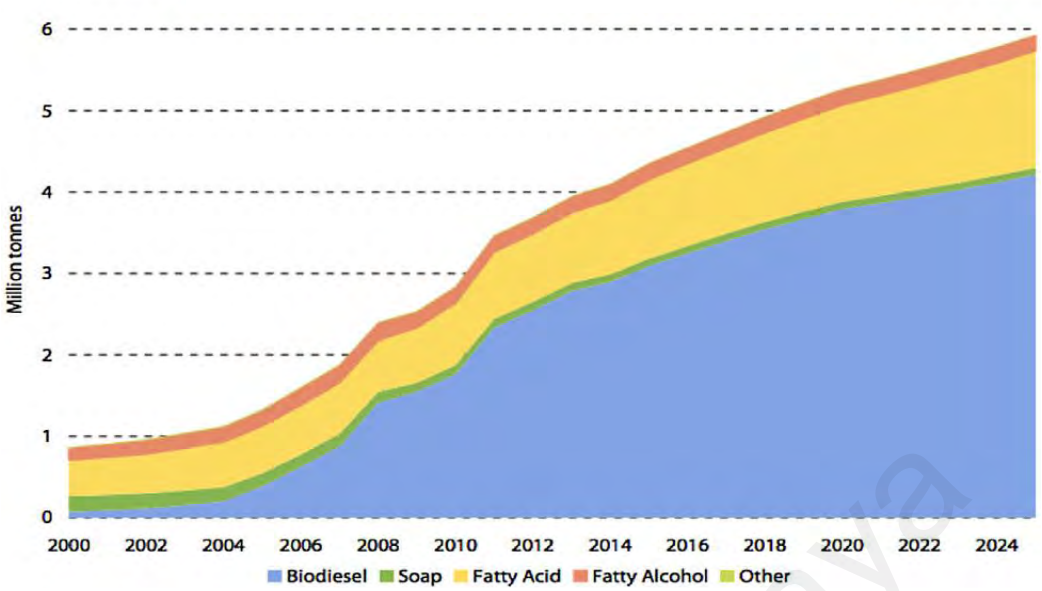


Figure 1.2 : World crude glycerine production by sector (as cited in Ciriminna *et al.*, 2014)

In the United States, the only US supplier of synthetic glycerol, Dow Chemical closed its Freeport, Texas plant in January 2006 due to influx of biodiesel-derived crude glycerol (Nilles, 2006). Today, the commercially available glycerol is mostly from natural glycerol which is purified from co-products generated by oleochemical industry (Patel *et al.*, 2006; Ayoub & Abdullah, 2012). Oleochemicals are chemicals derived from animal or vegetable fats and oils (Spitz, 2004). There are five chemicals considered as basic oleochemicals namely fatty acids, fatty amines, fatty alcohols, fatty esters and glycerol.

As mentioned earlier, the majority of commercially available glycerol results from the purification of the co-product obtained from oleochemical industry. Therefore, glycerol is a valuable co-product from oleochemical industry such as production of fatty acids, biodiesel and soap manufacturing. This is a good sign for oleochemical industry in order to replace a petroleum-based product in possible applications where the oleochemical-based product is claimed to be more environmentally friendly and it is a renewable resources compared to petroleum-based product. Being one of the basic

oleochemicals, glycerol plays an important role in influencing the market of oleochemical industries worldwide. Palm oil is one of the most important vegetable oils in the world besides soybean oil (Spitz, 2004). Malaysia as one of the world major producers of palm oil has also been looking into this oleochemical industry since palm oil is one of the sources of natural glycerol.

There are five grades of glycerine produced in Malaysia. The crude glycerine (80-86 % and 88 % glycerine) are further refined and only three grades of refined glycerine (99.5 %, 99.7 % and 99.8 % glycerine) are traded (Basiron *et al.*, 2000). Figure 1.3 shows the export of glycerine in Malaysia from 2004 to 2015 and it is expected to increase in the future if the biodiesel plants in Malaysia work as planned.

According to ABG Inc. Company as cited in Ayoub and Abdullah, it is estimated that the production of glycerol would reach 5.8 billion pounds in 2020. This is due to the demand of biodiesel that is projected at 8 billion gallons in 2020 (Ayoub and Abdullah, 2012).

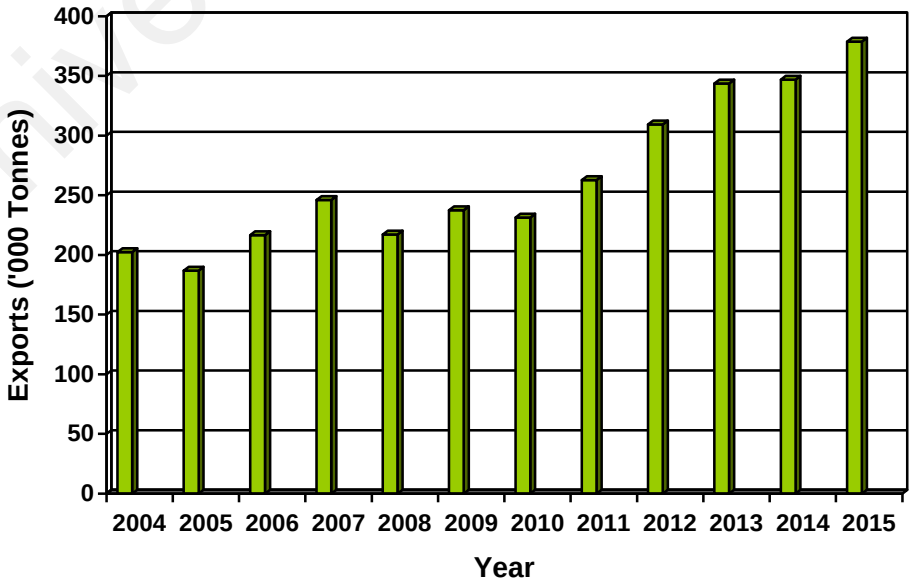


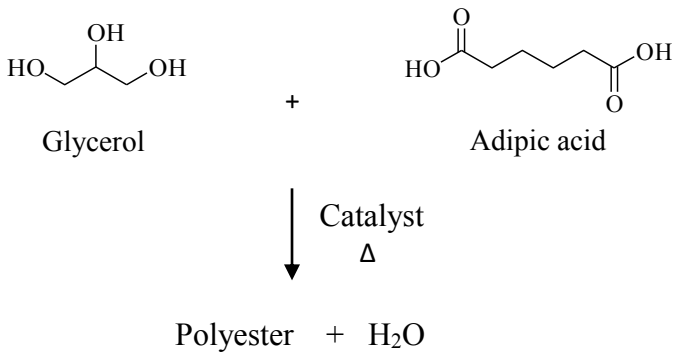
Figure 1.3 : Export volume of glycerine from Malaysia (MPOB, 2005-2016)

Glycerol is used in cosmetics, shampoos, soaps, herbal remedies, pharmaceuticals and other household products (Heming, 1999). It is versatile and is used in more than 1000 different products because it is non-toxic and is safe to use in foods, as it is easily digested, as stated by SDA Science.org (Peterman, 2011). Therefore, it is used both in food and non-food industry. In fact, more potential uses of glycerol are being investigated and still in research and development stage in order to add value to glycerol. Besides being used directly in consumer product formulations, glycerol has been used as a starting material to produce other intermediate compounds or products with other possible applications. This will add value to glycerol by varying its applications.

1.2 Chemistry of Glycerol

Having three free hydroxyl groups makes glycerol an ideal starting material for a very large number of derivatives. Chemical modification of glycerol is an important route to obtain industrial products using renewable feedstock. Glycerol can undergo esterification process, etherification or oxidation in the presence of suitable chemical substances and reaction conditions.

Glycerol esters can be produced by esterification of glycerol with carboxylic acids or by transesterification with fatty esters. For example, glycerol was esterified with decanoic acid in the presence of lipase as catalyst. The effect of organic solvents on the equilibrium position of the esterification process was investigated (Janseen *et al.*, 1993). On the other hand, Brioude *et al.* (2007) demonstrated the production of polyesters from the reaction of glycerol with adipic acid by using dibutyltin dilaurate as catalyst (Scheme 1.5).

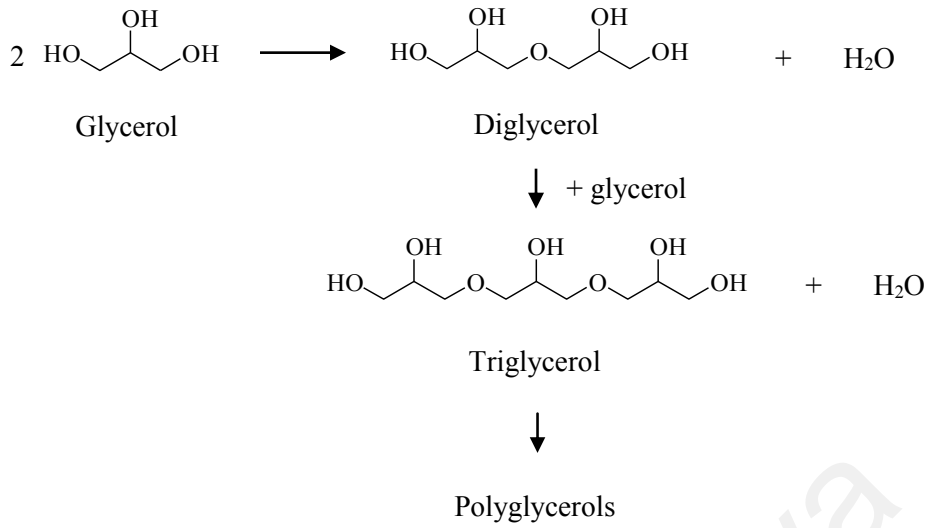


Scheme 1.5: Esterification of glycerol with adipic acid in the presence of dibutyltin dilaurate

In addition, glycerol *tri*-acetate was prepared from the esterification of glycerol with acetic acid. By using acidic functionalized ionic liquids as catalyst, the yield of the product produced was above 95 % (Li *et al.*, 2009). Other important glycerol ester is the cyclic ester of glycerol which is discussed in section 1.2.2.

According to Jerom *et al.*, the transesterification of glycerol with fatty methyl esters had formed glycerol esters with over 95 % yield and about 60 % selectivity to *mono*-glycerides. The one-pot reaction was catalyzed by guanidine derivatives in the absence of solvent (as cited in Barrault & Jerome, 2008).

Glycerol can be linked *via* ether bonding to linear or cyclic compounds. A higher number of glycerol molecules is linked together forming polyglycerols. The direct and selective synthesis of polyglycerols having a low polymerization degree from self-etherification of glycerol was demonstrated by Claceus *et al.* (2002). The reaction was conducted in the presence of solid mesoporous catalyst prepared by caesium impregnation and in the absence of solvent. Scheme 1.6 shows the etherification of glycerol to polyglycerols.

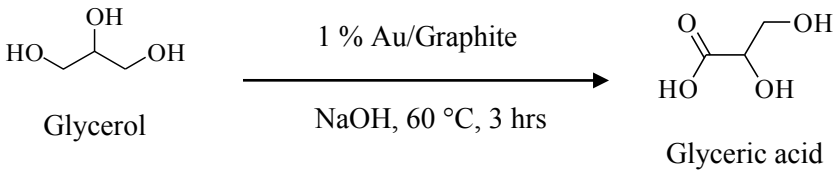


Scheme 1.6 : The etherification of glycerol to polyglycerols

Stuhler (1985) claimed the condensation of glycerol to polyglycerol at a temperature of 190 °C to 250 °C in the presence of catalyst containing a particular ratio of phosphorus and an alkali metal. Meanwhile, Seidin and Martin (1976) claimed the etherification process was conducted under reduced pressure and catalyzed by the addition of sulphuric acid and a low molecular weight glyceride to produce polyglycerol. Furthermore, Usha and Maitra (2016) demonstrated the preparation of polyglycerol using conventional heating at 270 °C for 5 hrs. Other etherified compound of glycerol is glycerol alkyl ether. The preparation of alkyl ethers of glycerol is discussed in section 1.2.1.

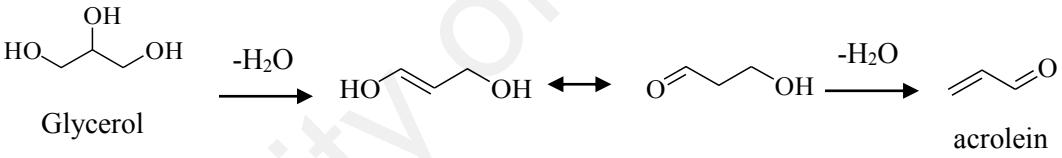
As mentioned earlier, glycerol can also undergo oxidation process. Glycerol can be oxidized to glyceraldehydes, dihydroxyacetone or glyceric acid. Glycerol conversion to 1,3-dihydroxyacetone by glycerol dehydrogenase co-expressed with an NADH oxidase for cofactor regeneration was reported by Zhang *et al.* (2016). Meanwhile, Lari *et al.* (2016) investigated the glycerol oxidation to dihydroxyacetone over MFI-type iron zeolite catalysts.

Carrettin *et al.* (2002) demonstrated the oxidation of glycerol to glyceric acid using gold catalyst (Scheme 1.7) under mild condition. The selectivity to glyceric acid was 100 %.



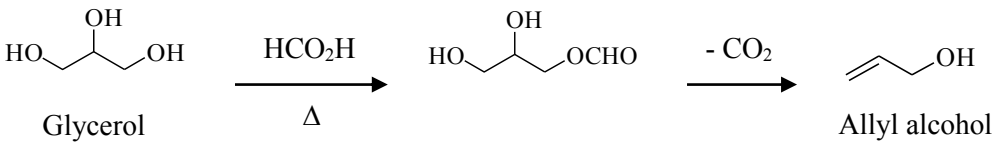
Scheme 1.7 : Oxidation of glycerol to glyceric acid

Claceus *et al.* (2002) reported the dehydration of glycerol to acrolein in the presence of lanthanum or magnesium containing catalyst. Scheme 1.8 shows the formation of acrolein from glycerol.



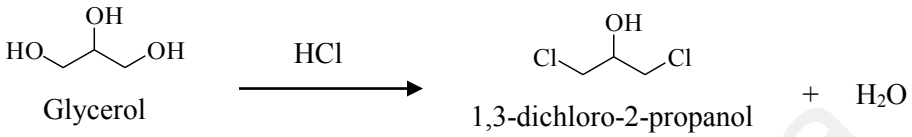
Scheme 1.8 : Double dehydration of glycerol to form acrolein

Other compound that can be produced from glycerol is allyl alcohol. Bergman *et al.* (2008) investigated the production of allyl alcohol by heating glycerol and carboxylic acid preferably formic acid under an inert atmosphere (Scheme 1.9). Distillation process of the reaction product yielded about 80 % or greater of allyl alcohol.



Scheme 1.9 : Production of allyl alcohol from glycerol

In addition, Luo *et al.* (2009) reported the chlorination of glycerol in which 1,3-dichloro-2-propanol was directly prepared from the reaction of glycerol with hydrochloric acid in the presence of acetic acid as catalyst (Scheme 1.10). Further reaction of the chlorinated product can be done to obtain epichlorohydrine.



Glycerol 1,3-dichloro-2-propanol

Scheme 1.10 : The formation of 1,3-dichloro-2-propanol from glycerol

Meanwhile, the production of propylene glycol from glycerol was reported where the preparation involved two-step reaction. The first step was the hydrogenation of glycerol to acetol using copper-chromium catalyst where the conversion of glycerol was 90 %. Then hydrogenolysis of acetol to propylene glycol with the same type of catalyst resulted in 93.5 % conversion of acetol (Suppes, 2006).

Other than that, glycerol can be used as carbon and energy sources replacing other carbohydrates in fermentation processes. Therefore, it can also be subjected to biotransformation process. According to Baumann and co-workers, 1,3-propanediol, dihydroxyacetone, glyceraldehyde, 3-hydroxypropyl aldehyde, pyruvic acid and 3-hydroxypropionic acid are among fermentation products obtained from glycerol (as cited in Basiron *et al.*, 2000).

As reported by Chen *et al.* (2003), glycerol was converted to 1,3-propanediol through a bioconversion by *Klebsiella pneumonia* under microaerobic conditions. Meanwhile, Malaoui and Marczak (2001) investigated the use of *Clostridium butyricum* in the preparation of 1,3-propanediol from glycerol. Furthermore, Mirończuk *et al.* (2016)

reported a novel strain of *Yarrowia lipolytica* as a platform for synthesis of erythritol and citric acid from glycerol.

In latter sections (1.2.1 – 1.2.5), topics on the conversion of glycerol to its derivatives that are relevant to this research project are discussed in more details.

1.2.1 Glycerol to Glyceryl Ether

As mentioned earlier, glycerol can be etherified by suitable chemical agent to produce glyceryl ether. Referring to that, Klepáčová *et al.* (2005) had studied a *tert*-butylation of glycerol with isobutylene without solvent in the presence of strong acid ion-exchange resins Amberlyst type to produce glyceryl ether. The reaction was also done in the presence of two large-pore zeolites H-Y and H-Beta. The highest glycerol conversion of 100 % was obtained over strong acid ion-exchange resin A35 at 60 °C.

di- and *tri*-*tert*-butyl ethers of glycerol (higher ethers of glycerol) have potential as oxygenate additives to diesel fuels because of their blending with diesel. From the work done by Klepáčová and co-workers, the highest yield of 88.7 % of desired *di*- and *tri*-ethers of glycerol was obtained over catalyst A35 at 60 °C. The reaction of glycerol with isobutene in the presence of catalyst was first investigated by Behr and Lohr at the Henkel Company (as cited in Behr *et al.*, 2008).

A technical process for the production of the higher butyl ethers of glycerol was developed by Behr in 1994 (as cited in Behr & Gomes, 2010). In a stirred-tank reactor, the etherification of glycerol and isobutene takes place under mild conditions (70-110 °C, 20-30 bar) in the presence of *p*-toluenesulfonic acid.

In 1995, Gupta patented (US5476971) the use of *p*-toluenesulfonic acid or methane sulfonic acid as a catalyst for the production of glyceryl ether compound. The etherifications were carried out at the surplus of isobutylene at the temperatures from 50 ºC to 100 ºC (Gupta, 1995).

On the other hand, Kesling and co-workers reported an improved diesel fuel which contains an ether derivative of glycerol in amount sufficient to reduce particulate matter emissions (Kesling *et al.*, 1994). The mixture of glycerol alkyl ethers was prepared by etherification of glycerol by alkenes and preferentially by isobutylene at molar ratio of glycerol/isobutylene is 1:2 and above, on strongly acidic catex Amberlyst. The temperature used was from 50 ºC to 150 ºC. The reduction of carbon oxide, hydrocarbons, aldehydes and particulate matters was proven after application of the glycerol alkyl ethers to diesel fuel. The composition comprised at least 70 % diesel hydrocarbons together with 1-30 % of said glyceryl ether or the combination of methyl soyate and said ether.

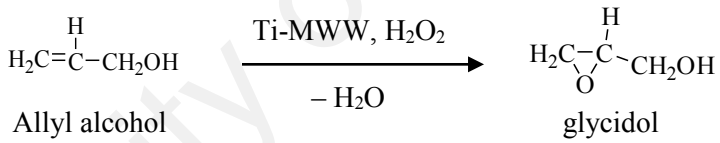
In addition, Janaun and Ellis (2010) investigated the application of sulfonated carbon catalyst for the production of glyceryl ethers. The sulfonated carbon catalyst was prepared by sulphonation of the sugar char and it is capable to promote the production of glyceryl ethers.

1.2.2 Glycerol to Glycidol

Glycidol which is an epoxide compound can be obtained by several routes. It is commonly obtained by the epoxidation of allyl alcohol. As patented in US3,625,981, the epoxidation of allyl alcohol was done with organic hydroperoxide in the presence of an inorganic vanadium compound as catalyst. The allyl alcohol used was obtained from

isomerization of propylene oxide in the presence of lithium phosphate catalyst. The work demonstrated the outstanding superiority of vanadium catalysts in the conversion of allyl alcohol to glycidol (Kollar, 1971).

There are several published papers reporting on the epoxidation of allyl alcohol with a hydrogen peroxide in the presence of a catalyst. Hutchings *et al.* (1996) investigated the epoxidation of allyl alcohol with hydrogen peroxide as the oxidant over titanium silicalite catalyst TS-1. They found that the acidity of the TS-1 is a crucial controlling parameter for the selectivity to glycidol. In recent study, Fajdek *et al.* (2011) demonstrated the epoxidation of allyl alcohol with 30 % hydrogen peroxide (H_2O_2) in the presence of MWW type titanosilicalite catalyst as shown in Scheme 1.11. The process was done under atmospheric pressure in the presence of methanol as a solvent.



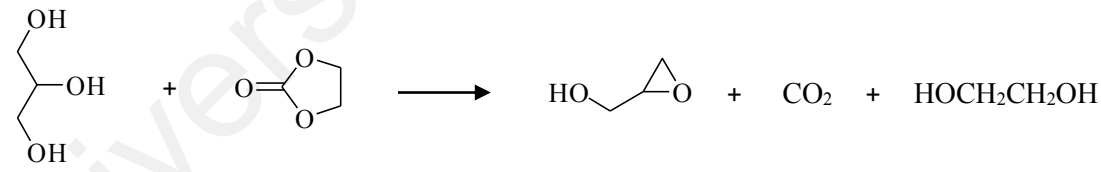
Scheme 1.11 : Epoxidation of allyl alcohol with hydrogen peroxide to form glycidol

Furthermore, other titanium silicalite catalyst namely Ti-SBA-15 which was prepared by direct hydrothermal synthesis was also used in the epoxidation process for testing its catalytic properties towards the formation of glycidol (Wróblewska & Makuch, 2012). Besides that, Danov *et al.* (2011) patented the epoxidation process of allyl alcohol with hydrogen peroxide using titanium containing zeolite as catalyst. The maximum yield of glycidol achieved was 93.6 %.

In addition, Payne & Sullivan (1961) claimed in US3,005,832 patent the process of preparing glycidol by hydrogenating glycidaldehide at a temperature of about 50 °C to

about 200 °C and at a pressure from about 500 to 1400 p.s.i.g. in the presence of copper chromite. On the other hand, Yu *et al.* (2007) prepared the glycidol from the reaction of 3-chloro-1,2-propanediol with NaOH and obtained the yield of 84.5 % under the optimal reaction conditions. Other than that, glycidol can also be produced from epichlorohydrin or acrolein.

The raw materials mentioned previously in the production of glycidol are of petroleum based. As an alternative, glycidol is also produced from glycerol which is a renewable source compared to petroleum-based raw materials. Bruson *et al.* (1953) patented the method for the production of glycidol by heating glycerol with a cyclic alkylene carbonate. It is believed that the glycerol will transform into an intermediate compound namely glyceryl carbonate in the presence of carbonating agent in the first step before the formation of glycidol in the second step with CO_2 liberation. The reaction may be formulated to its simplest form as in Scheme 1.12.



Glycerol | ethylene carbonate | glycidol | carbon dioxide | ethylene glycol

Scheme 1.12 : Production of glycidol from glycerol and ethylene carbonate

As glyceryl carbonate acts as an intermediate compound in the production of glycidol, some research works on the production of glyceryl carbonate from glycerol will be discussed simultaneously with the formation of glycidol. In fact, synthesis works on the preparation of glycidol from glyceryl carbonate as a starting material have also been investigated.

Glyceryl carbonate can be obtained from the reaction of glycerol with carbonating agent in the absence or presence of catalyst. Yoo and Mouloungui (2003) reported the preparation of glyceryl carbonate through the reaction of glycerol with urea in the presence of zinc mesoporous system. Heterogeneous catalyst system investigated included metallic sulfates or zinc organometallic sulfates. A molar yield of 86 % of glyceryl carbonate was obtained in the carbonylation of glycerol with urea at 140 °C, 30 mbar for two hrs in the presence of zinc sulfate. On the other hand, Wang *et al.* (2011) reported the carbonylation process in the presence of lanthanum oxide catalyst.

Other heterogeneous catalyst which is gold-based catalyst where the support was magnesium oxide was also used in the preparation of glyceryl carbonate (Hammond *et al.*, 2011). Besides that, the glycerolysis of urea was done in the presence of zirconium phosphate catalysts where *γ*-zirconium phosphate exhibited a good activity with 80 % conversion of glycerol. The recoverable and reusable of the catalyst in subsequent cycle of reaction were also investigated (Aresta *et al.*, 2009). Furthermore, the carbonylation process was also carried out in the presence of ionic liquid immobilized onto a structurally modified Merryfield peptide resin (MPR) and proved to be effective heterogeneous catalyst for that particular reaction (Kim *et al.*, 2011).

Besides urea, other carbonating agents such as carbon monoxide, carbon dioxide, alkyl carbonate and dialkyl carbonate are also used in the production of glyceryl carbonate. The carbonylation of glycerol with carbon monoxide and K_2CO_3 in the presence of selenium catalyst under 0.1 MPa in DMF followed by oxidation of selenocarbonate salt resulted in 83-84 % yield of glyceryl carbonate (Mizuno *et al.*, 2010).

Meanwhile, Ochoa-Gomez *et al.* (2011) reported the preparation of glyceryl carbonate through the reaction of 3-chloro-1,2-propanediol with carbon dioxide in the presence of triethylamine. A complete conversion of 3-chloro-1,2-propanediol and 90 % yield of glyceryl carbonate were obtained at 100 °C, using a carbon dioxide pressure of 25 bar and 60 minutes. 3-chloro-1,2-propanediol which was used as the starting material in the process is derived from glycerol. Before that, the same group of researchers investigated the reaction in the presence of K_2CO_3 where the yield of glyceryl carbonate was 80 % together with a simultaneous substantial production of glycidol (Gomez-Jimenez-Aberasturi et. al., 2010).

Huang *et al.* (2008) discovered the addition of acetonitrile in the reaction of glycerol with carbon dioxide in the presence of inorganic bases and organic bases catalysts improve the glycerol conversion and the yield of glyceryl carbonate. The acetonitrile not only acts as a solvent but also as a dehydrating agent to take away partially water formed in the reaction, thus improve the yield. In 1999, the use of supercritical CO_2 as a reaction medium and as a source of carbonate for the production of glyceryl carbonate was done where the product could be obtained by direct reaction of glycerol with CO_2 using ethylene carbonate co-reactant in the presence of zeolites and ion exchange resins (Vieville *et al.*, 1999).

The most widely carbonating agent investigated in the production of glyceryl carbonate from glycerol is dimethyl carbonate. Du *et al.* (2012) found that K_2CO_3/MgO was the most efficient catalyst in the production of glyceryl carbonate compared to CaO/MgO, KNO_3/MgO or KOH/MgO. The glyceryl carbonate yield of approximately 99 % was obtained when the reaction of glycerol with dimethyl carbonate was carried out in the presence of 1 % of K_2CO_3/MgO at 80 ºC for two hrs. Ochoa-Gomez *et al.* (2009)

reported the use of CaO in the transesterification process resulted in a 100 % conversion of glycerol with more than 95 % yield of glyceryl carbonate at 90 minutes reaction period. Furthermore, Simanjuntak *et al.* (2011) also used CaO catalyst in the transesterification process of glycerol with dimethyl carbonate.

In addition, other researchers carried out a coupling reaction and azeotropic distillation for the synthesis of glyceryl carbonate in the presence of CaO catalyst. At a molar ratio of 1 : 1 of dimethyl carbonate to glycerol, the yield of glyceryl carbonate can be as high as 98 % with benzene as azeotropic agent (Li & Wang, 2010). A mixed metal oxide catalyst namely CaO-PbO was also used in the production of glyceryl carbonate (Kang *et al.*, 2011). The glycerol conversion and the yield of glyceryl carbonate could reach 98.5 % and 97.8 % respectively under the appropriate reaction conditions.

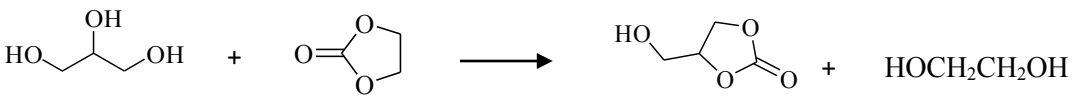
Bai *et al.* (2011) demonstrated the use of hydroxyapatite (HAP) modified with different metal salts as a catalyst for the synthesis of glyceryl carbonate. The glycerol conversion and the yield of glyceryl carbonate reached 99.3 % and 99.0 % respectively when the reaction was conducted at 78 ºC for 50 minutes with the molar ratio of dimethyl carbonate/glycerol of 2:1 in the presence of 3 % of KF/HAP catalyst.

The transesterification process was also carried out in the presence of Mg/Al/Zr mixed oxide base catalyst. As the transesterification process depends on the Mg/Al/Zr molar ratio, the catalyst with Mg/Al/Zr molar ratio of 3:1:1 exhibited an excellent activity towards the formation of glyceryl carbonate (Malyaadri *et al.*, 2011). Besides that, Takagaki *et al.* (2010) reported the use of an uncalcined Mg-Al hydrotalcite as a highly active base catalyst for the production of glyceryl carbonate under moderate reaction conditions. A greener approach which is the use of 1-butyl-3-methylimidazolium-2-

carboxylate as a catalyst for the production of glyceryl carbonate was described by Naik *et al.* (2009). A 1 % of catalyst dosage was sufficient to yield quantitative conversions of glycerol.

The synthesis of glyceryl carbonate from glycerol and dimethyl carbonate has also been developed through a biocatalytic route. A lipase from *Aspergillus niger* was found to be the most efficient biocatalyst from the enzyme screening process where the optimization of the reaction conditions resulted in a 74 % conversion of glycerol with 59.3 % yield of glyceryl carbonate (Tudorache *et al.*, 2012). Other lipase namely Novozyme 435 was reported to exhibit higher catalytic activity towards the formation of glyceryl carbonate (Lee *et al.*, 2010) and the use of Novozyme 435 as biocatalyst in the transesterification process was also investigated by Kim *et al.* (2007).

Ethylene carbonate has also been used as a carbonylation agent in the production of glycerol instead of dimethyl carbonate. Climent *et al.* (2010) demonstrated the synthesis of glyceryl carbonate by reacting glycerol with ethylene carbonate catalyzed by basic oxides (MgO and CaO) and mixed oxides (Al/Mg and Al/Li) derived from hydrotalcites as shown in Scheme 1.13. Meanwhile, Cho *et al.* (2010) investigated the reaction of glycerol with ethylene carbonate in the presence of ionic liquid immobilized on mesoporous MCM41 as a catalyst.

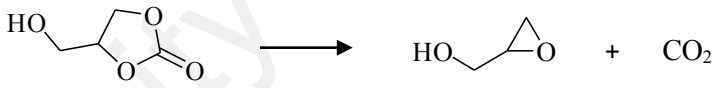


Glycerol ethylene carbonate glyceryl carbonate ethylene glycol

Scheme 1.13 : Synthesis of glyceryl carbonate from glycerol and ethylene carbonate in the presence of a base

Dadsaheb and Arvind (2010) patented a process for the production of glyceryl carbonate and glycidol from the reaction of glycerol with dimethyl carbonate over heterogeneous solid base catalyst comprising supported mixed oxide base catalyst. Furthermore, Liu *et al.* (2009) demonstrated the synthesis of glyceryl carbonate through the reaction of glycerol with urea in the presence of $ZnSO_4$ as a catalyst and the release of CO_2 from the former compound catalyzed by sodium phosphate formed the glycidol in 83.8 % yield.

In addition, several patents have been granted for the production of glycidol from glyceryl carbonate. In 1958, Malkemus and co-workers patented the preparation of glycidol from the decomposition of glyceryl carbonate. The glyceryl carbonate is heated in the presence of a metal salt forming glycidol and carbon dioxide as shown in Scheme 1.14 (Malkemus *et al.*, 1958).



Glyceryl carbonate glycidol carbon dioxide

Scheme 1.14 : Decomposition of glyceryl carbonate to glycidol and carbon dioxide

In US6,316,641B1 patent, Yoo *et al.* (2001) demonstrated the production of glycidol by heating the glycerol carbonate under reduced pressure in the presence of a solid catalyst consisting of a type A zeolite or *γ*-alumina. In another patent, the glyceryl carbonate was subjected to decarboxylation reaction in the presence of a catalyst comprising a neutral salt and a solvent containing no active hydrogen in order to obtain a high selectivity to glycidol (Seki *et al.*, 2011).

1.2.3 Ring Opening of Glycidol

The strained three-membered ring of epoxide of glycidol (Figure 1.4) makes it highly susceptible to ring opening reactions. The cleavage of one of the carbon-oxygen bonds opens the ring of the epoxide. The process can be initiated by either electrophiles or nucleophiles, or catalyzed by either acids or bases.

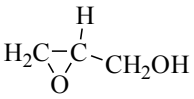
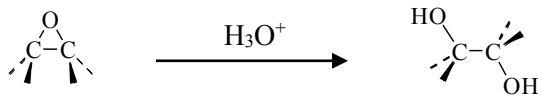


Figure 1.4 : Chemical structure of glycidol

Like other ethers, epoxides are cleaved by acid treatment but under much milder conditions because of the ring strain. For example, dilute aqueous acid at room temperature is sufficient to catalyze the hydrolysis of the epoxides to 1,2-diols, also called vicinal glycol (McMurry, 2008). Scheme 1.15 shows the ring opening of epoxides with water in the presence of acid where the epoxide is protonated to increase its reactivity, followed by nucleophilic addition of water. In acid-catalyzed ring opening, the nucleophile attacks the protonated epoxide ring from the side opposite the epoxide group. The carbon being attacked undergoes an inversion of configuration. The new C-O bond is always formed from the side opposite that of the original epoxide ring because the ring-opening reaction is an S_N2 reaction.



Scheme 1.15 : Ring opening of epoxides with water in the presence of acid

The nucleophiles for ring opening reaction of epoxide could be water or alcohol resulting in the formation of substances bearing vicinally arranged substituents of which one is a hydroxyl group (Daute *et al.*, 1995).

In 1983, Merk and co-workers patented the synthesis of guaiacol glyceryl ether by ring opening process of glycidol with *o*-methoxyphenol. The reactions were carried out at 80° C to 150 °C in the presence of an alkali hydroxide, an alkali alcoholate, an alkali cyanate or an alkali thiocyanate. The product was obtained in very good yield and in very high purity (Merk *et al.*, 1983). Twenty five years later, the ring opening process of glycidol was also patented but the epoxide ring of glycidol was opened by menthol to obtain L-menthoxypropyleneglycol. The process was done in organic solvent and in the presence of Lewis acid as catalyst (Zhang *et al.*, 2008).

Epoxide rings can be cleaved by base as well as by acid. The strain of the three-membered ring causes epoxides to react with hydroxide ion at elevated temperatures eventhough an ether oxygen is normally a poor leaving group in an S_N2 reaction. Attack of the nucleophile takes place at the less hindered epoxide carbon in base-catalyzed ring opening of epoxide (McMurry, 2008).

1.2.4 Glycerol to Solketal

Solketal is a ketals which can be obtained from the reaction of glycerol with acetone in the presence of an acid catalyst. The use of *p*-toluenesulfonic acid in the reaction of glycerol and acetone to produce solketal was investigated (Newman and Renoll, 1945; Bruchmann *et al.*, 1999). Bruchmann *et al.* demonstrated that more than 90 % yield of solketal was obtained from the process. Solketal can also be produced using calcium

carbide as catalyst instead of *p*-toluenesulfonic acid as reported by Maglio and Burger (1946). The maximum yield of 84 % of solketal was obtained from the process.

Solketal which is also known as 1,2-*O*-isopropylidene glycerol is a well-known protected glycerol. This was shown by Yu and co-workers (2003) where they synthesized the 1,2-*O*-isopropylidene glycerol as an intermediate in order to produce glycerol *mono*-stearate. The solketal can be obtained by refluxing glycerol with excess of acetone in the presence of an acid catalyst. However, on a large scale, the amount of acetone can be reduced by using chloroform as a solvent (Scheme 1.16). After neutralizing process of *p*-toluenesulfonic acid, the reaction mixture was distilled and resulted in 94 % yield of pure solketal. Besides that, Jing *et al.* (2011) had also used the protected glycerol, solketal as an intermediate to produce a high purity of *α*-glycerophosphoric acid.

Scheme 1.16 : Production of solketal from glycerol and acetone

Nowadays, the use of heterogeneous catalyst is preferable to homogeneous catalyst due to easier and better separation of the catalyst from the reaction product. In fact, some of the catalysts could be reused for several times with no significant difference in product yield. As reported by Roldán (2009), a heterogeneous catalyst namely K10 montmorillonite was used in the production of solketal. With a help of zeolite membrane to remove water formed during the reaction, there was a significant improvement in glycerol conversion with a reduction in the excess acetone needed. On

the other hand, by using that cheap and reusable catalyst, the ketals was synthesized in a good yield and can be used in biodiesel blends as reported by Miriam *et al.* (2012). In addition, titanium cation-exchanged montmorillonite exhibited high catalytic activity for the reaction of glycerol with ketones or aldehydes to produce cyclic acetals and the catalyst could be easily separated and reused (Takato *et al.*, 2012).

The acetalisation of glycerol was also catalyzed by heteropolyacids, immobilized in silica. A sol-gel method was used to immobilized tungstophosphoric (PW), molybdophosphoric (PMo), tungstosilisic (SiW) and molybdosilisic (SiMo) acids in silica. The catalytic activity decreased in the order of PW_S > SiW_S > PMo_S > SiMo_S. However, all catalysts exhibited good selectivity to solketal (Ferreira *et al.*, 2010). Other than that, the acetalisation of glycerol with acetone was performed over zirconia and promoted zirconia catalysts to produce solketal as reported by Padigapati S *et al.* (2011). The promoted zirconia catalysts exhibited promising catalytic activity and showed an excellent selectivity towards solketal.

A fully continuous process for the production of solketal was also developed where a heterogeneous catalyst namely Amberlyst DPT-1 was used in the process. The semi-continuous reactor experiment and sealed-tube experiment were also conducted as a model reaction (Clarkson *et al.*, 2001).

Other approach to prepare the solketal was made by Baohua *et al.* (2011) where the condensation reaction of glycerol with acetone was carried out in the presence of sulfonate-type ionic liquid catalysts. The simple procedures, high selectivity with a short reaction time (15 minutes) had resulted in 85.1 % yield of solketal.

Besides using high purity of glycerol, an attempt to use crude glycerine mixture from biodiesel production to prepare the solketal was made by Dengzheng *et al.* (2011). The crude glycerine was reacted with an aldehyde, a ketone and/or an acid at 20-110 °C for 3-10 hrs to give the glycerol ketal and glycerol fatty acid ester, *etc*.

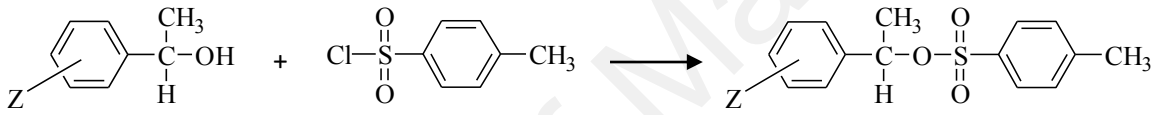
1.2.5 Transformation of Hydroxyl Group of Alcohol into a Good Leaving Group

Alcohols are very important compounds for synthesis. However, the hydroxide ion of alcohol is a very poor leaving group, thus decrease the reactivity of the alcohol (McMurry, 2008). There are several ways or methods that could be used in transforming the hydroxyl group of alcohol into a good leaving group where the product obtained could be used as an intermediate for further reaction. An effective way of installing the reactive leaving group is the preparation of sulfonate esters from alcohols. There are tosylation, mesylation, *etc*. Different process involves different substrate which producing different intermediate. For example, the tosylation process involves a reaction between the alcohol with tosylation agent namely *p*-toluenesulfonyl chloride (tosyl chloride) to produce tosylated compound (sulfonate ester).

The common method for introducing tosyl groups is to allow the alcohol to react with *p*-toluenesulfonyl chloride in the presence of pyridine. Tipson (1944) describes the treatment of alcohols such as ethanol, benzyl alcohol and 2,4-dinitrophenol with *p*-toluenesulfonyl chloride in pyridine. Meanwhile, Brown *et al.* (1967) demonstrated the preparation of 3-*p*-anisyl-3-methyl-2-butyl tosylate from the reaction of 3-*p*-anisyl-3-methyl-2-butanol with *p*-toluenesulfonyl chloride in pyridine. 2-*p*-anisylethanol, 2-*p*-tolylethanol and 2-*m*-tolylethanol were also converted to the corresponding tosyl esters by the usual pyridine procedure.

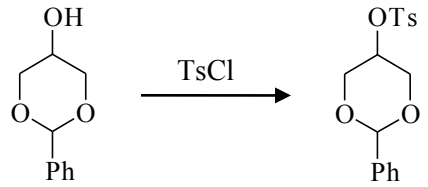
Kabalka and co-workers investigated the tosylation of decyl alcohol with *p*-toluenesulfonyl chloride in pyridine and chloroform was used as a solvent. The general procedure was used to produce 10-undecyn-1-yl *p*-toluenesulfonate, 12-tridecyn-1-yl *p*-toluenesulfonate, 4-undecyn-1-yl *p*-toluenesulfonate, 4-*tert*-butylcyclohex-1-yl *p*-toluenesulfonate and 4-hexyn-2-yl *p*-toluenesulfonate (Kabalka *et al.*, 1986).

Furthermore, Lee *et al.* (2000) describes the preparation of substituted 1-phenylethyl tosylates by reacting substituted 1-phenylethyl alcohols and *p*-toluenesulfonyl chloride as shown in Scheme 1.17.



Scheme 1.17 : Reaction of substituted 1-phenylethyl alcohols and *p*-toluenesulfonyl chloride where Z is *p*-NO_2, *m*-NO_2, *m*-Cl, *p*-Cl, H, *m*-Me

According to Wang *et al.* (2003), 2-phenyl-5-tosyloxy-1,3-dioxane was obtained from the tosylation process of 5-hydroxyl-2-phenyl-1,3-dioxane as illustrated in Scheme 1.18.

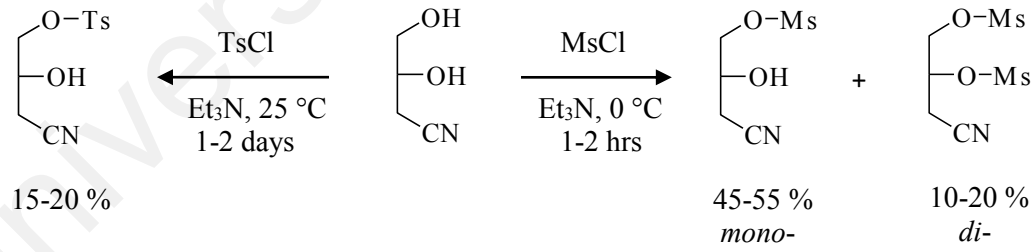


Scheme 1.18 : Tosylation process of 5-hydroxyl-2-phenyl-1,3-dioxane

Meanwhile, Ding *et al.* (2011) reported that benzyl tosylate was produced in 53 % yield when benzyl alcohol without any electron withdrawing substituent on benzene ring reacted with tosyl chloride.

In addition, Razieh *et al.* (2006) demonstrated the tosylation of some alcohols and phenols with *p*-toluenesulfonyl chloride using heteropolyacids as catalyst under solvent-free conditions. The method consistently has the advantage of excellent yield and short reaction time. On the other hand, Kazemi *et al.* (2007) investigated the tosylation of alcohols using a solid base as catalyst under solvent-free conditions.

Besides tosyl group, mesyl group can also be introduced to the alcohols by allowing the alcohols to react with methanesulfonyl chloride. As reported by Jung and Shaw (1980), the formation of both sulfonate esters from the reaction of cyanodiol with *p*-toluenesulfonyl chloride and methanesulfonyl chloride were investigated. Reaction of cyanodiol with *p*-toluenesulfonyl chloride resulted in a low yield of desired *mono*-tosylate. However, reaction of cyanodiol with methanesulfonyl chloride formed *mono*-mesylate in reasonable yield along with *di*-mesylate. Both reactions are illustrated in Scheme 1.19.



Scheme 1.19 : Reaction of cyanodiol with *p*-toluenesulfonyl chloride and methanesulfonyl chloride in the presence of triethylamine

In addition, Crossland and Servis (1970) also investigated the use of methanesulfonyl chloride in the preparation of sulfonate esters of alcohols.

Another useful sulfonate ester is *tri*-fluoromethanesulfonates (triflates). Beard *et al.* (1973) demonstrated the preparation of triflates of alkyl and allylic alcohols from the reactions of corresponding alcohols with *tri*-fluoromethanesulfonic acid anhydride. Isopropyl alcohol, 2-fluoro-2,2-dinitroethanol and allyl alcohol were converted to its' triflates in halogenated solvents in the presence of pyridine.

1.3 Applications of Glycerol and Its Derivatives

Nowadays, a number of applications of glycerol and its derivatives have been highlighted. Some of them are already in the stage of implementation by the industry and some are still under investigations. The applications of glycerol and its derivatives are very important in order to add value to the glycerol itself by expanding the use of glycerol. The uses of glycerol and its derivatives will be discussed in this chapter including the uses in food industry, cosmetic and personal care products besides other applications.

1.3.1 Food Industry

Glycerol is useful as a food additive since it has a sweet taste and low level of toxicity (Robinson, 2010). Glycerol is used to sweeten as well as to add a chewy texture in the food industry as it is about 60 % as sweet as sucrose (Haycock, 2001). It is a common sugar substitute, especially for diabetics (Robinson, 2010). The manufacturer of glycerin in the U.S., Procter & Gamble (P&G) Chemicals has long served the food industry with its quality line of Superol^{TM}, Moon^{TM} and Star^{TM} glycerin brands. P&G's glycerin functions as a humectants, solvent, sweetener and preservative in foods and beverages. It acts as a humectants and softening agent in candy, cakes and casings for meats and cheeses. It also acts as a solvent for flavours and food colours in soft drinks

and confections. Besides that, it helps to retain moisture and enhance palatability in dry pet foods (P&G Chemicals, 2012).

Glycerol itself is not a surfactant but by modifying its molecule, the glycerol derivatives could be used as surfactant or emulsifier in food and non-food applications. For example, polyglycerol esters are non-ionic surfactants that have been used as emulsifiers in food for many years (Solvay Chemicals International, 2008). Glycerol esters of fatty acids, also known as *mono*-glycerides are used as emulsifiers and stabilizers for many food products. Edible *mono*-glycerides are added to dough mixes to promote dispersion of fat and to margarine to increase plasticity. They help to maintain moisture balance in a product and permit richer formulations with longer shelf life. They are also used in frozen desserts, candy and salad dressings (Soap and Detergent Association, 1990).

1.3.2 Cosmetic and Personal Care Products

Glycerol is widely used in cosmetic and personal care products. It serves as a humectants, solvent and lubricant in personal care products such as toothpaste, mouthwashes, skin care products, hair care products and soaps. It is a major ingredient in toothpaste that preventing drying out and hardening in the tube and around the cap threads. It is also used in skin creams and lotions, make up, shaving preparations and deodorants (Soap and Detergent Association, 1990). As reported by Aghel *et al.* (2007), glycerin was used as viscosity modifier in the formulation of a clear liquid shampoo base. Glycerol derivatives namely glycerol esters of fatty acids are used as replacements for waxes in lipstick, mascara and other non-greasy emulsions besides as emulsifiers in creams and lotions (Soap and Detergent Association, 1990).

A mixture consisting at least of *mono*- and *di*-alkyl ethers of glycerol was found to have extremely good emulsifying and consistency-imparting properties which suitable as (co)emulsifier or consistency factor in cosmetic compositions (Neuss *et al.*, 2005).

Beilfuss *et al.* (1996) patented the application of glycerol alkyl ether in cosmetic formulations where the alkylation agents are branched or unbranched C_8 to C_{12} group. It was surprisingly found that the glycerol alkyl ether showed a good deodorizing effect. The glyceryl ethers produced have an antimicrobial effect, a good effect being achieved in particular with gram-positive, odor-causing bacteria. They also display good adhesion property to skin, excellent stability (hydrolysis stability, thermal stability and pH stability) and compatible with other active ingredients and auxiliaries support for the dispersing and co-emulsifying action in cosmetic preparations.

In 1987, Takaishi and co-workers patented the use of *α-mono*(methyl-branced alkyl)glyceryl ether as emulsifier for a skin care cosmetic composition where it has superior emulsifying power and non-irritant to the skin (Takaishi *et al.*, 1987). Meanwhile, Sebag had patented the process for preparing polyglycerolated nonionic surfactant which is usable in cosmetic compositions (Sebag, 1989). In addition, polyglycerol esters are being used in personal care products for many years besides their application in food products (Solvay Chemicals International, 2008).

A family of glycerol ether surfactants having a hydrophilic moiety of amino acid and a hydrophobic alkyl chain was reported (Pegiadou *et al.*, 2000). Those surfactants were proven to have good surface properties. They are an interesting alternative to conventional synthetic surfactants in pharmaceutical and cosmetic applications. In

conjunction to that, a homologous series of 1-*N*-L-phenylalanine-glycerol ether surfactants were synthesized by other group of researchers (Varka *et al.*, 2004).

1.3.3 Other Uses

Wolfson *et al.* (2007) reported that glycerol which is non-toxic, biodegradable and recyclable liquid has a high potential as alternative green solvent for organic reactions. As discussed by Gu *et al.* (2008), the use of glycerol as solvent was able to considerably accelerate the reaction rate of an organic reaction. Furthermore, Nascimento and co-workers describes the use of glycerol as an efficient, safe and recyclable solvent in the one-pot hetero-Diels-Alder reaction of (*R*)-citronellal with substituted arylamines (Nascimento *et al.*, 2011).

Glycerol is widely used in drugs and pharmaceutical products. It is a common ingredient in liquid products such as ointments and cough syrups. It is also a common holding agent in tablets for medicinal use and vehicles for antibiotics and anticeptics. Furthermore, it is a standard ingredient in tinctures. Nitroglycerine, which is one of the glycerol derivatives is a chemical well known as an explosive but also having medical uses. It is a coronary vasodilator and is used medically especially in treatment of angina (Soap and Detergent Association, 1990).

In the area of diesel application, some countries produce a special diesel fuel to help reduce the air pollution. These reformulated fuels have some improved qualitative parameters. However, reformulated diesel fuel is more expensive than conventional fuel, but its use as diesel fuel can reduce emissions of particulate matters. Diesel fuel from renewable sources especially from vegetable oils is already being globally produced. There are methyl esters of rapeseed in Europe whereas in the USA, they are

made from soybean oil. The methyl esters of vegetable oils are produced by transesterification of *tri*-glycerides in the presence of an alkaline catalyst. The main products are methyl esters of fatty acids from the vegetable oil and glycerol is obtained as a co-product.

It was found that a mixture of glycerol alkyl ethers is suitable as an additive for diesel fuel. The addition of these ethers to the fuel formula gave effects on the quality of the diesel fuel (high cetane number). The presence of ethers can reduce particulate matters, carbon oxides and carbonyl compounds in exhausts of vehicles. It was also reported that the addition of glycerol alkyl ethers (*di*- and *tri*-) decreased the cloud point of diesel or biodiesel (Klepáčová *et al.*, 2003). Furthermore, Bradin had patented the use of glyceryl ethers as fuel additive in 1996 (Bradin, 1996).

In other applications, the use of glycerol derivatives which are *di*-alkylglyceryl ethers and *di*-acylglycerols in detergent composition was patented by Hamada and co-workers. The preferred glycerol derivatives are those which are liquid at 20 °C or higher (Hamada *et al.*, 1997).

In 1990, the preparation of glycerol ether sulfates from the reaction of glycerol ethers with sulfur trioxide and subsequent neutralization with aqueous bases was reported. The glycerol ether sulfates obtained are suitable to be used in manual dishwasing detergents, liquid and powdered laundry detergent formulations (Fabry *et al.*, 1990). Furthermore, a sulfate salts of C_{10}-C_{20} linear alkyl glyceryl ether alcohols were produced where those compounds are useful as biodegradable surfactants in detergent formulations (Berkowitz, 1980). In addition, a detergent composition comprising from about 0.1 % to about 10 % by weight of the composition of an alkyl glyceryl sulfonate surfactant, an

alkyl glyceryl sulfate surfactant and mixtures thereof was patented. Those surfactants comprise an alkyl chain length from C_{10-44} (Hecht *et al.*, 2009).

Other than that, polyglycerol and their derivatives play a role as emulsifiers, stabilizers, dispersants or humectants in inks and agrochemical formulations. They also act as antifoaming agents in the paper industry or in wastewater treatment plant (Solvay Chemicals International, 2008). Glycerol is a standard component in the manufacture of alkyds resins which are used in surface coatings. The alkyds resins may be modified to meet a wide range of coating applications. Glycerol also serves as the fundamental block in polyether for urethane foam (Soap and Detergent Association, 1990).

1.3.4 Future Prospects of Glyceryl Ether

Glyceryl ethers could be used in cosmetics and personal care products or as a solvent in a number of applications. This can substitute ethylene glycol ethers which are now banned in cosmetic application. An increased by approximately 4.25 % in 2006, the European Cosmetic Industry remained the world market leader. Globally, the cosmetics industry has recorded sales in the amount of $250 billion (Morante, 2007). Asia Pacific is the second biggest personal and home care market after Western Europe. According to Kline & Company, sales of cosmetics and toiletries in India grew by 12.6 % in 2006. The Japanese cosmetics market remains stable, while China cosmetics market is witnessing increased demand due to improving lifestyles of the Chinese population. Meanwhile, South Korean cosmetic market is reportedly growing at a faster rate than the developed regions. In Taiwan, the skin care products dominate the cosmetic market, followed by color cosmetics and hair care products. Despite the current market restraints, ASEAN has already been holding more than a quarter of the percent share in the global market as of 2013 (Loh, 2016). Its retail sales have been expected to grow to

360 billion USD by the year 2017, and its worth has been predicted to reach 27 billion USD by 2020, anchoring it as a focused region in the area of personal care.

According to Kline and Company, the hair care market was among the fastest-growing cosmetic and personal care categories, slightly behind skin care in 2006 with growth of 5-6 %. According to Euromonitor, the hair care markets retail value in the US is said to have grown 3.2 % to $10.3 billion in 2006. The market grew 2 % to $9.92 billion in 2005 (Guzman, 2007). In 2013, the two products of the products on the market to have reached the highest end-use have been hair care products and skin care products, and these hold more than half (55 %) of the market share together (Loh, 2016). Besides the potential use in cosmetic and personal care products, glyceryl ether could be used as a solvent in a number of applications such as in industrial products namely agrochemical products, liquid cleaners, *etc*.

Therefore, based on the information, the preparation of glyceryl ether compounds has been explored by several possible routes and evaluations on its application in possible fields have been investigated in this study.

1.4 Research Objectives

The main objective of this study is to produce glyceryl ether and other related compounds namely glyceryl esters and epoxide that could be used as an intermediate in producing glyceryl ether. It is also an object of this study to evaluate the antibacterial activity and dermal irritation potential of the compounds produced, and to conduct a study on the applications of glyceryl ether in macroemulsion, microemulsion and transparent soap making.

1.5 Research Scope

The subject of this thesis is the chemical modification of glycerol to its derivatives specifically to glyceryl ether through direct etherification process and also involves other possible routes or processes when the direct method is not possible to produce the desired glyceryl ether. Besides the synthesis, the research also covers the application evaluations of the compounds produced in possible area.

In the introduction of this thesis which is the first chapter, a general overview on glycerol is presented. Topics such as chemical nature, origin, market view and glycerol applications are revealed. The main chemical reactions of glycerol to its derivatives are disclosed, emphasizing those closely related to this thesis, i.e. direct etherification, epoxidation and ring opening process of the epoxide. This chapter also includes a discussion on the protection and tosylation process of alcohol which is related to this thesis. In addition, the potential applications of glycerol derivative compounds are also disclosed in this chapter.

Chapter 2 of this thesis describes the results and discussion on one-pot synthesis of glyceryl ether. The reaction was focused on the etherification of glycerol with *tert*-butanol as preliminary experiments showed that other short chain alcohols did not react with glycerol using the one-pot method in the range of the experimental study. Scaling up of the reaction of glycerol with *tert*-butanol was also done in order to provide sufficient amount of product for its application evaluations.

The results and discussion on the production of an epoxide from glycerol through an intermediate compound namely glyceryl carbonate and also the ring opening of the epoxide to form glyceryl ethers are described in Chapter 3.

Chapter 4 contains the results and discussion on the protection and tosylation of hydroxyl groups of glycerol which the products could be used as an intermediate to form the desired glyceryl ether.

Results obtained from the potential application evaluations of glyceryl ether in macroemulsions, microemulsions and transparent soap making besides the antibacterial activity and *in vitro* dermal irritation potential are discussed in Chapter 5. Meanwhile, Chapter 6 consists of experimental details and Chapter 7 is the conclusion of this thesis.

The proposed modifications of the glycerol are an important manner to obtain potentially useful products using a renewable feedstock. This will enhance and add value to glycerol by varying its uses in a variety of possible applications.

CHAPTER 2: ONE-POT SYNTHESIS OF GLYCERYL ETHER

2.1 Introduction

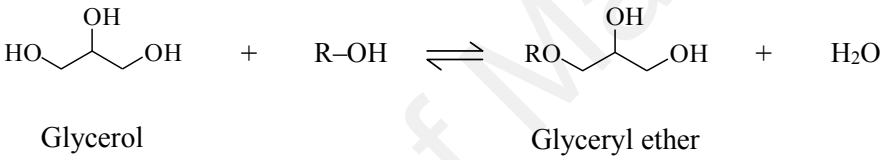
One of the important derivatives of glycerol is the glyceryl ether where glycerol can be linked via ether bonding to linear or cyclic compounds. A number of glycerol molecules link together to form polyglycerols. Self-etherification of glycerol in the presence of solid mesoporous catalyst prepared by caesium impregnation and in the absence of solvent that yielded polyglycerols was reported by Claceus *et al.* (2002). In addition, the condensation of glycerol to polyglycerol at a temperature of 190 °C to 250 °C in the presence of catalyst was carried out by Stuhler (1985).

The other etherified compound of glycerol is glyceryl alkyl ether. It can be obtained from the reaction of glycerol with alkylation agent. *tert*-Butylation of glycerol with isobutylene in the presence of strong acid ion-exchange resins Amberlyst type and zeolites for producing glyceryl alkyl ether was studied by Klepáčová *et al.* (2005). In 1994, a process for the production of the butyl ethers of glycerol was developed by Behr in the presence of *p*-toluenesulfonic acid (as cited in Behr & Gomes, 2010). The etherification process was also patented by Gupta (Gupta, 1995).

Glyceryl ether could be produced by several routes but in this chapter, the plan was to produce ethers of glycerol by direct etherification of glycerol with alcohol. Screening process of the production of glyceryl ethers from one-pot reaction of glycerol with short chain primary alcohols (C_1-C_4), secondary alcohols and tertiary alcohol were carried out.

2.2 Direct Etherification of Glycerol with Alcohols

As mentioned earlier, one-pot etherification reaction of glycerol with alcohols such as methanol, ethanol, 1-propanol, 1-butanol, 2-propanol, *iso*-butanol and *tert*-butanol were carried out. According to Le Chatelier's principle, reversible reactions are self-correcting; when the equilibrium is disturbed by changing the conditions, the position of equilibrium moves to counteract the change (Boundless, 2016). Based on the Le Chatelier's principle, the equilibrium of reaction of glycerol with alcohols can be illustrated as in scheme 2.1.



Scheme 2.1 : General reaction scheme of the reaction of glycerol with alcohols wherein R = alkyl

Table 2.1 shows the expected glyceryl ethers produced from reactions of glycerol with different alcohols. The reactions were done in the presence of acidic catalysts (Amberlyst 15 and Montmorillonite K10). The progress of the reactions was monitored by gas chromatography (GC) and the results are summarized in Table 2.2. As tabulated in Table 2.2 only reaction of glycerol with *tert*-butanol that produced etherified products.

Table 2.1: Products expected from etherification of glycerol with different alcohols

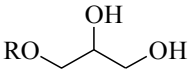
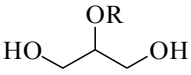
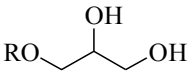
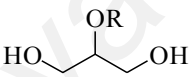
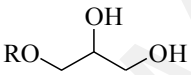
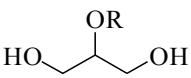
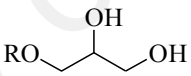
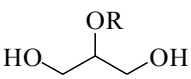
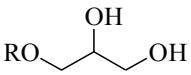
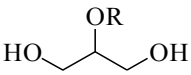
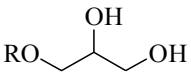
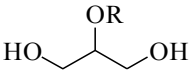
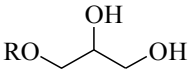
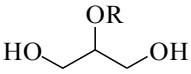
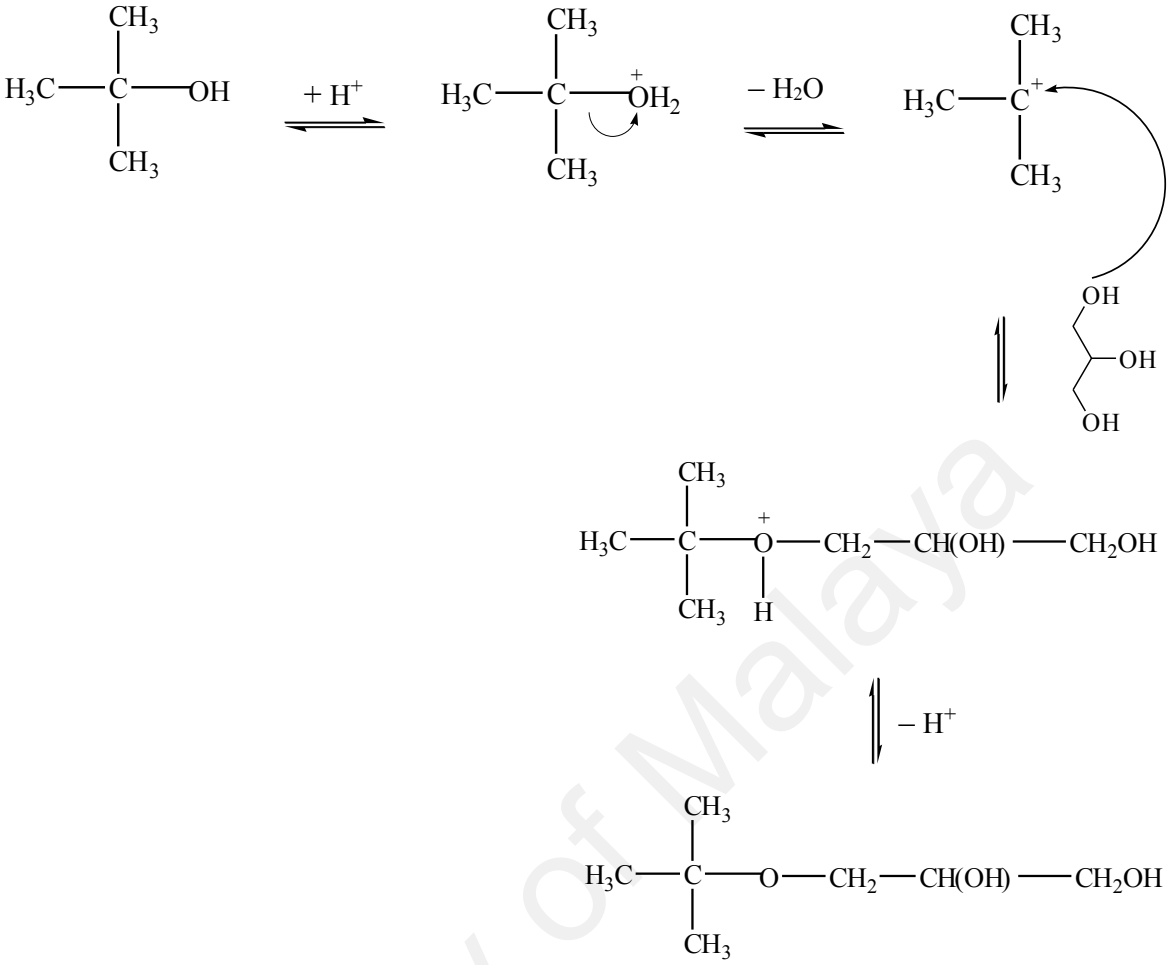
R-OH	Product	
R = –CH_3		
	2.1a	**2.1b**
R = –CH_2CH_3		
	2.2a	**2.2b**
R = –$CH_2CH_2CH_3$		
	2.3a	**2.3b**
R = –$CH_2CH_2CH_2CH_3$		
	2.4a	**2.4b**
R = –$CH(CH_3)_2$		
	2.5a	**2.5b**
R = –$CH_2CH(CH_3)_2$		
	2.6a	**2.6b**
R = –$C(CH_3)_3$		
	2.7a	**2.7b**

Table 2.2 : Reactions of glycerol with different alcohols

R-OH	Glycerol conversion	Product
R = $–CH_3$	No	No
R = $–CH_2CH_3$	No	No
R = $–CH_2CH_2CH_3$	No	No
R = $–CH_2CH_2CH_2CH_3$	No	No
R = $–CH(CH_3)_2$	No	No
R = $–CH_2CH(CH_3)_2$	No	No
R = $–C(CH_3)_3$	Yes	Yes

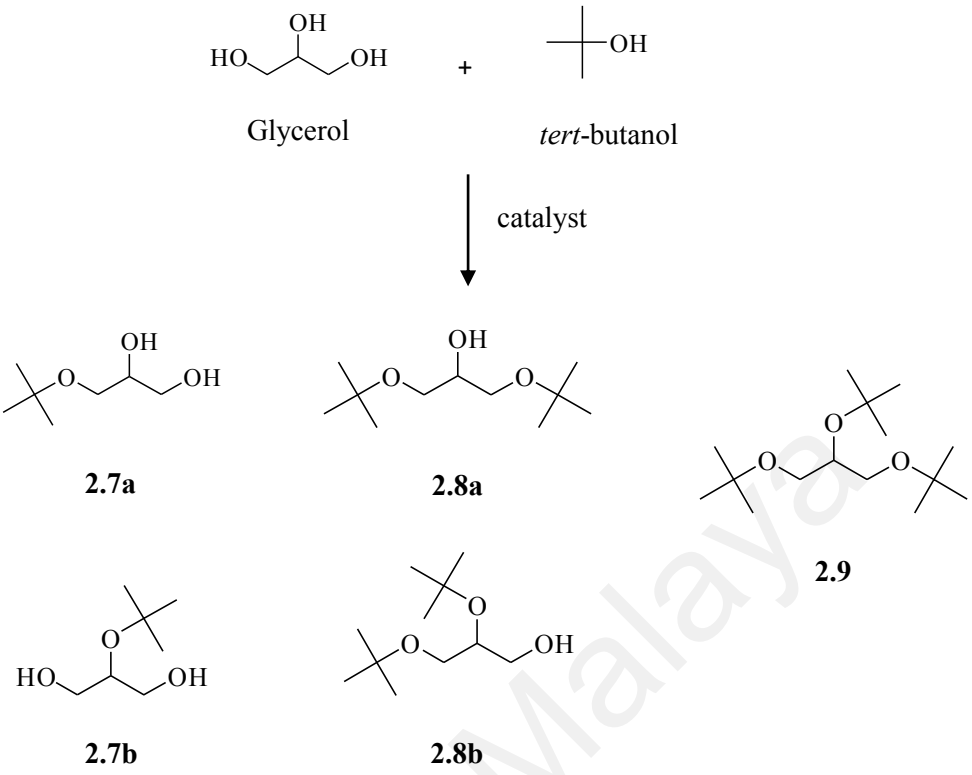
The product formation from the reaction of glycerol with *tert*-butanol can be explained as in scheme 2.2. *tert*-Butanol formed the carbocation as an intermediate before the formation of the ethers, indicating the reaction was S_N1 reaction. Meanwhile, primary and secondary alcohols did not form the carbocation intermediate because of the lower reactivity compared to tertiary alcohol. Thus, the ether formation was not possible from direct reaction of glycerol with primary or secondary alcohols in the range of the experimental study. This shows the etherification occurred is one hundred percent through S_N1 reaction.



2.7a

Scheme 2.2: Reaction of glycerol with *tert*-butanol in the presence of acidic catalyst

As shown in previous experiments, only reaction of glycerol with *tert*-butanol occurred through the one-pot method and this was being investigated further in this chapter. A number of etherification reactions of glycerol with *tert*-butanol were conducted at atmospheric pressure, in a reflux condition. The reactions were carried out in the presence of an acidic catalyst. The progress of the reactions was monitored by using thin layer chromatography (TLC) and GC. Scheme 2.3 shows the possible products formation when glycerol is reacted with *tert*-butanol in the presence of an acidic catalyst where water forms as a by-product. However, the desired compound in this study was compound **2.7** which represents an isomer mixture of **2.7a** and **2.7b**.



wherein **2.7a** = 1-*mono*-*tert*-butoxypropane-2,3-diol
2.7b = 2-*mono*-*tert*-butoxypropane-1,3-diol
2.8a = 1,3-*di*-*tert*-butoxypropane-2-ol
2.8b = 1,2-*di*-*tert*-butoxypropane-3-ol
2.9 = 1,2,3-*tri*-*tert*-butoxypropane

Scheme 2.3 : Possible products from reaction of glycerol with *tert*-butanol

The major component of the product mixture was isolated and subjected to ^{1}H and ^{13}C NMR analyses. The NMR spectra of the isolated component confirmed that the compound was **2.7a**. 2D NMR spectrum of compound **2.7a** is shown in Appendix 2.1. In compound **2.7a** molecule, the hydrogen atoms on the carbon attached to the oxygen are deshielded due to the electronegativity of the attached oxygen, and they appear in the range of 3.4-3.75 ppm. In addition, the hydrogen atoms from those three methyl groups having the similar electronic environment appear as a tall singlet at the chemical shift of 1.15 ppm. Meanwhile, carbon atom which is directly attached to the electronegative oxygen atom in compound **2.7a** molecule shows higher deshielding effect thus leading to a greater chemical shift in ^{13}C spectrum.

The second isolated compound from the product mixture was also subjected to ^{1}H and ^{13}C NMR analyses. The component was compound **2.8a** as expected. The ^{1}H and ^{13}C NMR spectra of compound **2.8a** are shown in Appendix 2.2.

An addition of one band at 2973.80 cm^{-1} in the IR spectrum of compound **2.7a** shows the sp^3-hybridized C–H stretching indicating the presence of methyl group in the compound **2.7a** molecule (Appendix 2.3). Furthermore, a new band at 1194.58 cm^{-1} which does not exist in the spectrum of glycerol (Appendix 2.4) indicates the presence of C–O ether bond in the compound **2.7a** molecule.

Appendix 2.5 shows the IR spectrum of isolated compound **2.8a** where the band of -OH region is less broad compared to glycerol and compound **2.7a** indicating the presence of only one –OH group in the molecule.

GC was used to characterize the reaction product besides TLC. Based on TLC analysis, the spot of products can be seen clearly on the plate under iodine vapour. Glycerol remains at the initial spot as expected because of the mobile phase used (hexane/diethyl ether). From the gas chromatogram (Figure 2.1), the reaction product is a mixture consisting of five components including unreacted glycerol.

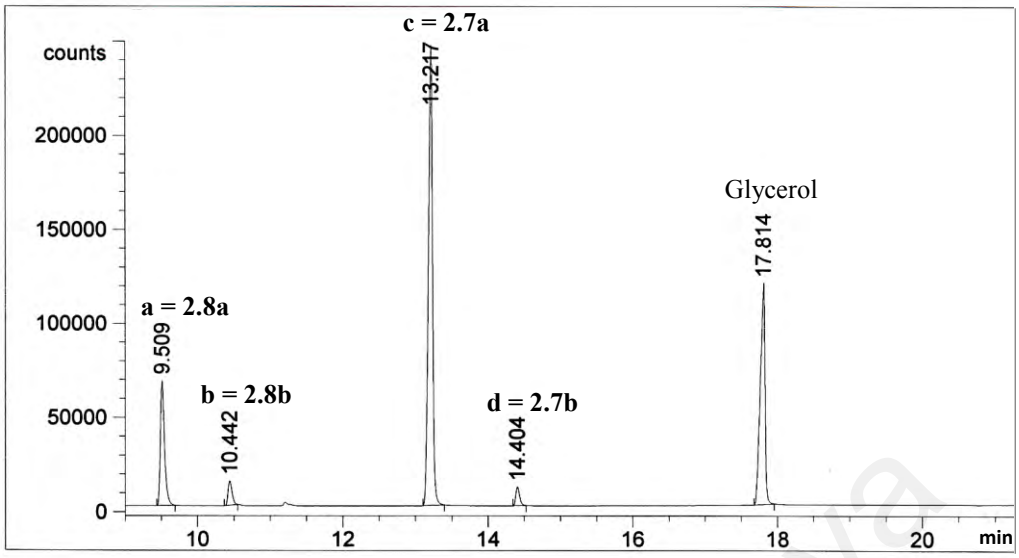


Figure 2.1 : Chromatogram of the product obtained from the etherification of glycerol and *tert*-butanol at 80 °C for 5 hours in the presence of an acidic catalyst (Cat-D)

Mass spectra of components a, b, c and d (Appendices 2.6 to 2.9) showed that the compounds were **2.8a** (1,3-*di*-*tert*-butoxypropane-2-ol), **2.8b** (1,2-*di*-*tert*-butoxypropane-3-ol), **2.7a** (1-*mono*-*tert*-butoxypropane-2,3-diol) and **2.7b** (2-*mono*-*tert*-butoxypropane-1,3-diol), respectively.

With the purpose to obtain the optimum parameters for the production of glyceryl ether, GC was used in order to monitor the extent of the reaction through the determination of glycerol left unreacted in the reaction mixture. The effects of the following variables on the glycerol conversion were investigated: i) type and amount of catalyst, ii) reaction temperature, iii) reaction period and iv) mole ratio of reactants.

2.2.1 Effect of Catalyst : Type and Dosage

Six types of acid catalysts namely sulphuric acid (Cat-A), Amberlyst 15 (Cat-B), *p*-TsOH (Cat-C), Amberlite IR-120 (Cat-D), Amberlyst 36 (Cat-E) and Montmorillonite

K10 (Cat-F) were used in the etherification process. The performances of these catalysts were compared based on the percentage of glycerol conversion to the desired products. For this purpose, the amount of catalyst was varied from 2.5 % to 7.5 % and the results are shown in Figures 2.2 to 2.4. From the graphs, Cat-F exhibited the lowest activity. Cat-B and Cat-E were found to cause a reversible reaction after three hours of reaction time. The highest conversion of glycerol to product (about 80 %) was attained in the presence of Cat-A (5 %) or Cat-D (5 % or 7.5 %).

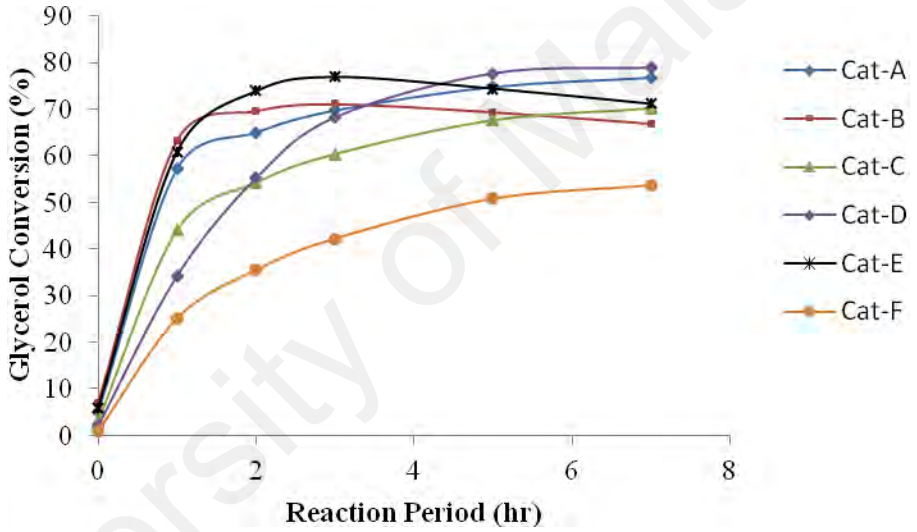


Figure 2.2 : Reaction of glycerol with *tert*-butanol at 85 °C in the presence of different catalyst (2.5 % catalyst dosage)

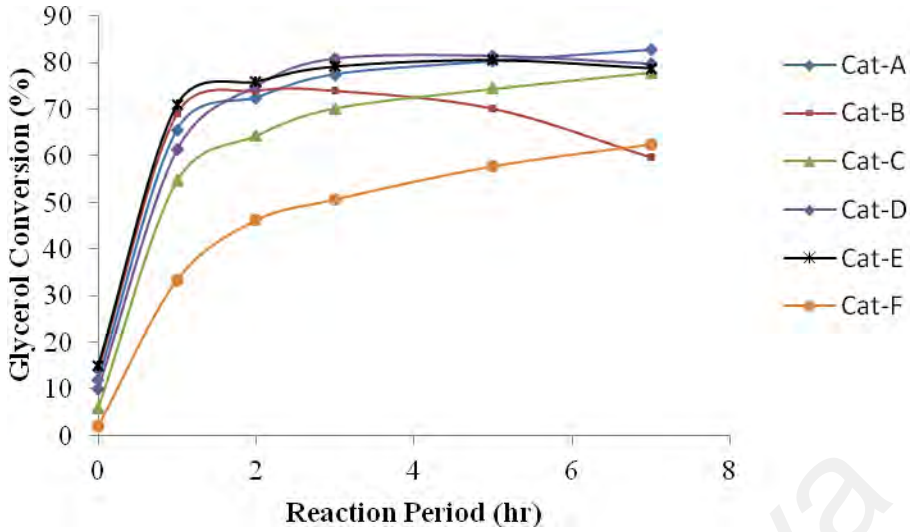


Figure 2.3 : Reaction of glycerol with *tert*-butanol at 85 °C in the presence of different catalyst (5 % catalyst dosage)

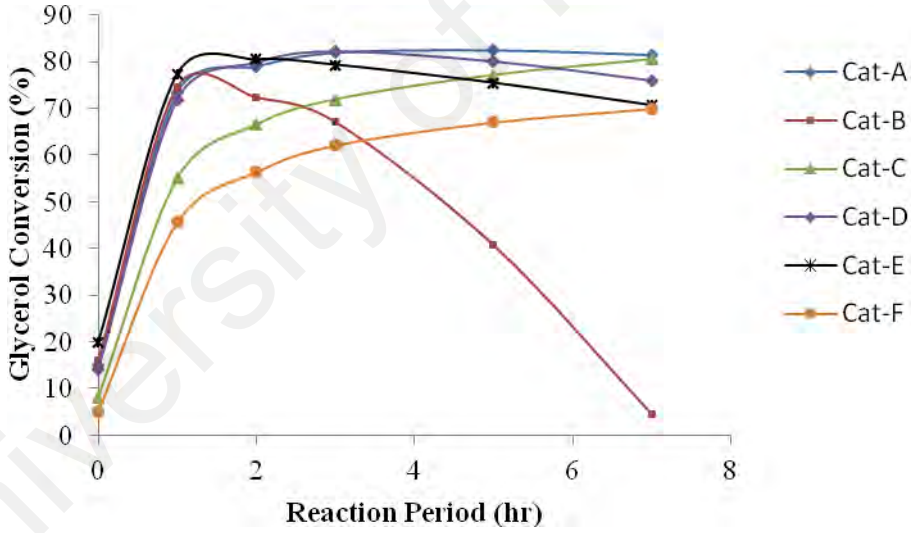


Figure 2.4 : Reaction of glycerol with *tert*-butanol at 85 °C in the presence of different catalyst (7.5 % catalyst dosage)

The ease of removing the catalyst from the product obtained depends very much on the type of catalyst used, whether it is homogeneous or heterogeneous. In this study, Cat-A and Cat-C were homogeneous catalysts and difficult to be removed. Therefore, the catalysts remained in the product (Table 2.3). Cat-B, Cat-D, Cat-E and Cat-F were

heterogeneous hence, it was easier to be removed using a filtration technique. Nevertheless, Cat-D is the preferred catalyst because the reaction time is shorter and the separation of Cat-D from the product is easier than Cat-A.

Table 2.3 : Product separation from catalyst and product appearance

Catalyst	Product separation from catalyst	Product appearance
Cat-A	Difficult	Colourless liquid
Cat-B	Easy	Slightly brownish liquid
Cat-C	Difficult	Colourless liquid
Cat-D	Easy	Slightly yellowish liquid
Cat-E	Easy	Colourless liquid
Cat-F	Easy	Colourless liquid

Note : Cat – A = Sulphuric acid, Cat – D = Amberlite IR-120
Cat – B = Amberlyst 15, Cat – E = Amberlyst 36
Cat – C = *p*-TsOH, Cat – F = Montmorillonite K10

2.2.2 Effect of Reaction Temperature

Reactions of glycerol with *tert*-butanol were then carried out at the temperatures from 40 ºC to 85 ºC, catalyzed by Cat-D. Eventhough the catalyst can withstand the temperature up to 120 ºC, the highest reaction temperature was 85 ºC as it was done in reflux condition. It can be seen that the highest conversion of glycerol (80 %) was achieved at 85 ºC in the presence of 5 % catalyst compared to 2.5 %, 7.5 % and 10.0 %

catalyst, where the conversions were 77 %, 73 % and 68 %, respectively (Figure 2.5). The conversion of glycerol decreased with the decreasing of reaction temperature, e.g. the highest conversion of glycerol was only 15 % at 40 ºC.

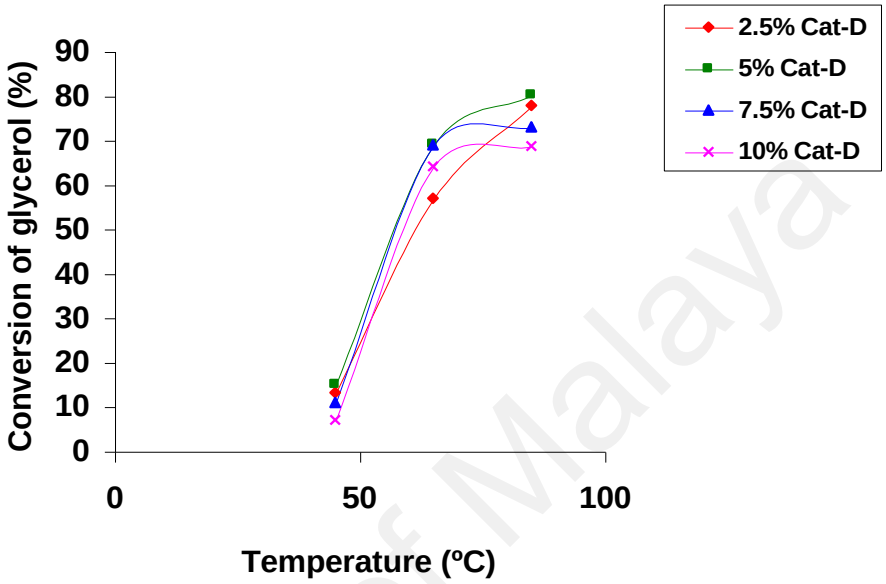


Figure 2.5 : Effect of reaction temperature on glycerol conversion at 5 hours reaction time in the presence of different amount of catalyst (Cat-D)

2.2.3 Effect of Mole Ratio

The effect of mole ratio of reactants (glycerol and *tert*-butanol) on the glycerol conversion was also investigated. Figures 2.6 and 2.7 show that at lower temperatures (40 ºC-65 ºC), the conversion of glycerol to product did not increase much, even though the mole ratio of the reactants increased (an excess *tert*-butanol). However, when the reaction temperature of 85 ºC was applied, the conversion of glycerol to compounds **2.7a**, **2.7b**, **2.8a** and **2.8b** increased as the mole ratio of the reactants increased. Compound **2.7a** was the major component and there was no formation of compound **2.9** detected in the reaction product. A similar pattern was observed when the reaction was carried out in the presence of 5 % catalyst instead of 2.5 %. Therefore, the effect of

mole ratio of reactants on the conversion of glycerol is influenced by the reaction temperature.

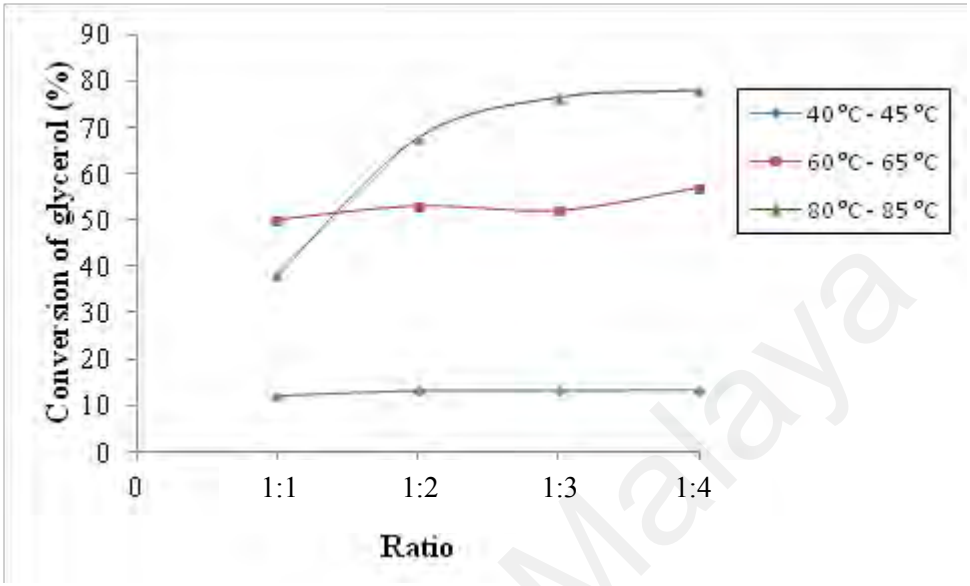


Figure 2.6: Effect of mole ratio of the reactants (Glycerol : *tert*-Butanol) at different reaction temperatures (2.5 % Cat-D)

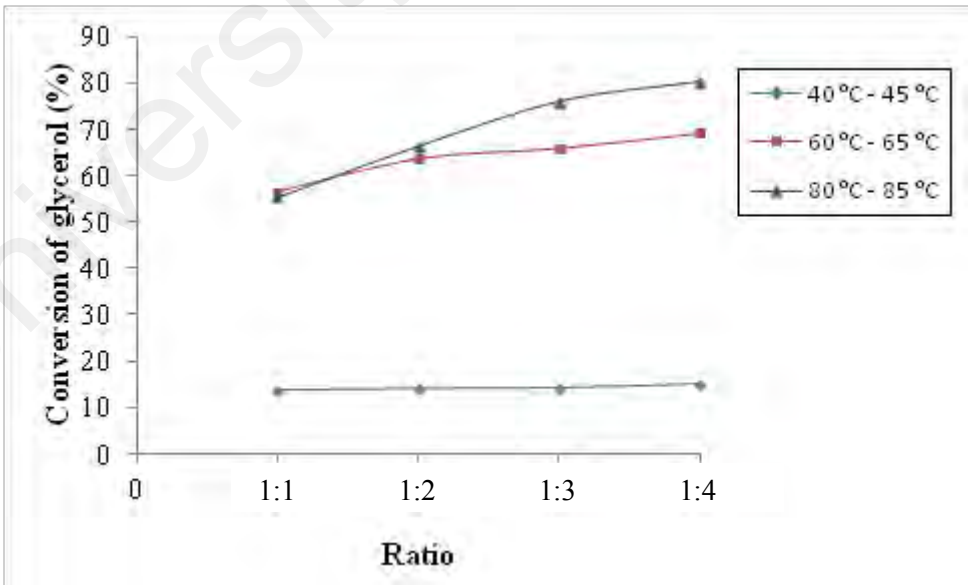


Figure 2.7 : Effect of mole ratio of the reactants (Glycerol : *tert*-Butanol) at different reaction temperatures (5 % Cat-D)

In the previous experiment, the highest conversion of glycerol to product achieved was 80 %, where the reaction was carried out at 80-85 °C for 5 hrs, whereby the mole ratio of glycerol to *tert*-butanol was 1:4. Efforts were made to increase the conversion of glycerol by using excess *tert*-butanol to glycerol at ratios 6 : 1 and 8 : 1. However, as clearly shown by Figures 2.8 and 2.9, the increasing mole ratio of *tert*-butanol to glycerol (providing excess *tert*-butanol) did not increase the conversion of glycerol even after seven hours of reaction.

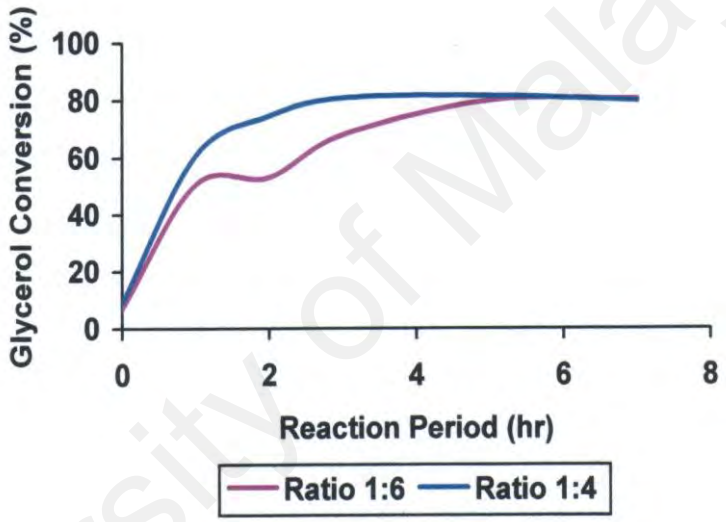


Figure 2.8 : Reaction of glycerol and *tert*-butanol at 1:6 mole ratio of glycerol to *tert*-butanol compared to 1:4 in the presence of 5 % Cat-D at 80-85 °C

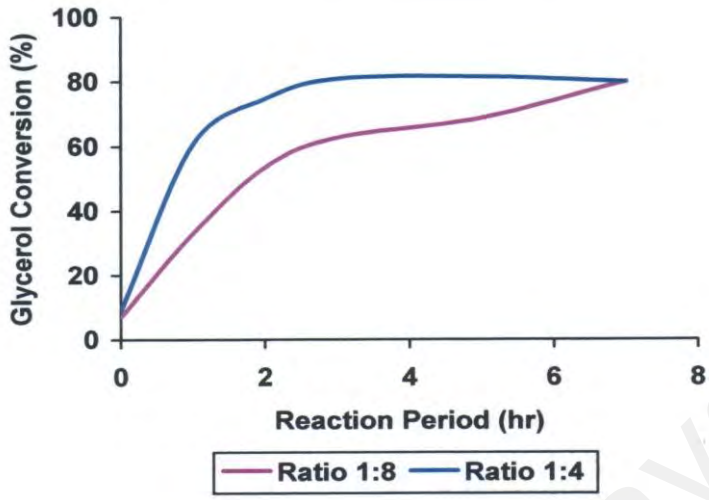


Figure 2.9 : Reaction of glycerol and *tert*-butanol at 1:8 mole ratio of glycerol to *tert*-butanol compared to 1:4 in the presence of 5 % Cat-D at 80-85 °C

From all reactions of glycerol with *tert*-butanol in the presence of different types of catalyst, there was no formation of compound **2.9** detected in the product mixture. Those reactions only produced compounds **2.7** and **2.8**. This is in agreement with the work done by Janaun and Ellis (2010). Water formed as byproduct during the etherification reaction may inhibit the formation of compound **2.9**. As suggested by Frusteri and co-workers, it is necessary to remove water formed from the reaction medium for the formation of *di*- and *tri*-ethers (Frusteri *et al.*, 2009). When the reaction was carried out using Dean Stark set-up to remove water formed from the reaction, the glycerol conversion achieved was in the range of 70-80 %. This may be due to the lower composition of water in azeotropic mixture of *tert*-butanol/water and there was no separation between water and *tert*-butanol. Therefore, attempt to use petroleum ether to aid the process of removing water formed from the reaction was made. It was successful where the conversion of glycerol reached 90-93 % with compound **2.7** as the major product.

The preparation of glyceryl ether had involved six acidic catalysts and the effect of these catalysts on the conversion of glycerol was studied. The type and amount of catalysts used affect the conversion of glycerol and some of the catalysts may have caused reversible reaction. Nevertheless, reaction temperature, mole ratio of the reactants and reaction period are also important factors that influence the percentage of glycerol conversion. An 80 % conversion of glycerol using the studied parameters can now be achieved at the reaction temperature of 85 ºC for five hours in the presence of 5 % Cat-D with 1:4 mole ratio of glycerol to *tert*-butanol through a reflux set-up. The product obtained is a liquid and slightly yellowish in colour. It is a water-soluble compound which consists of compound **2.7** (*mono-tert*-butoxypropanediol) as the major product.

2.2.4 Scale Up Production of Compound 2.7 (*mono-tert*-butoxypropanediol)

There is a need to produce the glyceryl *tert*-butyl ether particularly compound **2.7**, *mono-tert*-butoxypropanediol at a bigger volume in order to investigate its potential application in possible fields. Therefore, the preparation of glyceryl ether has been done at a larger scale. The process parameter at laboratory scale has been used as a basis to conduct a bigger reaction scale. A high purity of compound **2.7a** (purity > 99 %) was purified *via* column chromatography technique and it was used as a standard reference for the production of glyceryl ether specifically to determine the yield of compound **2.7** produced and its availability in product matrix.

Based on the optimized reaction condition obtained from previous experiments (sections 2.2.1-2.2.3), the production of glyceryl ether was done using 1 L and 2 L reaction flasks. The reaction was done in the presence of 5 % catalyst D with 1 : 4 mole ratio of glycerol to *tert*-butanol at 80 ºC-85 ºC for 5 hours. The product mixture consists

of about 55-57 % compound **2.7** with 70-75 % glycerol conversion. Then it was decided to produce the glyceryl ether at a bigger volume capacity where a 5 L reactor, equipped with propeller was utilized (Figure 2.10). The highest glycerol conversion was 58 % based on the gas chromatography analysis and the product was a mixture of five components including glycerol left in the mixture (Figure 2.11).

Figure 2.10 : Experimental set-up for etherification of glycerol and *tert*-butanol in 5 L reactor

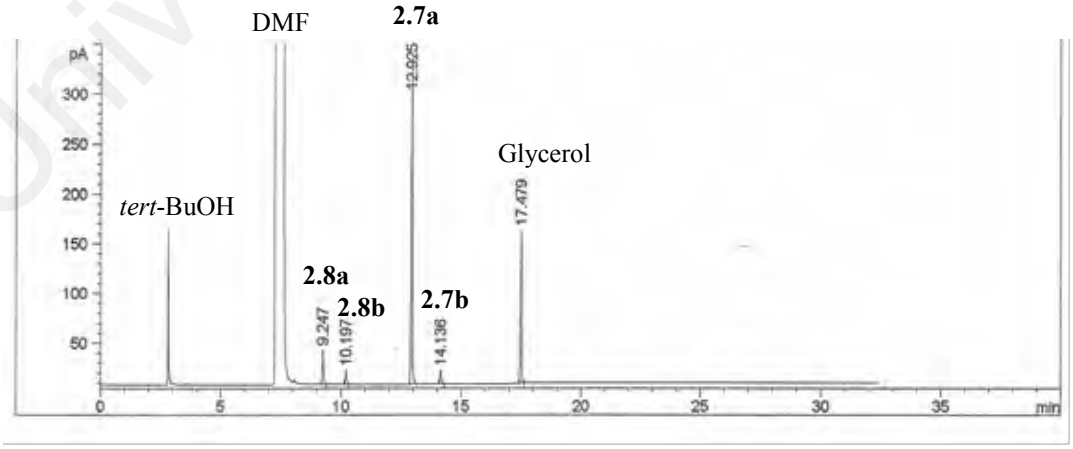


Figure 2.11 : Gas chromatogram of the product mixture obtained from the reaction of glycerol and *tert*-butanol

Since the product obtained is a mixture, research work has been focused on the separation and purification of the product mixture. No work was done on the optimization of reaction parameters at the 5 L reactor capacity since the objective was to obtain a sufficient volume of glyceryl ether for its potential application evaluation in possible fields. The separation work was also to recover the unreacted glycerol left in the product mixture. For the purpose of carrying out the separation process besides obtaining sufficient amount of purified glyceryl ether for its application evaluations, a number of batches of crude glyceryl ether were produced by using a 5 L reactor. The conversion of glycerol was 50 % to 58 % with the formation of 45 % to 49 % *mono-tert*-butoxypropanediol. Four separation methods were tested namely i) simple vacuum distillation, ii) fractional vacuum distillation, iii) liquid-liquid extraction and iv) short path distillation.

i) Simple vacuum distillation

The temperature and vacuum pressure were varied in order to obtain a good separation of the components. After several trials, 40 % yield of distillate containing less than 5 % glycerol was achieved when the distillation process was done at 2-2.5 mbar for 5-6 hours where the liquid vapour temperature was 56-60 °C. The percent yield and glycerol content of the distillates and residue are shown in Table 2.4.

Table 2.4 : Results obtained from simple vacuum distillation

Fraction	Yield (%)	2.7 (%)	2.8 (%)	Glycerol (%)
1st distillate	40	78	21	< 5
2nd distillate	25	43	2	~ 50
Residue	19	-	-	100

2.7 = **2.7a** + **2.7b** = *mono-tert*-butoxypropanediol
2.8 = **2.8a** + **2.8b** = *di-tert*-butoxypropanediol

In addition, Figures 2.12 to 2.15 show the composition of components in the product mixture before distillation, 1st distillate, 2nd distillate and residue. The amount of glycerol in the product mixture decreased to less than 5 % (1st distillate) from simple vacuum distillation process. The residue left after distillation process was only glycerol.

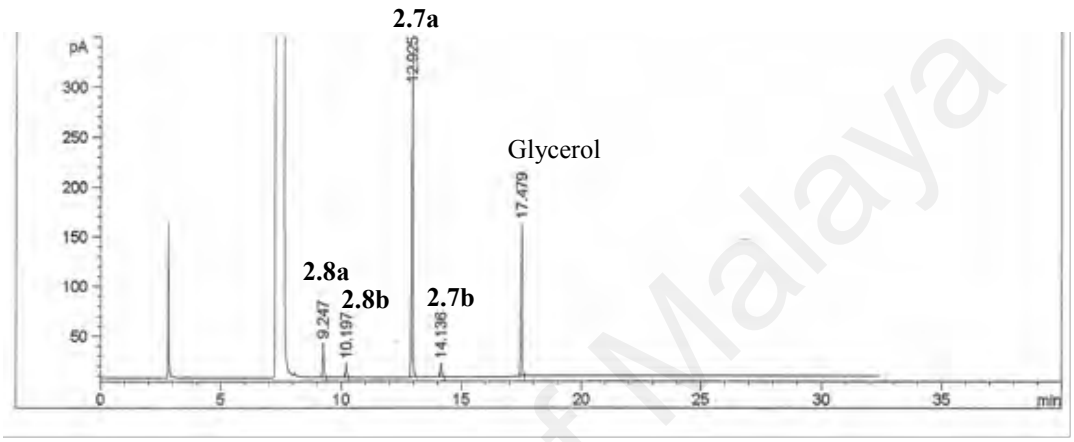


Figure 2.12 : Gas chromatogram of the product mixture before distillation process for comparison

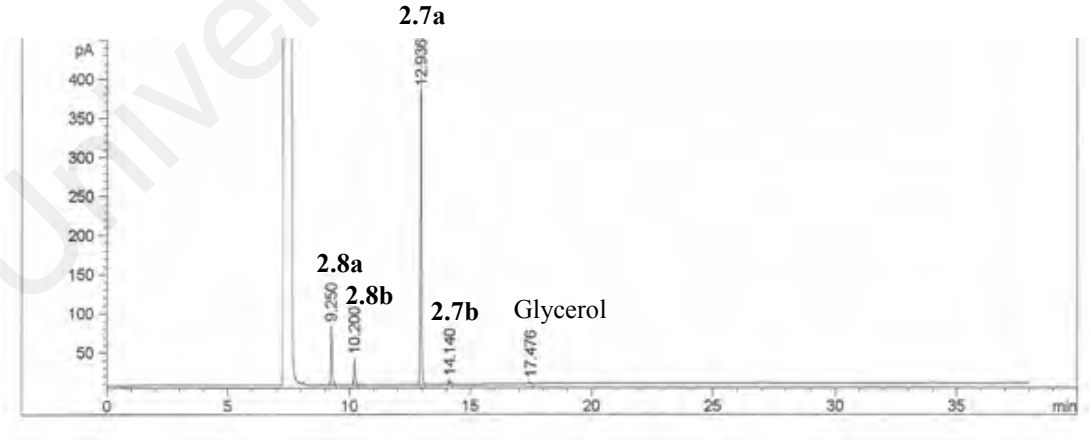


Figure 2.13 : Gas chromatogram of the 1st distillate obtained

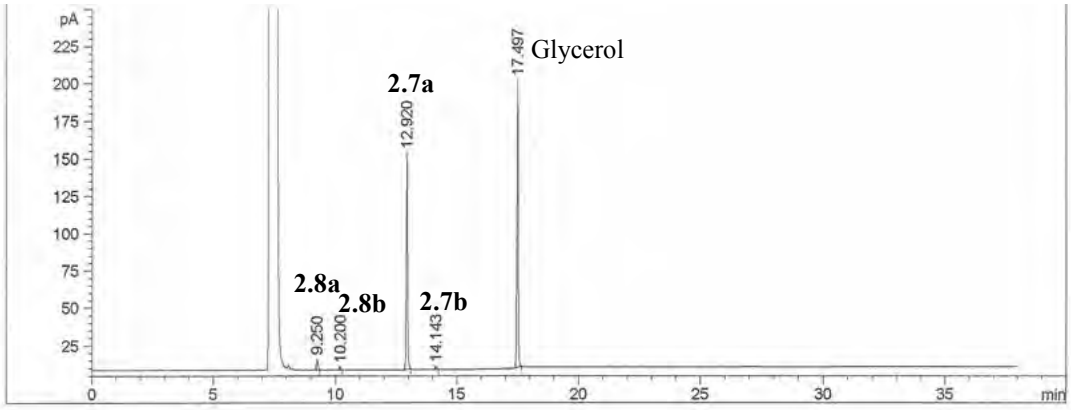


Figure 2.14 : Gas chromatogram of the 2nd distillate obtained

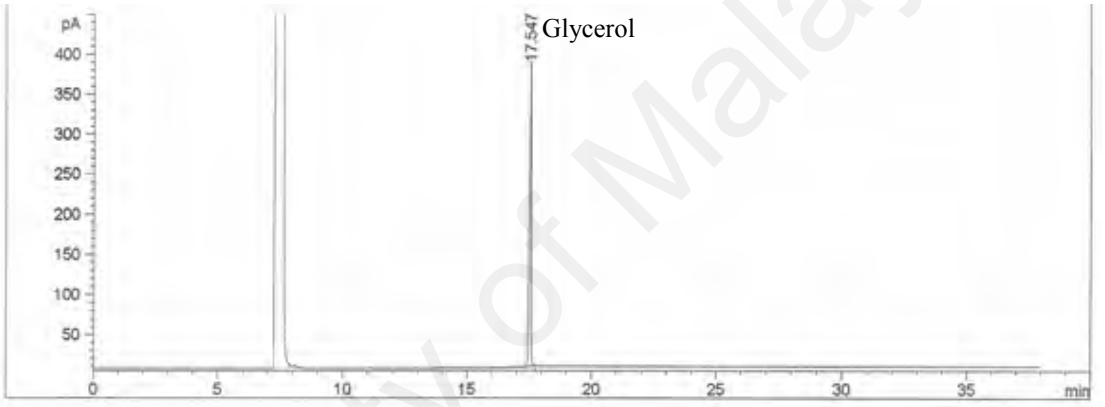
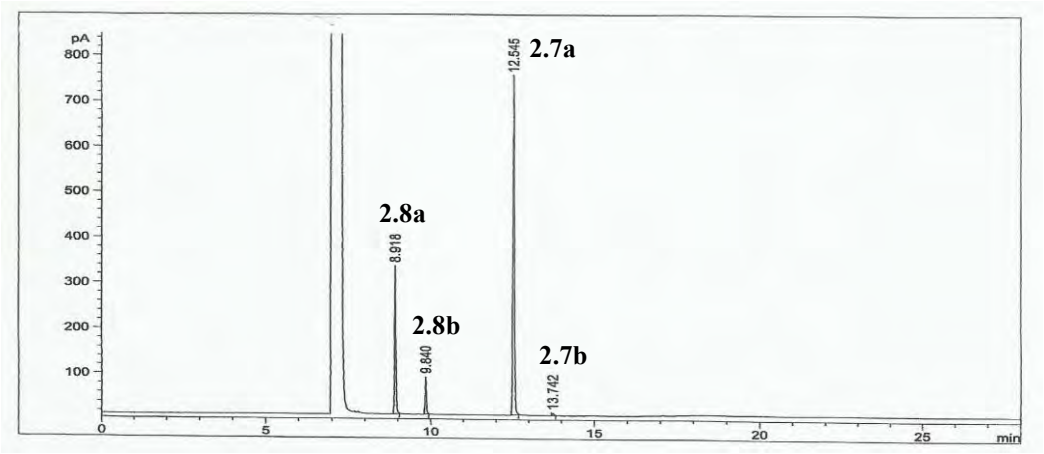


Figure 2.15 : Gas chromatogram of the residue left after distillation process

ii) Fractional vacuum distillation

A product mixture containing less than 5 % glycerol can be obtained *via* a simple vacuum distillation process. Fractional vacuum distillation of the product mixture at 0.5-1.0 mbar for 6-7 hours resulted in distillate containing four components with the absence of glycerol as shown in Figure 2.16. The temperature of liquid vapour was 55-60 °C. Therefore, fractional vacuum distillation process successfully removed the glycerol from the product mixture (distillate). The yield of the distillate was 48 %.



2.7 = **2.7a** + **2.7b** = 71 %
2.8 = **2.8a** + **2.8b** = 29 %

Figure 2.16 : Gas chromatogram of the distillate obtained from fractional vacuum distillation process

iii) Liquid-liquid extraction

The extraction was first carried out in a small scale. The liquid-liquid extraction was conducted with several solvents namely diethyl ether, ethyl acetate, toluene, chloroform and dichloromethane. The amount of organic extracts (expressed as percentage) and percent composition of the components extracted into organic solvents are shown in Figures 2.17 to 2.18. As shown in both figures, ethyl acetate, chloroform and dichloromethane extracted over 30 % amount of organic extracts containing more than 60 % *mono-tert*-butoxypropanediol compared to diethyl ether and toluene.

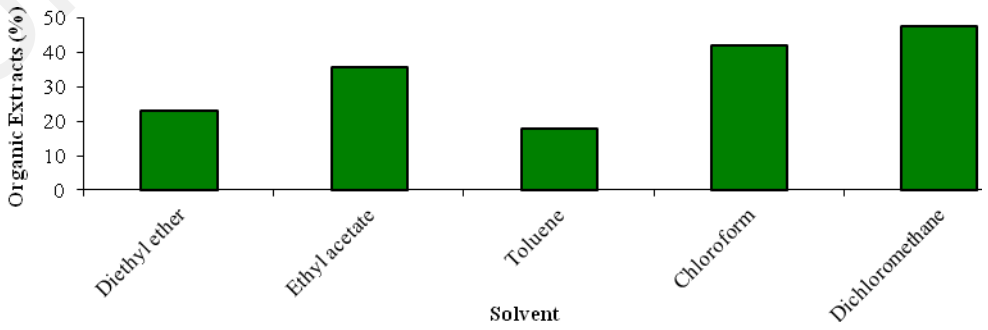


Figure 2.17 : The amount of organic extracts (expressed as percentage) extracted into organic solvents

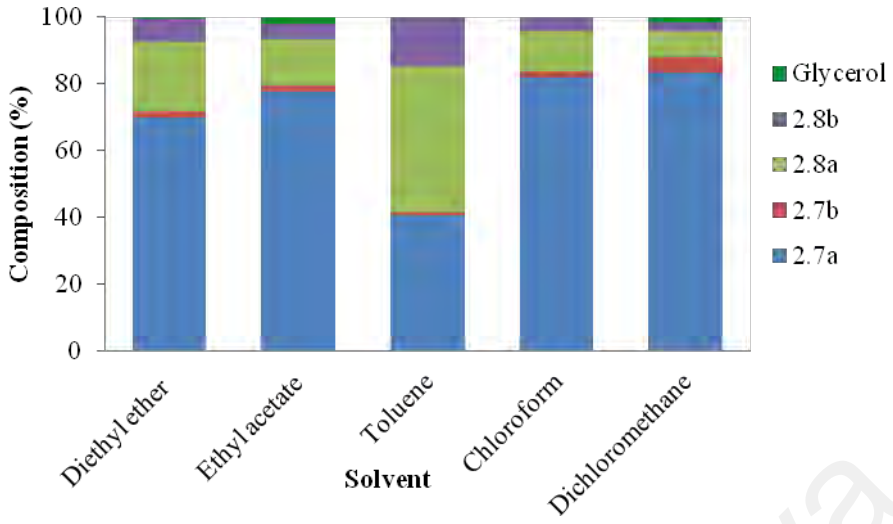


Figure 2.18 : Percent composition of components extracted into organic solvents based on area percent of gas chromatograph

It was found that dichloromethane can extract out almost 100 % of the glyceryl ether apart from glycerol. Therefore the extraction method was applied to a large scale sample. Larger volume of product needs to be extracted in order to obtain enough volume of glyceryl ether for possible application evaluation. 0.78 kg of product containing 50 % glycerol had undergone the liquid-liquid extraction process. The yield of the product mixture containing less than 5 % glycerol extracted into the organic solvent was 48 %, whereas the unreacted glycerol recovered in the aqueous system was 51 %. Therefore, the recovery was 99 %. Figures 2.19 and 2.20 show the gas chromatogram of the extracted components in organic and aqueous system using larger scale extraction after removing the organic solvent and water.

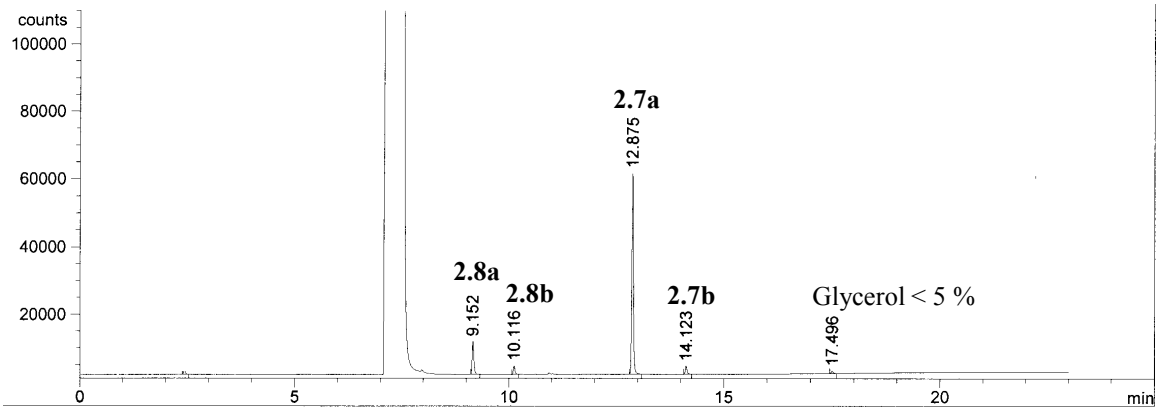


Figure 2.19 : Gas chromatogram of the extracted components in the organic solvent using large scale extraction

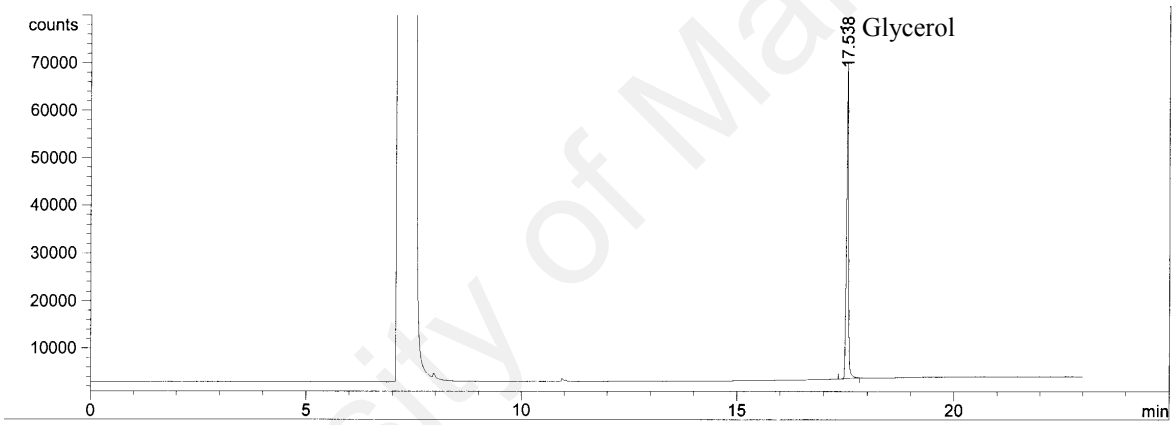


Figure 2.20 : Gas chromatogram of the extracted component in the aqueous system using large scale extraction

iv) Short Path Distillation Process

Besides those three previous methods, the separation method was also done *via* short path distiller. Several trials using short path distiller were conducted to separate glycerol from the reaction mixture.

The short path distillation method was used to isolate product from glycerol through gentle thermal separation method. The distillation process was carried out by varying the internal condenser and evaporator temperatures. The capacity of the distiller unit

was 1 L to 2 L. Table 2.5 shows the parameters set for the short path distiller. The aim was to obtain the glyceryl ether containing more than 60 % *mono-tert*-butoxypropanediol with less than 5 % glycerol.

Table 2.5 : Parameters for short path distillation process for purifying the product mixture

	Temperature setting (ºC)	
Sample	**Internal Condenser**	**Evaporator**
B1	0	45
B2	-5	45
B3	-10	45
B4	10	45
B5	0	60
B6	5	60
B7	-5	60
B8	-10	60

In attempts to obtain the desired composition of glyceryl ether containing more than 60 % *mono-tert*-butoxypropanediol with less than 5 % glycerol, the evaporator temperature was set at 60 °C while the internal condenser was set between -10 °C to 5 °C. Based on GC analysis, the yield of distillate containing glyceryl ether was expected to be around 50 % and the yield of residue which consists of the remaining glycerol was expected to be around 50 %. The distillation process of B5-B8 gave the distillate yields between 30 % to 50 % (Figure 2.21). Lower temperature setting (sample B7 and B8) of internal condenser (-5 °C and -10 °C) did not significantly increase the distillate yields as compared to the distillate yield of B6 obtained at higher temperature

(5 °C) of internal condenser. The lower temperature setting will increase the energy and cost where a cooling agent has to be used instead of water. In principle, the internal condenser temperature needs to be set as low as possible to prevent fraction vapor from disappearing into the trap and subsequently into the vacuum pump.

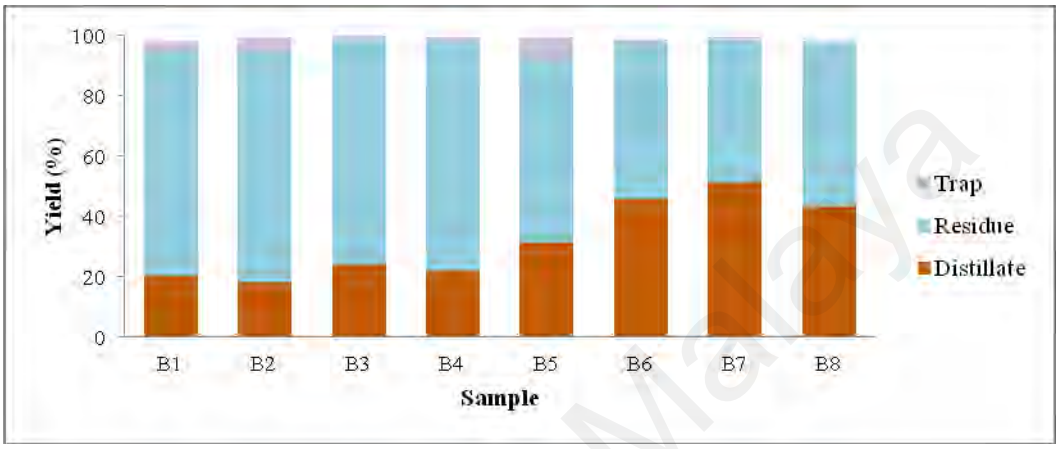


Figure 2.21 : The yields of residue and distillate obtained from short path distillation method

In order to determine which temperature setting will produce the desired product with a good balance in cost, the composition percentage of *mono-tert*-butoxypropanediol in the distillate was taken into consideration. Figure 2.22 shows the highest *mono-tert*-butoxypropanediol was recovered in distillates of B6-B8 with minimum amount of glycerol content (less than 2 %). The composition of components (expressed as percentage) in the residue fraction obtained from the distillation process is shown in Figure 2.23 where the highest amount of unreacted glycerol retained was 67 % with minimum amount of *mono-tert*-butoxypropanediol (B7). The unreacted glycerol retained in both B6 and B8 were less than 60 % with higher amount of *mono-tert*-butoxypropanediol than B7. Re-distillation process of the residue has successfully resulted in a high purity of *mono-tert*-butoxypropanediol (80-90 %) with less than 5 % glycerol.

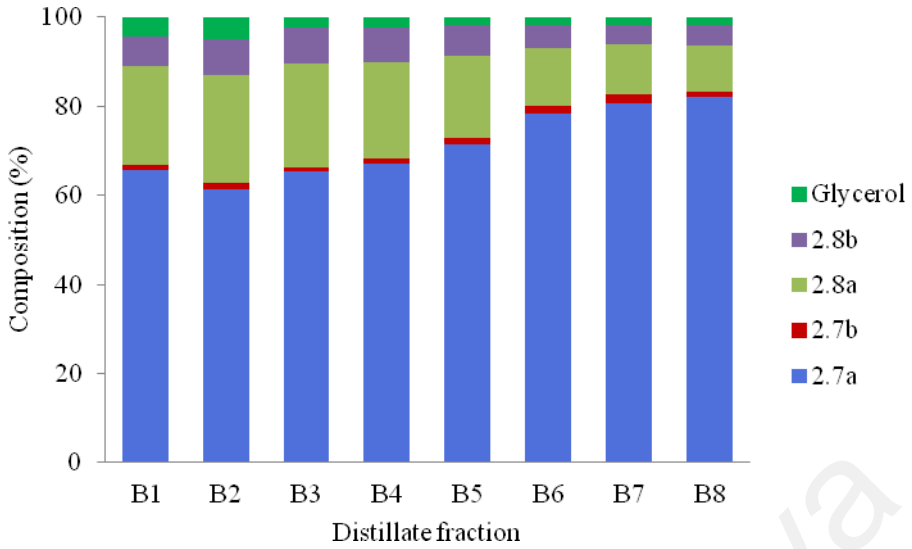


Figure 2.22 : Composition percentage of components in distillate fraction obtained from short path distillation method based on area percent of gas chromatograph

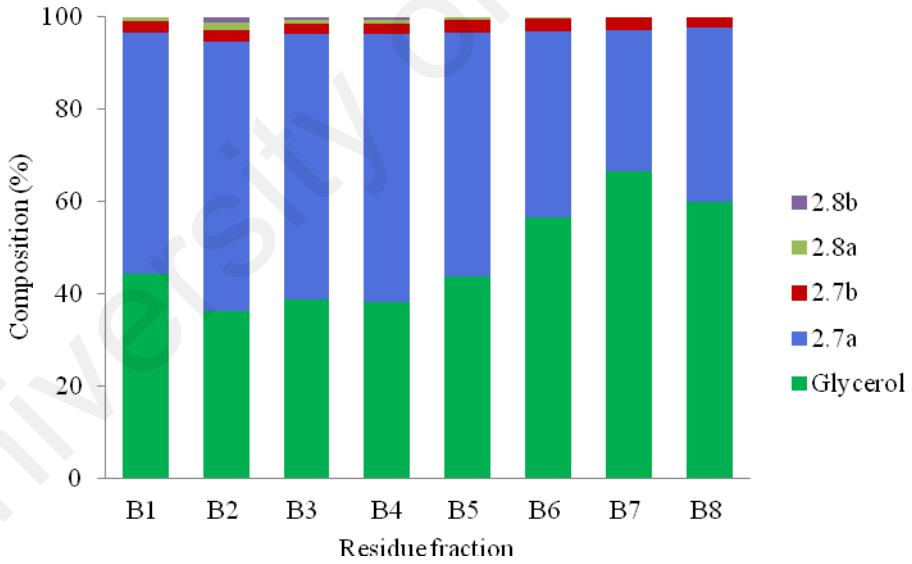


Figure 2.23 : Composition percentage of components in residue fraction obtained from short path distillation method based on area percent of gas chromatograph

To summarize, the results obtained from four separation methods are shown in Table 2.6. All four separation methods gave positive results where a product mixture consists of more than 60 % *mono-tert*-butoxypropanediol with less than 5 % glycerol content

can be isolated. In fact, there was no unreacted glycerol left in the product mixture obtained from fractional vacuum distillation.

Table 2.6 : Results obtained from four different separation methods for the purification of *mono-tert*-butoxypropanediol

Separation Method	Yield of product mixture (%)	Glycerol content in the product mixture after separation process (%)
Simple vacuum distillation	40	< 5
Fractional vacuum distillation	48	0
Liquid-liquid extraction	48	< 5
Short path distillation	40-50	< 5

Furthermore, a high purity of compound **2.7a** was obtained when the product mixture was extracted into DCM followed by short path distillation process. Figures 2.24 and 2.25 show the distillate and residue obtained from short path distillation, respectively. The residue from short path distillation consists of high purity of compound **2.7a** (94.6 %).

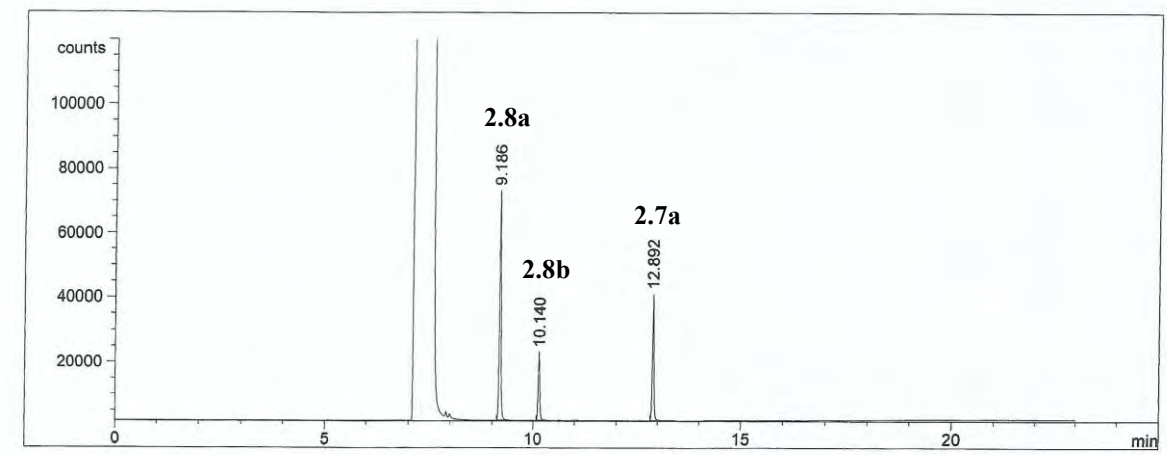


Figure 2.24 : Gas chromatogram of distillate obtained from short path distillation

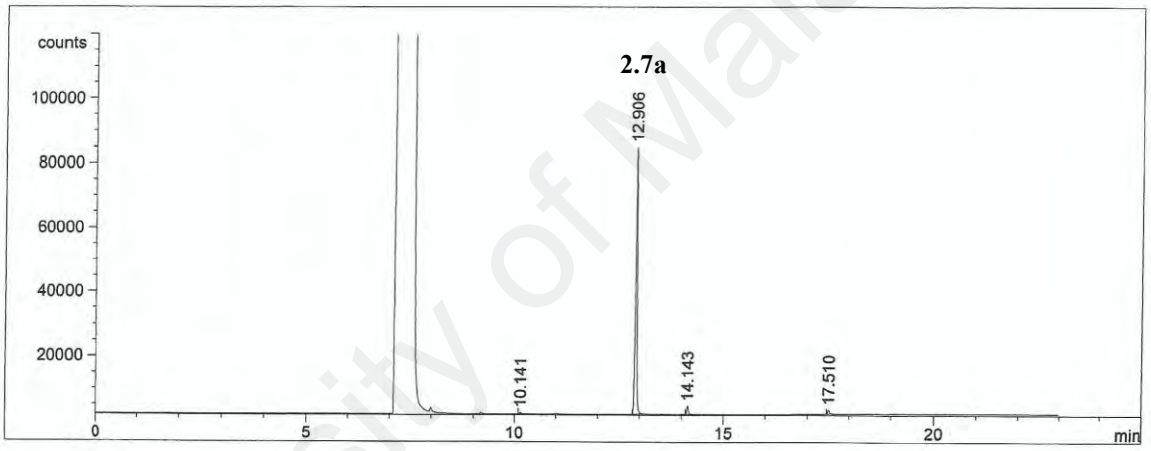


Figure 2.25 : Gas chromatogram of residue obtained from short path distillation

In summary, reactions of glycerol with *tert*-butanol were carried out using a 5 L reactor to produce glyceryl ether. The glycerol conversion was in the range of 50 % to 58 % based on gas chromatography analysis. This conversion was slightly low compared to small scale process conducted at similar reaction parameters where the glycerol conversion was about 75-80 %. However, attempts have been made in order to minimize and recover the glycerol left in the product mixture. The results showed that glycerol can be recovered from the product mixture by using vacuum distillation process and also liquid-liquid extraction where the product mixture containing less than 5 % glycerol can be obtained. Glyceryl ether containing more than 60 % *mono-tert-*

butoxypropanediol (compound **2.7a**) with less than 5 % glycerol is required for its application as a co-surfactant in microemulsion system and this product composition was used as a basis to investigate other potential applications of glyceryl ether, as discussed in Chapter 5.

CHAPTER 3: TWO-STEP REACTION FOR PRODUCING GLYCERYL ETHER

3.1 Introduction

Glycidol can be produced by several routes. It can be obtained from the conversion of allyl alcohol (Kollar, 1971). There are several published papers reporting on the epoxidation of allyl alcohol with a hydrogen peroxide in the presence of a catalyst (Hutchings *et al.*, 1996; Fajdek *et al.*, 2011; Danov *et al.*, 2011).

In addition, Payne & Sullivan (1961) claimed in US3,005,832 patent the process of preparing glycidol by hydrogenating glycidaldehyde in the presence of copper chromite. On the other hand, Yu *et al.* (2007) described the preparation of glycidol from the reaction of 3-chloro-1,2-propanediol with NaOH. Other than that, glycidol can also be obtained from epichlorohydrin or acrolein. In fact, the glycidol can also be prepared from glycerol. Bruson *et al.* (1953) patented the method for the production of glycidol by heating glycerol with a cyclic alkylene carbonate.

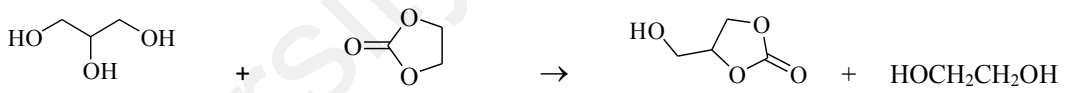
Epoxide rings can be cleaved by base as well as by acid. Merk and co-workers patented the synthesis of guaiacol glyceryl ether by ring opening process of glycidol with *o*-methoxyphenol in the presence of an alkali hydroxide, an alkali alcoholate, an alkali cyanate or an alkali thiocyanate. The product was obtained in very good yield and in very high purity (Merk *et al.*, 1983). Meanwhile, other researchers have patented the ring opening process of glycidol by menthol in the presence of Lewis acid as catalyst to obtain L-menthoxypropyleneglycol (Zhang *et al.*, 2008).

Efforts to produce glyceryl carbonate (compound **3.1**) by known method were made and further heating of compound **3.1** had led to the formation of compound **3.2** with liberation of CO_2. Then, compound **3.2** obtained was subjected to ring opening process in basic and acidic medium to yield glyceryl ethers with different alkyl chain length.

3.2 Synthesis of Glycidol from Glycerol

Attempts have been made to produce an epoxide from glycerol namely glycidol (compound **3.2**). The process involves two-step reaction. The first step is the preparation of the ester (4-hydroxymethyl-1,3-dioxolan-2-one) from glycerol and the second step is the decomposition of the ester into an epoxide compound. The epoxide obtained is further reacted with an alkylation agent to produce an ether compound.

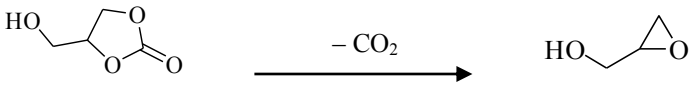
Reactions of glycerol and 1,3-dioxolan-2-one were conducted to produce compound **3.1**, 4-hydroxymethyl-1,3-dioxolan-2-one as shown in Scheme 3.1.



Glycerol 1,3-dioxolan-2-one **3.1** ethylene glycol

Scheme 3.1 : Reaction of glycerol and 1,3-dioxolan-2-one

Then 4-hydroxymethyl-1,3-dioxolan-2-one (compound **3.1)** decomposed to compound **3.2**, glycidol (Scheme 3.2) with CO_2 liberation. Glycidol is used in the synthesis of glyceryl ether.



3.1 **3.2**

Scheme 3.2 : Decomposition of 3.1 to 3.2 with CO_2 liberation

Firstly, the work had focused on the preparation of compound **3.1**. The reactions of glycerol and 1,3-dioxolan-2-one were carried out under reduced pressure ranging from 10 to 50 mbar in the absence of catalyst. The temperature of the reactions varied from 110 to 150 °C. The by-product, ethylene glycol was distilled off during the reaction.

The product mixture was analyzed by using FTIR and GC analyses. The infrared spectrum of product mixture obtained where the reaction was done at 140 °C under 30-50 mbar is shown in Appendix 3.1. A band at the wavenumber of 1780.8 cm^{-1} represents an ester bond in the molecule of compound **3.1**. Figure 3.1 shows the gas chromatogram of the product obtained.

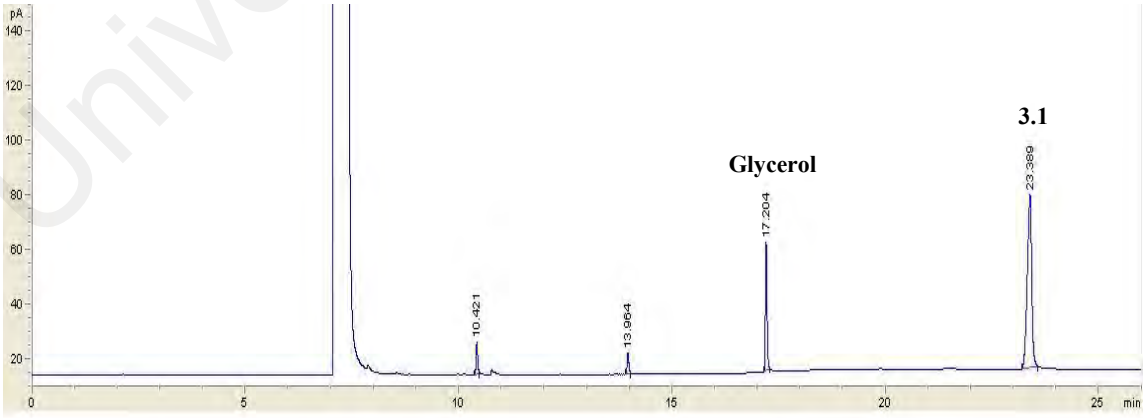
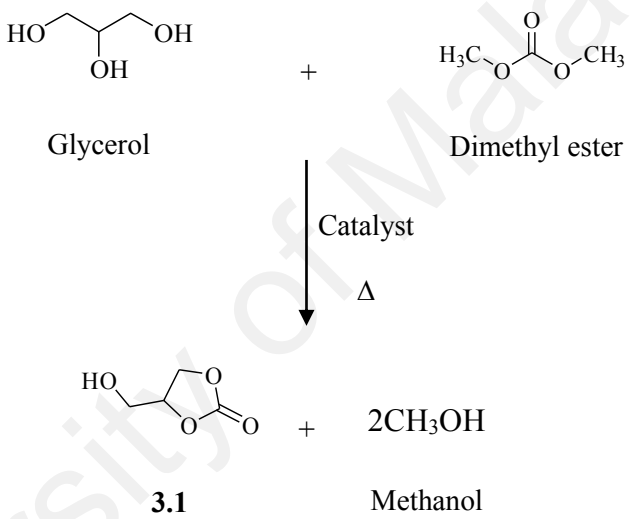


Figure 3.1 : Gas chromatogram of the product obtained from the reaction of glycerol and 1,3-dioxolan-2-one

Based on GC analysis, the percentage conversion of glycerol and the yield of the product were determined. The GC analysis was performed using an external standard calibration curve. From the reaction, the percentage yield of compound **3.1** produced was 86 %.

Other method to convert glycerol to compound **3.1** was also conducted according to Du *et al.* (2012) where dimethyl ester was reacted with glycerol in the presence of organometallic salt catalyst (Scheme 3.3).



Scheme 3.3 : Reaction of glycerol and dimethyl ester

Reaction of glycerol and dimethyl ester in the presence of K_2CO_3 was carried out under reflux condition. The by-product which is methanol was distilled off as well as unreacted dimethyl ester after the reaction process. The product mixture was extracted into ethyl acetate in order to discard the catalyst. Figure 3.2 shows the gas chromatogram of the product obtained from the reaction of glycerol and dimethyl ester, extracted into organic solvent.

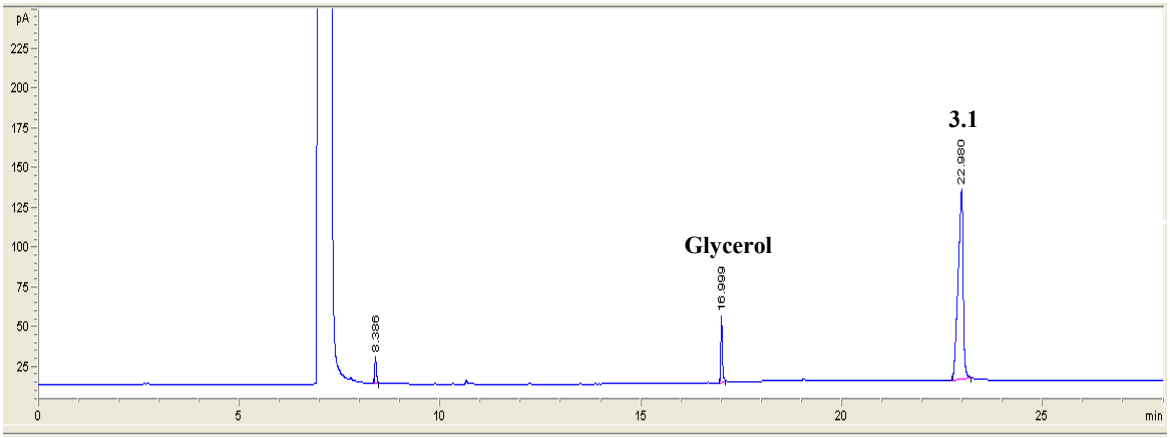


Figure 3.2 : Gas chromatogram of the product obtained from the reaction of glycerol and dimethyl ester

The yield and purity of compound **3.1** were determined quantitatively based on GC analysis. The GC analysis was performed using an external standard calibration curve. The yield of compound **3.1** obtained from the reaction was 70 %.

The results obtained from both methods for the production of compound **3.1** can be summarized as follows:-

Method	Yield of the product obtained (%)	Purity of the product obtained (%)
A) Glycerol + 1,3-dioxolan-2-one	86	80
B) Glycerol + dimethyl ester	70	94

Reactions to produce compound **3.2**, glycidol were done using compound **3.1** obtained from previous experiment (Method B) where the purity of compound **3.1** was 94 %. The reactions were done in the presence of sodium sulfate under reduced pressure (10-40 mbar) where compound **3.1** decomposed to liberate CO_2 besides compound **3.2**. Appendix 3.2 shows the infrared spectrum of the product obtained. A band at the wavenumber 1780 cm^{-1} of compound **3.1** was no longer present in the spectrum of

compound **3.2,** indicating the breakage of the ester bond. It is the most important band of distinguishing compound **3.1** from compound **3.2**.

Gas chromatogram (Figure 3.3) showed that the product contains one major component which was compound **3.2** based on the standard chemical.

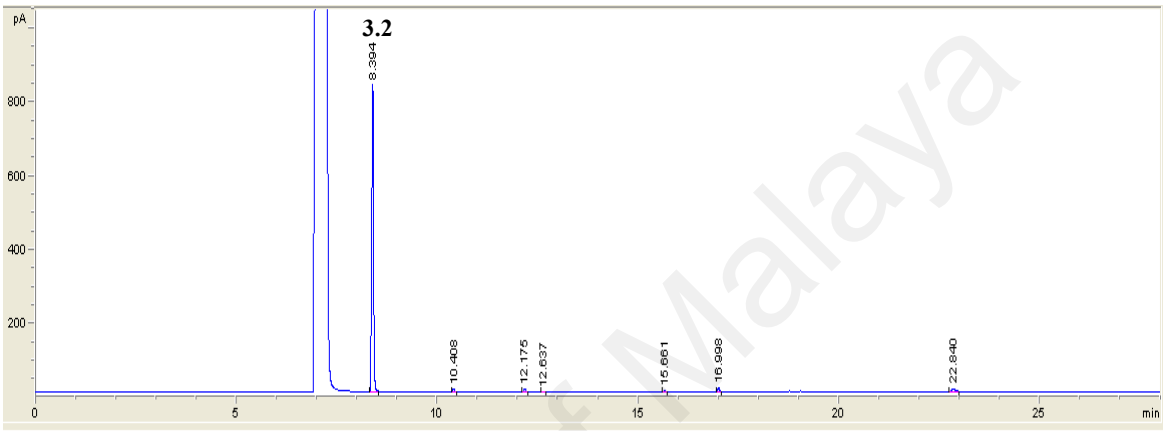


Figure 3.3 : Gas chromatogram of the product (compound 3.2)

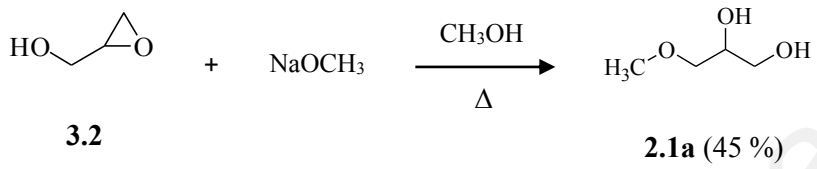
The highest yield of the product (compound **3.2**) obtained was 50 % based on GC analysis. The GC analysis was performed using an external standard calibration curve.

3.3 Glyceryl Ethers Formation by Ring Opening of Glycidol (Compound 3.2) under Basic Medium

Glyceryl ether was prepared from ring opening of compound **3.2** in a basic medium. According to McMurry, the attack of nucleophile takes place at the less hindered epoxide carbon in base-catalyzed ring opening of an epoxide (McMurry, 2008). Therefore, the possible product is in 1-position of carbon if the ring opening is done in basic medium.

3.3.1 Synthesis of 1-*mono*-methoxypropane-2,3-diol (compound 2.1a)

Reactions of compound **3.2** with sodium methoxide (30 % in MeOH) were carried out to produce compound **2.1a**, 1-*mono*-methoxypropane-2,3-diol (Scheme 3.4). The highest yield of product obtained was 45 %.



3.2 **2.1a** (45 %)

Scheme 3.4 : Reaction of 3.2 with sodium methoxide to obtain 2.1a, *mono*-methoxypropanediol

From the reaction, compound **2.1a** was successfully obtained as expected. The NMR spectra (Figures 3.4 and 3.5) confirmed that the structure was compound **2.1a**.

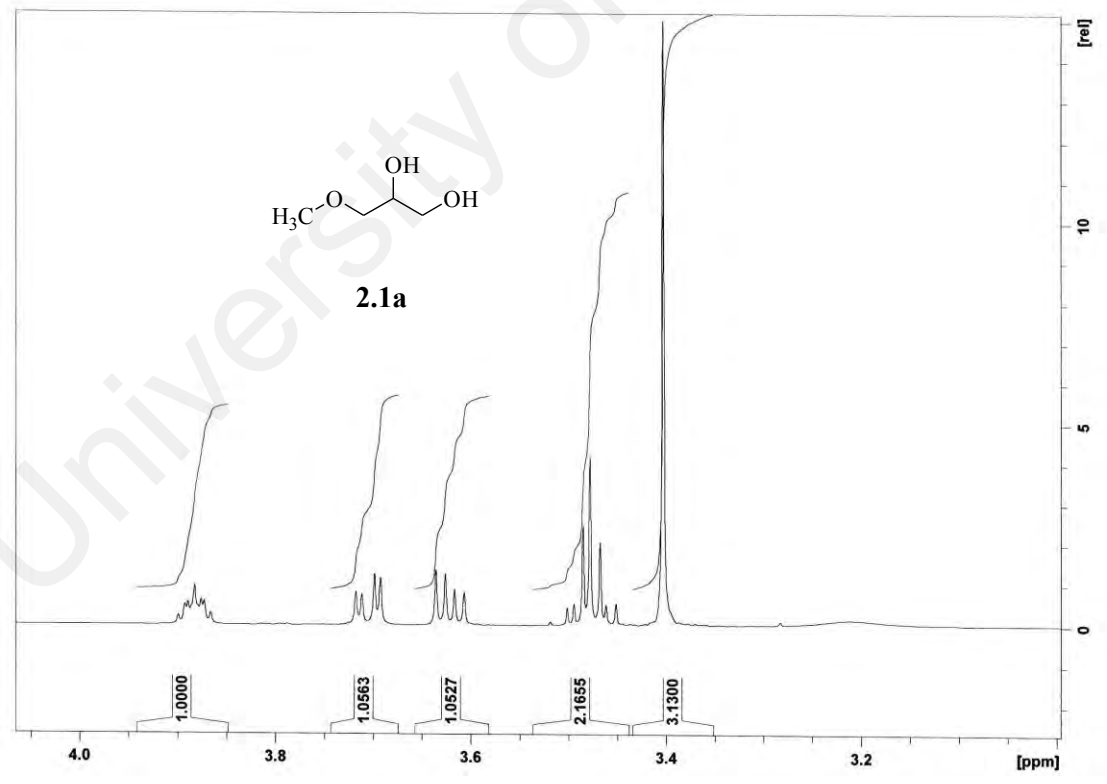


Figure 3.4 : ^{1}H NMR of compound 2.1a

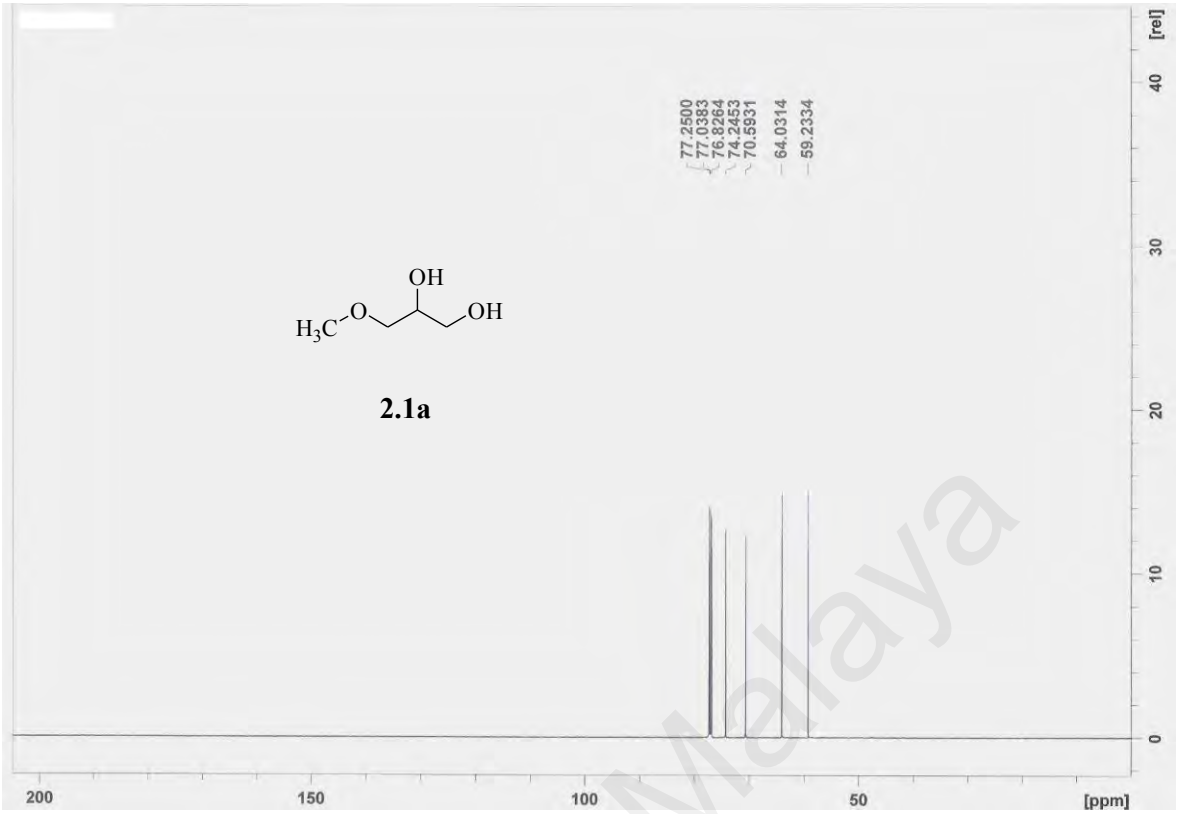


Figure 3.5 : ^{13}C NMR of compound 2.1a

The IR spectrum of compound **2.1a** is shown in Appendix 3.3. Figure 3.6 shows the gas chromatogram of compound **2.1a** with high purity.

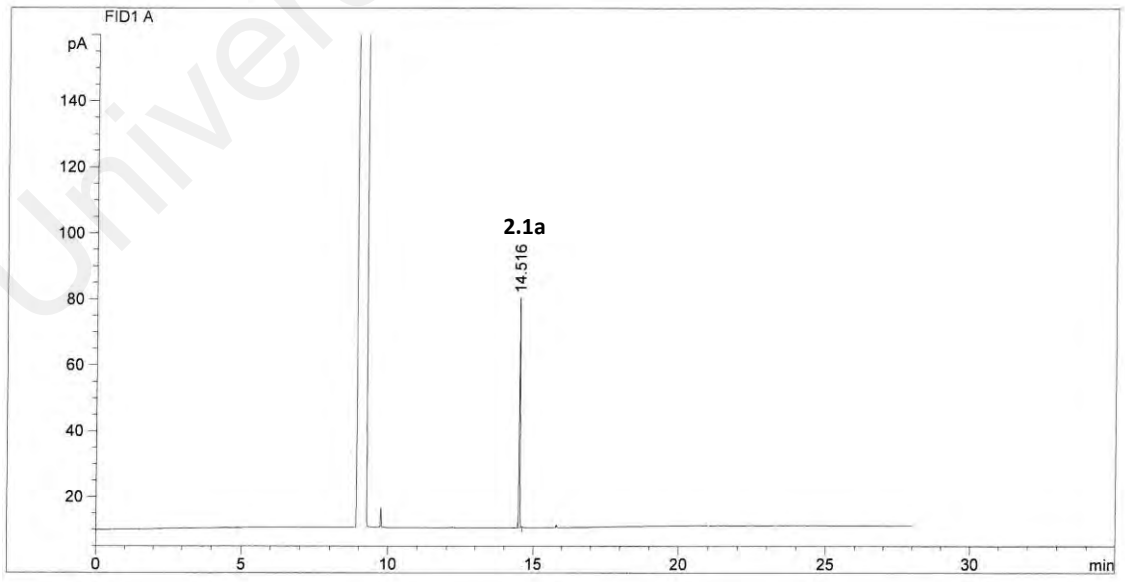
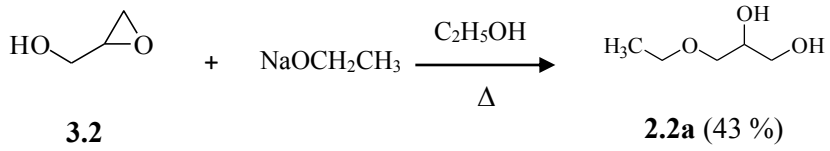


Figure 3.6 : Gas chromatogram of compound 2.1a

3.3.2 Synthesis of 1-*mono*-ethoxypropane-2,3-diol (compound 2.2a)

Reaction of compound **3.2** with sodium ethoxide had formed compound **2.2a** as depicted in Scheme 3.5.



3.2 **2.2a** (43 %)

Scheme 3.5 : Reaction of 3.2 with sodium ethoxide to obtain 2.2a, *mono*-ethoxypropanediol

In this study, sodium metal or sodium hydroxide was used to produce sodium ethoxide before proceed to the reaction with compound **3.2**. Both methods were compared and the highest crude yield was obtained when sodium metal was used to initiate the ethoxide. The product was successfully purified and from NMR spectra, compound **2.2a** was determined as expected. The ^{1}H and ^{13}C NMR spectra of compound **2.2a** are shown in Figures 3.7 and 3.8, respectively. In compound **2.2a** molecule, hydrogen atoms on the carbon attached to the oxygen are deshielded due to the electronegativity of the attached oxygen, and they appear in the range 3.55-3.89 ppm. Meanwhile, hydrogen atoms from methyl group appear as triplet at the chemical shift of 1.24 ppm. In addition, carbon atom which is directly attached to the electronegative oxygen atom in compound **2.2a** molecule shows higher deshielding effect thus leading to a greater chemical shift in ^{13}C spectrum.

An addition of one band at 2976.20 cm^{-1} in the IR spectrum of compound **2.2a** shows the sp^3-hybridized C–H stretching indicating the presence of methyl group in the compound **2.2a** molecule (Appendix 3.4). Figure 3.9 shows the gas chromatogram of compound **2.2a** with more than 99 % purity.

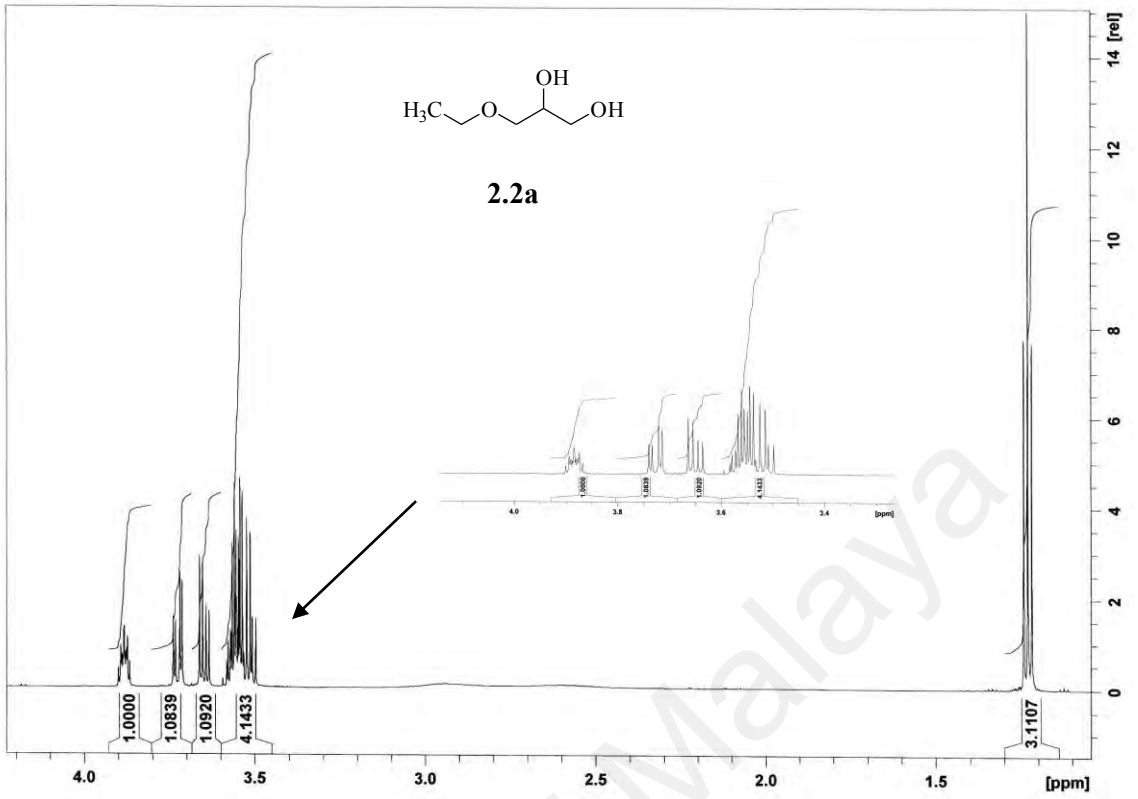


Figure 3.7 : ^{1}H NMR of compound 2.2a

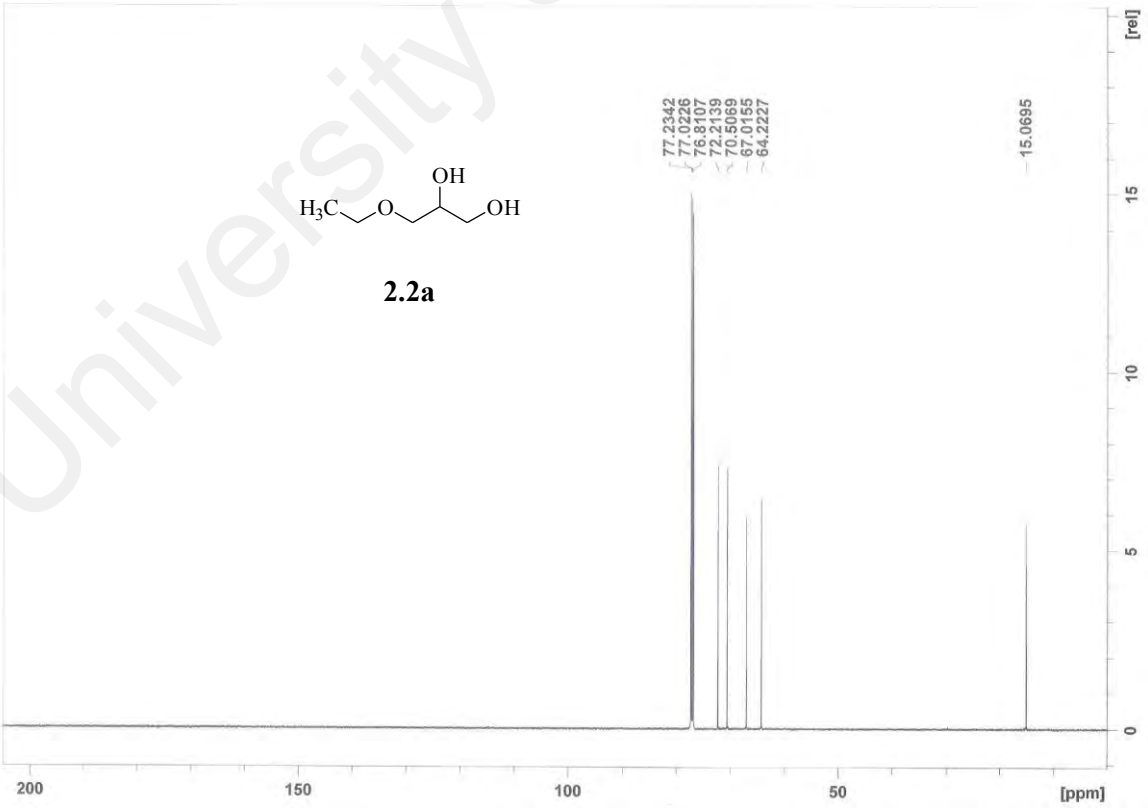


Figure 3.8 : ^{13}C NMR of compound 2.2a

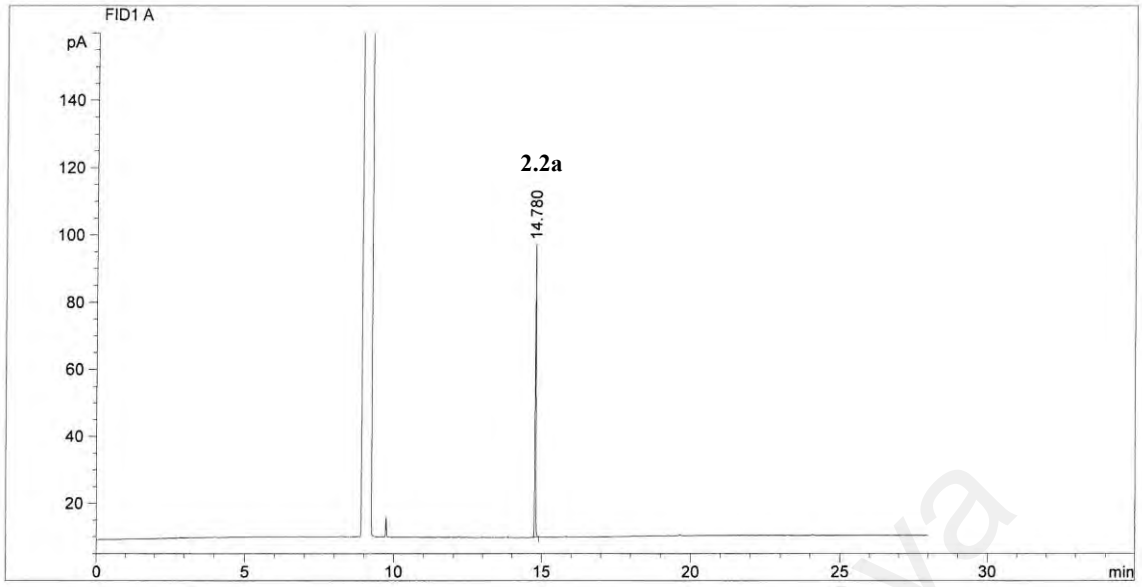
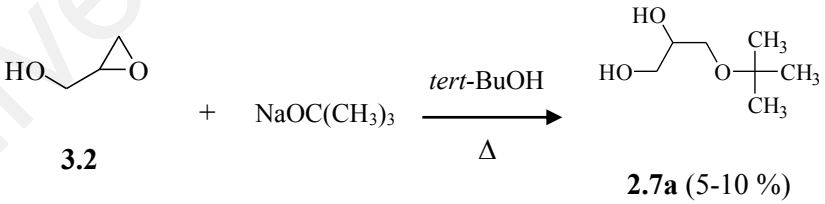


Figure 3.9 : Gas chromatogram of compound 2.2a

3.3.3 Synthesis of 1-*mono-tert*-butoxypropane-2,3-diol (compound 2.7a)

Reactions of compound **3.2** with sodium *tert*-butoxide were conducted and the reaction products were analyzed by GC analysis. Based on the standard compound synthesized in Chapter 2, the product obtained was compound **2.7a**. Even though the yield was low (5-10 %), but compound **2.7a** can be synthesized from this reaction route.



Scheme 3.6 : Reaction of 3.2 with sodium *tert*-butoxide to obtain 2.7a, *mono-tert*-butoxypropanediol

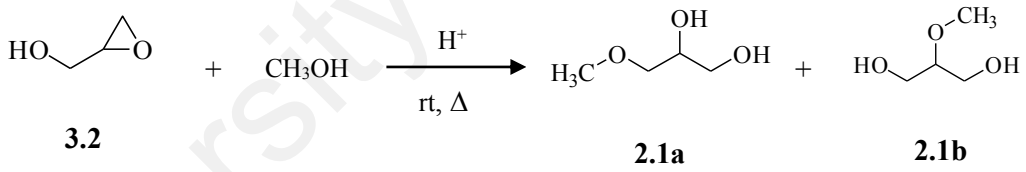
3.4 Glyceryl Ethers Formation by Ring Opening of Glycidol (Compound 3.2) under Acidic Medium

Ring opening of compound **3.2** can occur under a basic or acidic medium. Section 3.3 reports the ring opening reactions of compound **3.2** with alkoxide ions namely

methoxide, ethoxide and *tert*-butoxide to form the glyceryl ethers. Nevertheless, attempts to find a simple way without using alkoxide ion were made by performing the ring opening reactions of compound **3.2** in the presence of an acidic catalyst.

3.4.1 Ring opening of glycidol (compound 3.2) with methanol

Reaction of compound **3.2** with methanol was carried out and it was used as a model reaction to other alcohols. The optimum reaction parameters for synthesizing high yield of product were investigated. When compound **3.2** reacts with methanol in acidic medium, the expected product is compound **2.1b.** However, it was found that two isomers of *mono*-methoxypropanediol were formed as shown in Scheme 3.7. In fact, the isomer (compound **2.1a**) was the major component, as depicted in gas chromatogram (Figure 3.10).



3.2 **2.1a** **2.1b**

Scheme 3.7 : Reaction of compound 3.2 with methanol in acidic medium

Janaun and Ellis (2010) reported that the isomers of product produced from the etherification of glycerol give different RT in gas chromatogram. As clearly shown by Figure 3.10, the RT of compounds **2.1b** and **2.1a** in gas chromatogram is not similar. Compounds **2.1a** and **2.1b** were formed in a 3.4:1 mole ratio from the reaction of compound **3.2** with methanol in the presence of an acid catalyst.

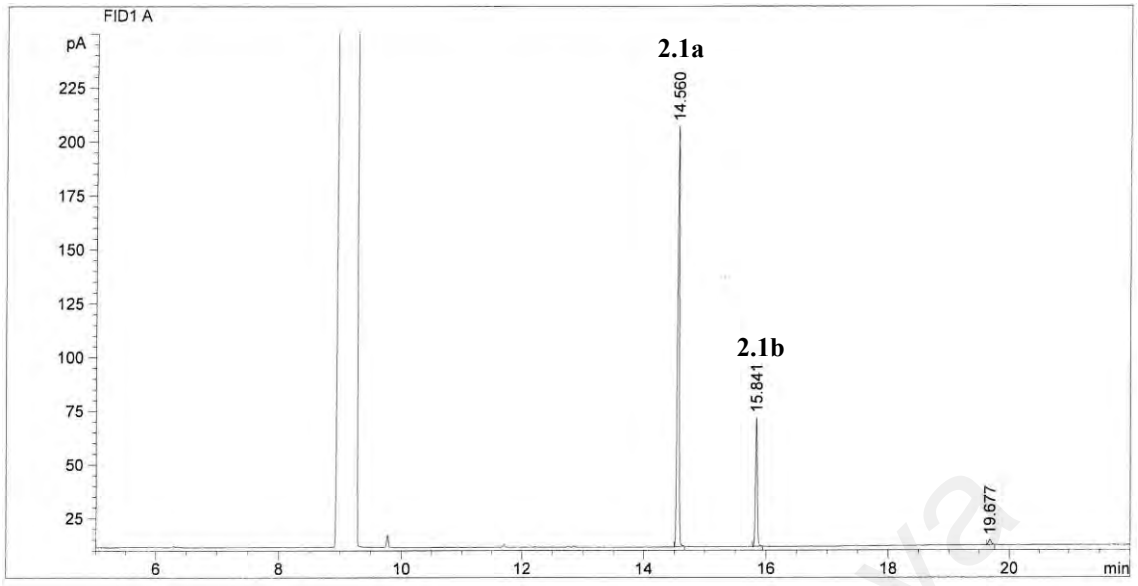
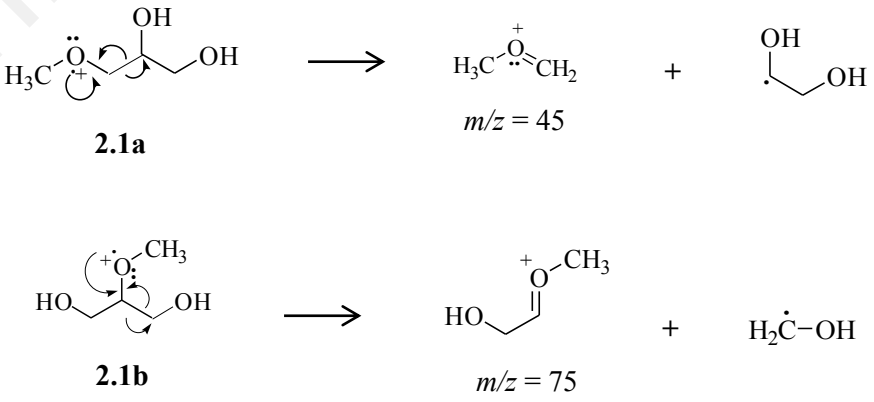


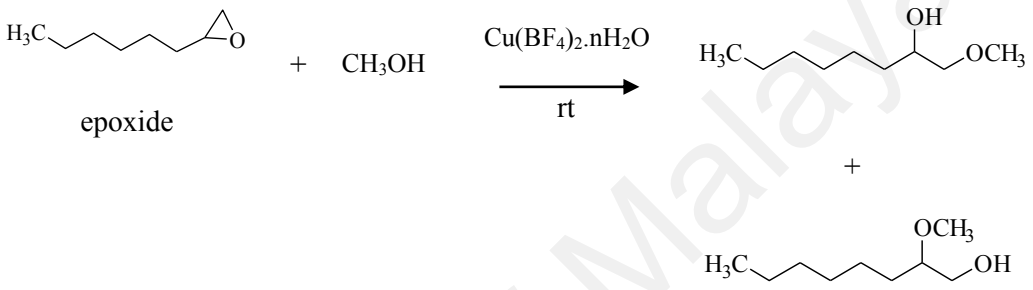
Figure 3.10 : Gas chromatogram of product obtained from the ring opening process of compound 3.2 with methanol in the presence of acid catalyst

Mass spectra (Appendix 3.5) of compounds **2.1a** and **2.1b** are nearly identical. It is identified that both compounds are isomers with molecular ion of *m/z* 106. The difference is the base peaks of *m/z* 45 and *m/z* 75 which correspond to *α*-cleavage of compound **2.1a** and compound **2.1b**, respectively (Scheme 3.8). When referring to both compounds **2.1a** and **2.1b**, we will use the term compound **2.1**.



Scheme 3.8: *α*-cleavage of isomers 2.1

Surprisingly, the formation of **2.1a** is more favorable than **2.1b** from the reaction. It reflects the expected competition of opening pathways. This finding is in agreement with Barluenga *et al.* (2002), where they reported that two adducts were obtained upon reaction of epoxide with methanol as shown in Scheme 3.9. Generally attack of the nucleophile takes place at the less hindered epoxide carbon in base-catalyzed ring opening of epoxide (McMurry, 2008).



Scheme 3.9 : Reaction of epoxide with methanol

Two types of acidic catalyst namely Amberlyst 15 and Montmorillonite K10 were used in the ring opening reaction of compound **3.2** with methanol. Both catalysts yielded isomers of *mono*-methoxypropanediol. However, there was a limitation when using Amberlyst 15 where it leached into the reaction mixture when the reaction was carried out at more than two hrs. Therefore, Montmorillonite K10 is preferable to Amberlyst 15 and it is used for latter study. The effect of the amount of Montmorillonite K10 on the yield of product (compound **2.1**) was investigated and the results are depicted in Figure 3.11.

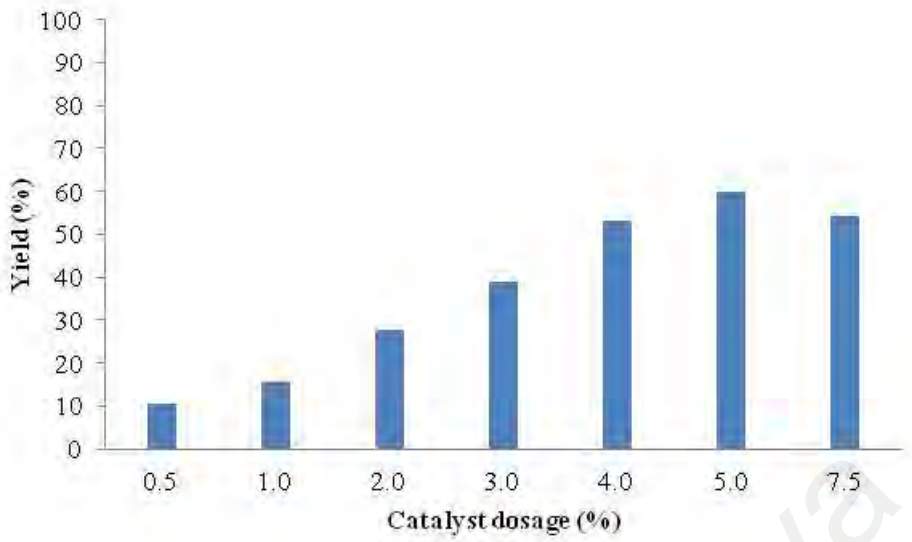


Figure 3.11: Product obtained from the reaction of compound 3.2 with methanol in the presence of different amount of catalyst, Montmorillonite K10

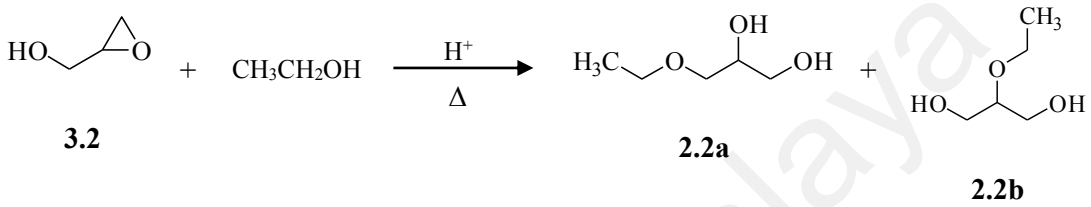
The highest yield of *mono*-methoxypropanediol (compound **2.1**) was obtained in the presence of 5 % catalyst. The mole ratio of 1:10 compound **3.2** to methanol produced higher yield compared to mole ratio of 1:5. The methanol may have acted as a solvent besides the reactant in the reaction, thus increase the yield. Furthermore, three hours reaction time had resulted in the highest yield of *mono*-methoxypropanediol. The optimum reaction temperature was 55-60 °C. The yield was low when the reaction was carried out at room temperature. When the reaction was carried out in the presence of 7.5 % catalyst, the conversion of compound **3.2** was high but the yield of targeted compound was lower than the yield obtained from the reaction conducted in the presence of 5 % catalyst. Compound **3.2** is very reactive and it may convert to undesirable side product besides the targeted compound in the presence of 7.5 % with heating.

The highest yield of 60 % compound **2.1** was obtained at the mole ratio of 1:10 (compound **3.2** : methanol) when the reaction was done at 55-60 °C for three hrs in the presence of Montmorillonite K10. The optimum reaction parameters were used as a

basis to conduct reactions of compound **3.2** with other alcohols in the presence of acidic catalyst.

3.4.2 Ring opening of glycidol (compound 3.2) with ethanol

Reaction of compound **3.2** with ethanol in acidic medium also formed an isomer of compound **2.2b** as shown in Scheme 3.10.



3.2 **2.2a** **2.2b**

Scheme 3.10 : Reaction of compound 3.2 with ethanol in acidic medium

The yield of the product was 49 % with compound **2.2a** as the major component (Figure 3.12). The phenomenon is similar to the reaction of compound **3.2** with methanol where the formation of compound **2.2a** is more favorable than **2.2b** even though it is done in acidic medium.

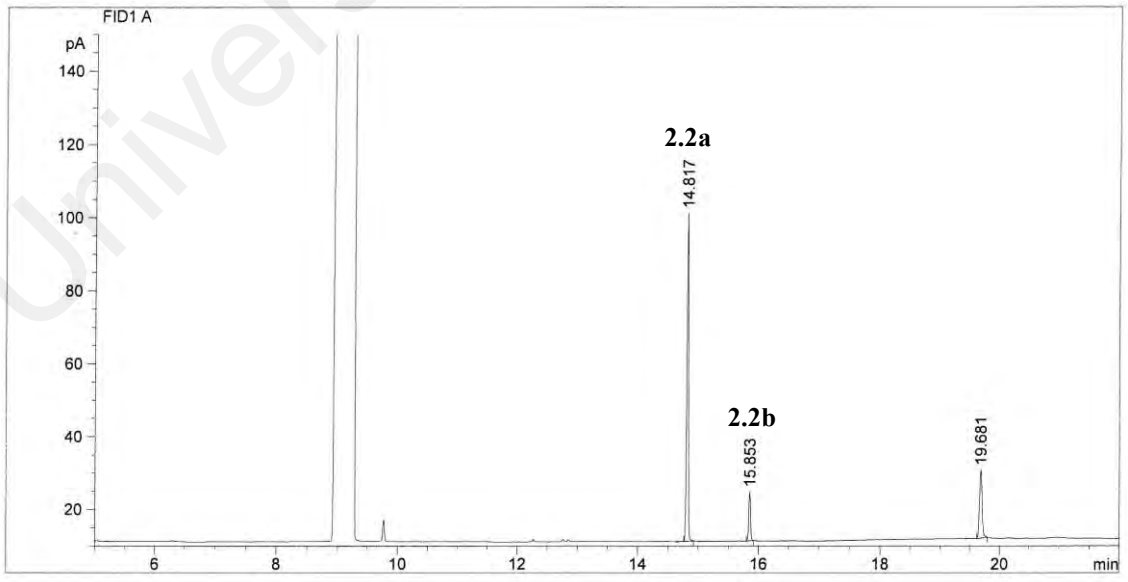
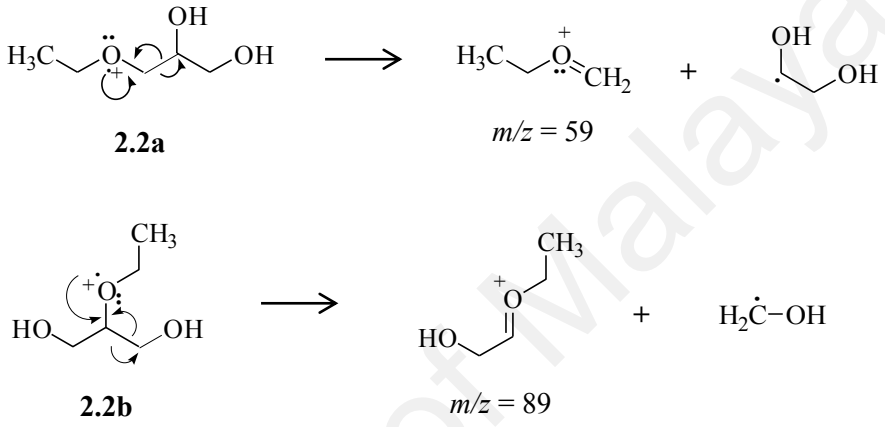


Figure 3.12 : Gas chromatogram of product obtained from the ring opening process of compound 3.2 with ethanol in the presence of acid catalyst

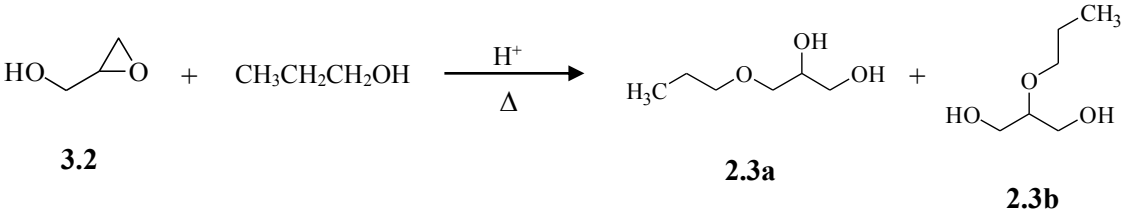
It is identified from the mass spectra (Appendix 3.6) that both compounds **2.2a** and **2.2b** are isomers with molecular ion of *m/z* 120. The difference is the base peaks of *m/z* 59 and *m/z* 89 which correspond to *α*-cleavage of compound **2.2a** and compound **2.2b**, respectively (Scheme 3.11). Furthermore, the *m/z* 59 is absent in the mass spectrum of compound **2.2b**. When referring to both compounds **2.2a** and **2.2b**, we will use the term compound **2.2**.



Scheme 3.11: *α*-cleavage of isomers 2.2

3.4.3 Ring opening of glycidol (compound 3.2) with propanol

Reaction of compound **3.2** with propanol in the presence of acidic catalyst also resulted in two components as shown in Scheme 3.12. The yield of the product was 24 %. Figure 3.13 shows the gas chromatogram of the product obtained consisting of compounds **2.3a** and **2.3b**.



Scheme 3.12 : Reaction of compound 3.2 with propanol in acidic medium

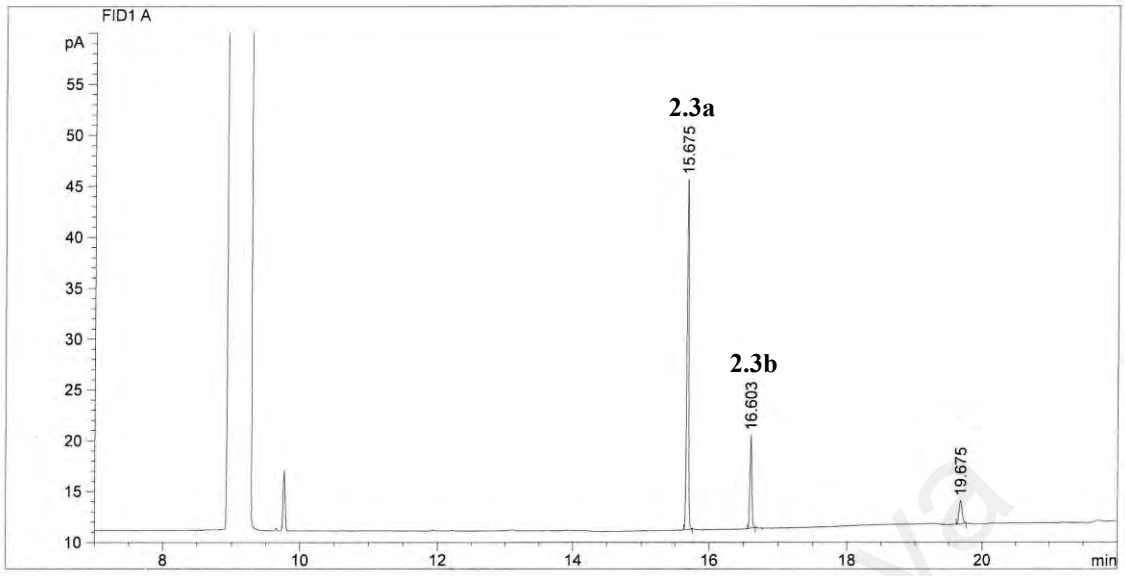
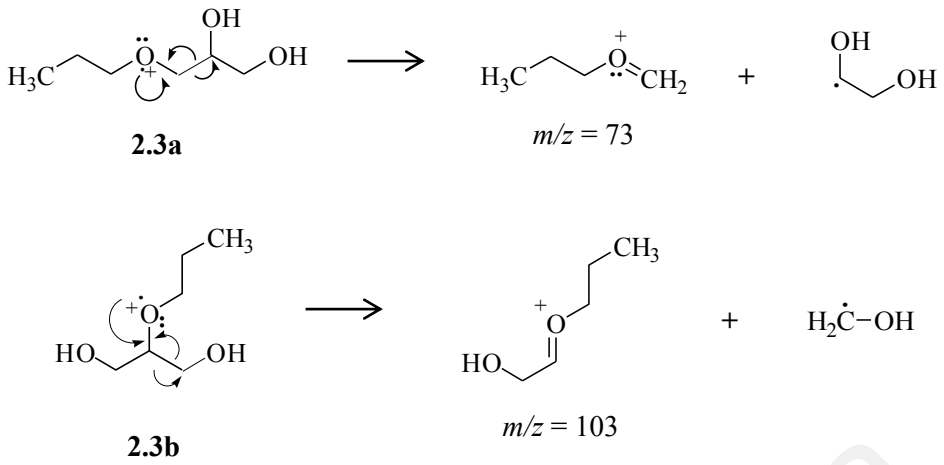


Figure 3.13 : Gas chromatogram of product obtained from the ring opening process of compound 3.2 with propanol in the presence of acid catalyst

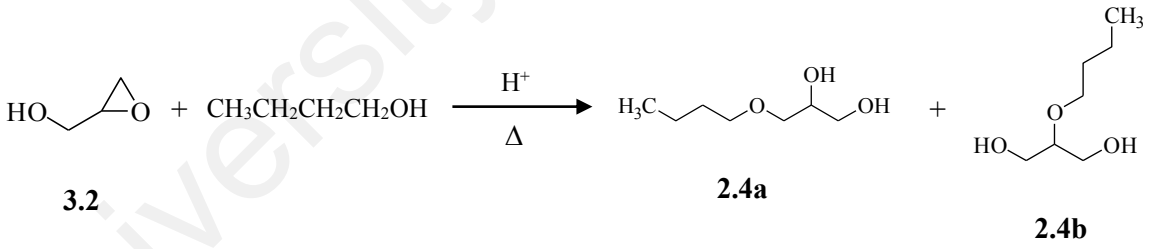
It is identified from the mass spectra (Appendix 3.7) that both compounds **2.3a** and **2.3b** are isomers with molecular ion of *m/z* 134. The carbon-carbon bond of both compounds **2.3a** and **2.3b** may be broken to yield fragment ions of *m/z* 73 and 103, respectively (Scheme 3.13). The presence of fragment ion of *m/z* 73, $[CH_2OCH_2CH_2CH_3]^+$ at modest abundance in the spectrum of compound **2.3a** is important in distinguishing both isomers. It is almost absent in the spectrum of compound **2.3b**. In addition, the α-cleavage gives rise to the fragment ion of *m/z* 103 in the mass spectrum of compound **2.3b**. When referring to both compounds **2.3a** and **2.3b**, we will use the term compound **2.3**.



Scheme 3.13: *α*-cleavage of isomers 2.3

3.4.4 Ring opening of glycidol (compound 3.2) with butanol

Similar phenomenon occurred in the reaction of compound **3.2** with butanol. In the presence of acidic catalyst, the formation of compound **2.4a** was detected along with compound **2.4b** as shown in Scheme 3.14. The yield of the product was 15 %.



3.2 **2.4a** **2.4b**

Scheme 3.14 : Reaction of compound 3.2 with butanol in acidic medium

Gas chromatogram (Figure 3.14) shows the product consists of both isomers (compounds **2.4a** and **2.4b**).

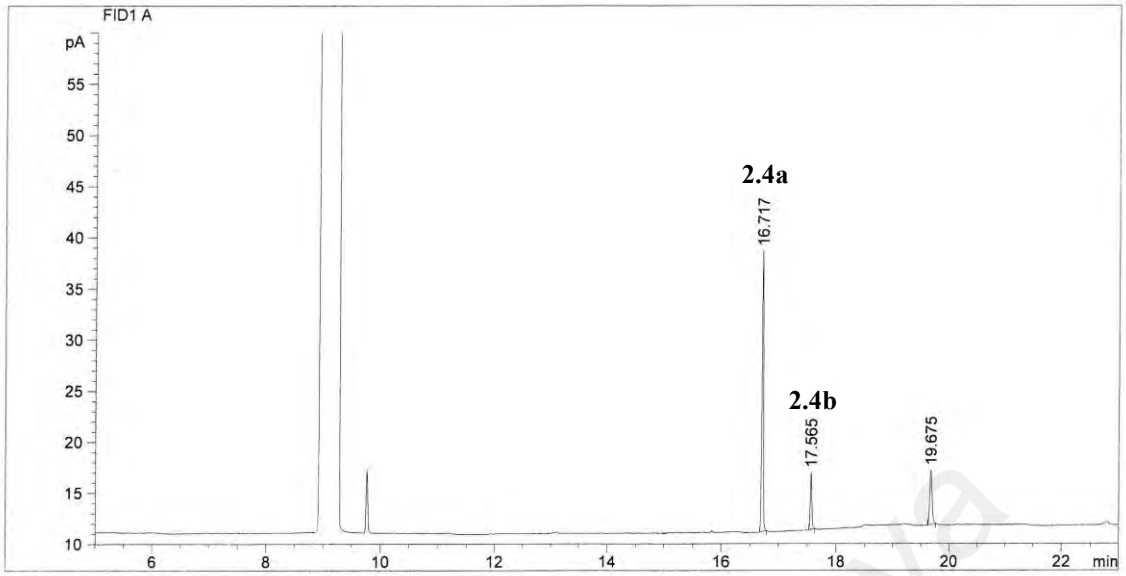
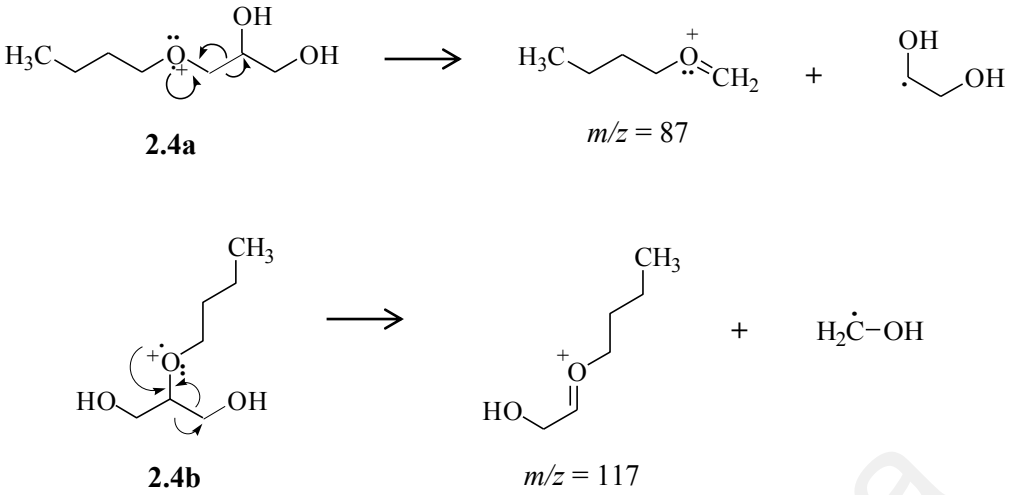


Figure 3.14 : Gas chromatogram of product obtained from the ring opening process of compound 3.2 with butanol in the presence of acid catalyst

It is identified from the mass spectra (Appendix 3.8) that both compounds **2.4a** and **2.4b** are isomers with molecular ion of *m/z* 148. Both isomers display similar *m/z* 57 base peaks. The presence of fragment ion of *m/z* 87, $[CH_2OCH_2CH_2CH_2CH_3]^+$ at high abundance in the spectrum of compound **2.4a** is important in distinguishing both isomers. It is absent in the spectrum of compound **2.4b**. In addition, the cleavage gives rise to the fragment ion of *m/z* 117 in the mass spectrum of compound **2.4b**.

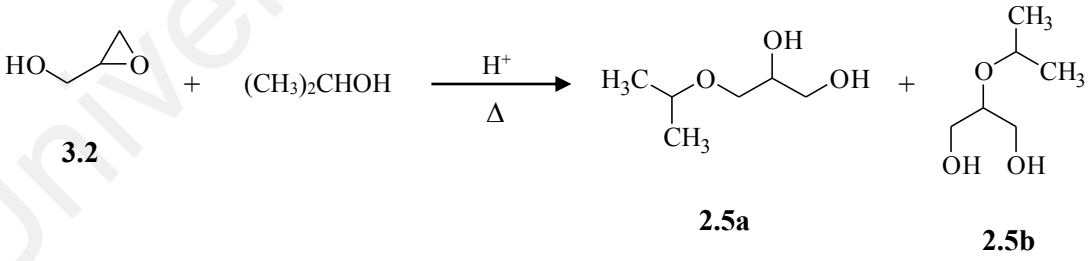
Fragment ions of *m/z* 87 and *m/z* 117 correspond to *α*-cleavage of compounds **2.4a** and **2.4b**, respectively (Scheme 3.15). When referring to both compounds **2.4a** and **2.4b**, we will use the term compound **2.4**.



Scheme 3.15: *α*-cleavage of isomers 2.4

3.4.5 Ring opening of glycidol (compound 3.2) with *iso*-propanol

Reaction of compound **3.2** with *iso*-propanol was faster than 1-propanol, indicated by the conversion of **3.2** and the yield of the product (45 %). However, similar phenomenon occurred where the reaction also produced compound **2.5a** besides **2.5b** as shown in Scheme 3.16.



Scheme 3.16 : Reaction of compound 3.2 with *iso*-propanol in acidic medium

Figure 3.15 shows the gas chromatogram of the product containing both isomers **2.5a** and **2.5b**.

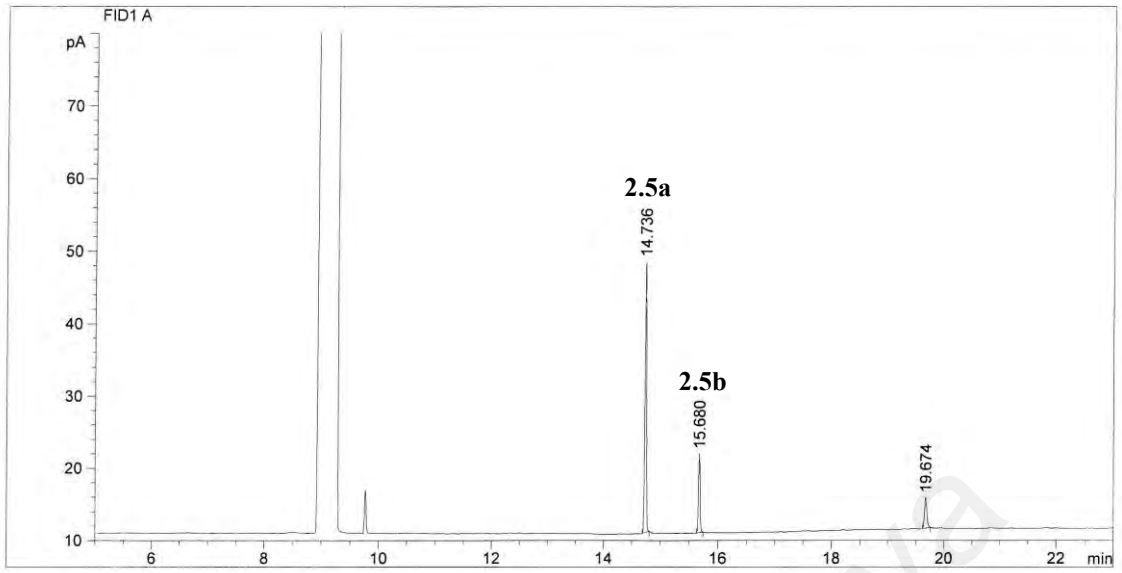
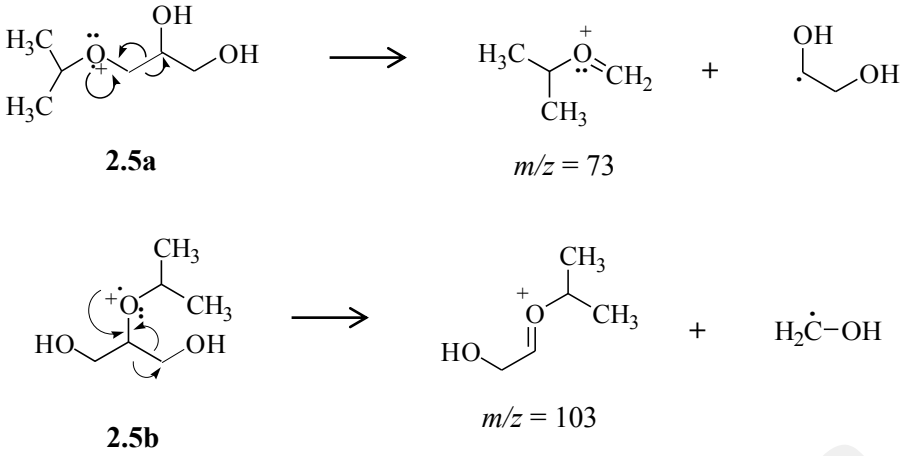


Figure 3.15 : Gas chromatogram of product obtained from the ring opening process of compound 3.2 with *iso*-propanol in the presence of acid catalyst

It is identified from the mass spectra (Appendix 3.9) that both compounds **2.5a** and **2.5b** are isomers with molecular ion of *m/z* 134. The presence of fragment ion of *m/z* 73, $[CH_2OCH(CH_3)_2]^+$ at modest abundance in the spectrum of compound **2.5a** is important in distinguishing both isomers. It is almost absent in the spectrum of compound **2.5b**. In addition, the cleavage gives rise to the fragment ion of *m/z* 103 in the mass spectrum of compound **2.5b**.

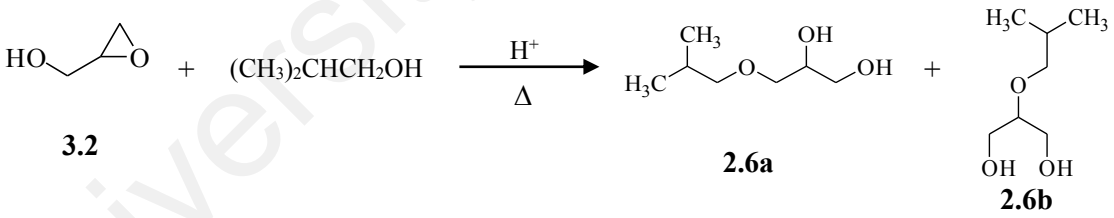
Fragment ions of *m/z* 73 and *m/z* 103 correspond to *α*-cleavage of compounds **2.5a** and **2.5b**, respectively (Scheme 3.17). When referring to both compounds **2.5a** and **2.5b**, we will use the term compound **2.5**.



Scheme 3.17: *α*-cleavage of isomers 2.5

3.4.6 Ring opening of glycidol (compound 3.2) with *iso*-butanol

Reaction of glycidol with *iso*-butanol produced a mixture of isomers of *mono-iso*-butoxy-propanediol (compounds **2.6a** and **2.6b**) as shown in Scheme 3.18 with 21 % yield.



Scheme 3.18 : Reaction of compound 3.2 with *iso*-butanol in acidic medium

Figure 3.16 shows the gas chromatogram of the product obtained containing both isomers **2.6a** and **2.6b**.

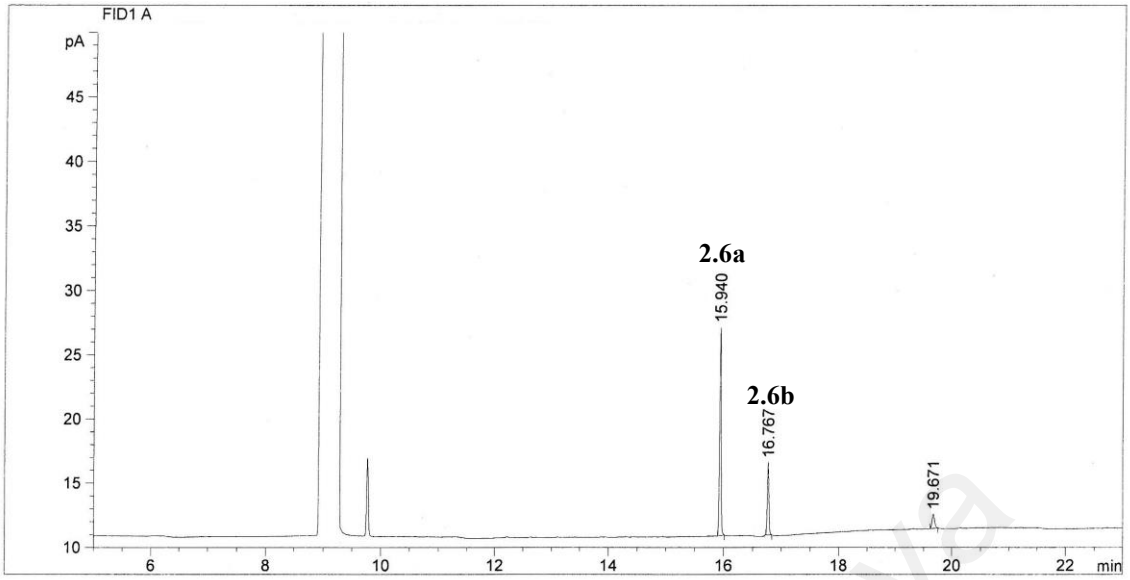
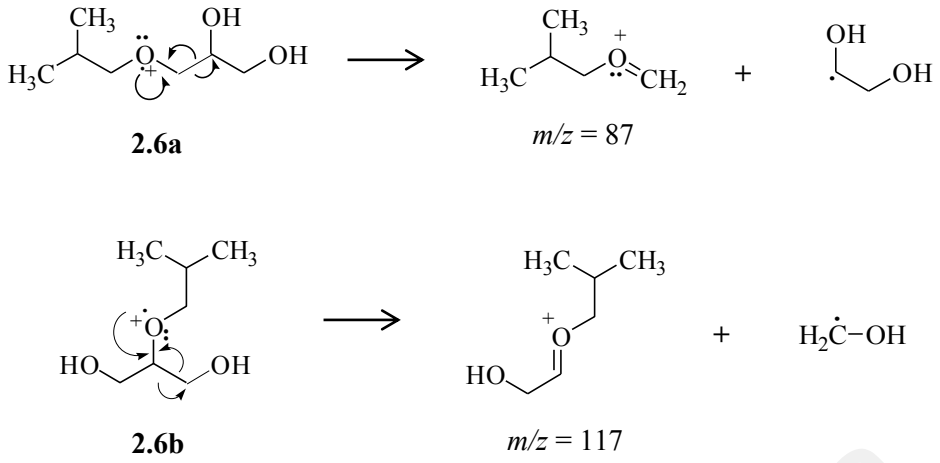


Figure 3.16 : Gas chromatogram of product obtained from the ring opening process of compound 3.2 with *iso*-butanol in the presence of acid catalyst

It is identified from the mass spectra (Appendix 3.10) that both compounds **2.6a** and **2.6b** are isomers with molecular ion of *m/z* 148. Both isomers display similar *m/z* 57 base peaks. The presence of fragment ion of *m/z* 87, $[CH_2OCH_2CH(CH_3)_2]^+$ at modest abundance in the spectrum of compound **2.6a** is important in distinguishing both isomers. It is absent in the spectrum of compound **2.6b**. In addition, the cleavage gives rise to the fragment ion of *m/z* 117 in the mass spectrum of compound **2.6b**.

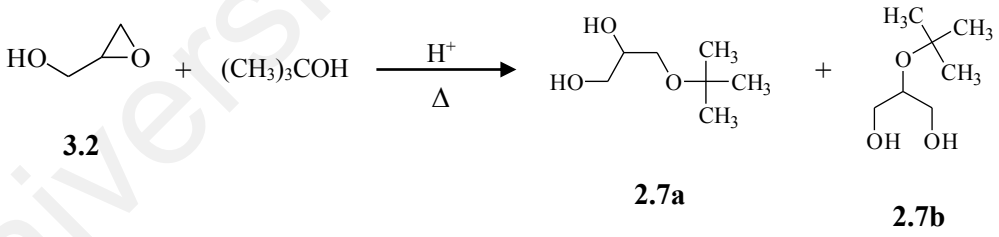
Fragment ions of *m/z* 87 and *m/z* 117 correspond to *α*-cleavage of compounds **2.6a** and **2.6b**, respectively (Scheme 3.19). When referring to both compounds **2.6a** and **2.6b**, we will use the term compound **2.6**.



Scheme 3.19: *α*-cleavage of isomers 2.6

3.4.7 Ring opening of glycidol (compound 3.2) with *tert*-butanol

It was found that compound **2.7** (compounds **2.7a** and **2.7b**) with 31 % yield can also be produced from the ring opening of compound **3.2** under acidic medium (Scheme 3.20) besides the direct etherification as in Chapter 2.



Scheme 3.20: Reaction of compound 3.2 with *tert*-butanol in acidic medium

When compound **3.2** is reacted with *tert*-butanol in the presence of acid, other reaction could occur with the formation of *tert*-butanol carbocation, thus retaining the epoxide structure. But in this case, no formation of the epoxide product detected in the reaction mixture indicating that the ring opening reaction of compound **3.2** occurred faster than the formation of the carbocation of *tert*-butanol, thus yielding only compound **2.7a** and **2.7b**.

Therefore, one can produce compound **2.7** using this reaction route if the target compound is only **2.7**. In contrast, compound **2.8** is also obtained along with **2.7** from the direct etherification reaction of glycerol with *tert*-butanol as reported in Chapter 2. Figure 3.17 shows the gas chromatogram of the product containing both isomers (compound **2.7a** and **2.7b**).

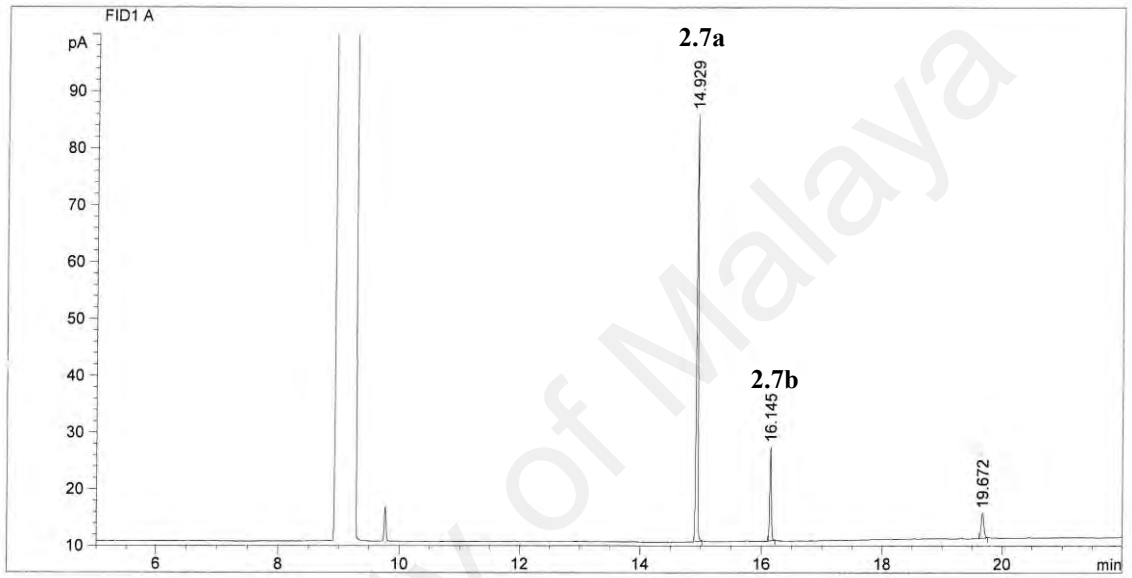
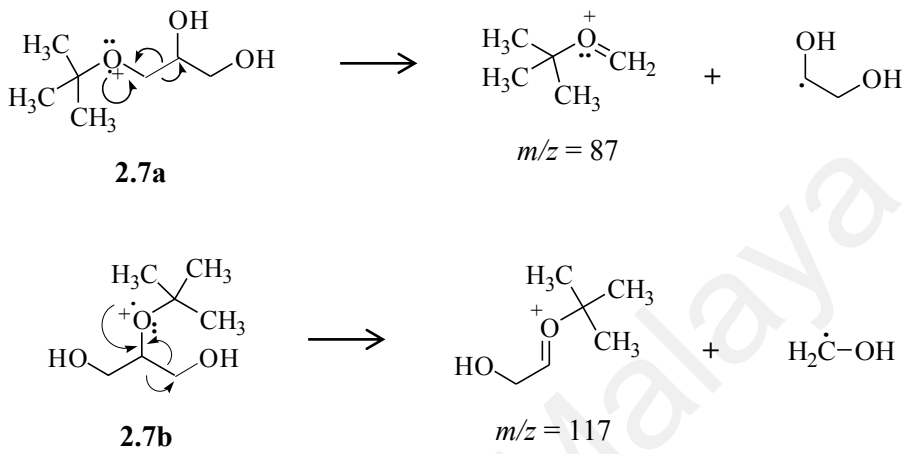


Figure 3.17 : Gas chromatogram of product obtained from the ring opening process of compound 3.2 with *tert*-butanol in the presence of acid catalyst

It is identified from the mass spectra (Appendix 3.11) that both compounds **2.7a** and **2.7b** are isomers with molecular ion of *m/z* 148. Both isomers display similar *m/z* 57 base peaks. The presence of fragment ion of *m/z* 87, $[CH_2OC(CH_3)_3]^+$ in the spectrum of compound **2.7a** is important in distinguishing both isomers. It is absent in the spectrum of compound **2.7b**. In addition, the cleavage gives rise to the fragment ion of *m/z* 117 in the mass spectrum of compound **2.7b**.

Fragment ions of *m/z* 87 and *m/z* 117 correspond to *α*-cleavage of compounds **2.7a** and **2.7b**, respectively (Scheme 3.21). When referring to both compounds **2.7a** and **2.7b**, we will use the term compound **2.7**.



Scheme 3.21: *α*-cleavage of isomers 2.7

In summary, ring opening of compound **3.2** with respective alcohols in acidic medium formed two adducts or isomers. We propose as a general mechanism that ring opening of compound **3.2** occurs, involving the attack of the nucleophile at both sides of epoxide carbon.

All glyceryl ethers produced (compounds **2.1**-**2.7**) were analyzed for their antibacterial activity and *in vitro* dermal irritation potential, as discussed in Chapter 5.

CHAPTER 4: TRANSFORMATION OF HYDROXYL GROUP OF GLYCEROL INTO A GOOD LEAVING GROUP

4.1 Introduction

It has been discovered that the hydroxyl group of alcohols can be transformed into a good leaving group by replacing the hydroxyl group with tosyl or mesyl group. The alcohol can also be transformed into triflates. Treatments of alcohols with *p*-toluenesulfonyl chloride (*p*-TsCl) have been widely investigated (Tipson, 1944; Brown *et al.*, 1967; Lee *et al.*, 2000; Wang *et al.*, 2003). The tosylation of alcohols with *p*-TsCl in the presence of a catalyst under solvent-free conditions was also reported (Razieh *et al.*, 2006; Kazemi *et al.*, 2007). Besides tosyl group, mesyl group can also be introduced to the alcohols by allowing the alcohols to react with methanesulfonyl chloride (Jung & Shaw, 1980). In addition, alkyl and allylic alcohols were converted to its' triflates by reacting the corresponding alcohols with trifluoromethanesulfonic acid anhydride (Beard *et al.*, 1973).

Tosylates contain an excellent leaving group and they are versatile substrates for nucleophilic substitution reactions (Ding *et al.*, 2011). The most widely used tosylating agent is the tosyl chloride (TsCl) which is more reactive than tosyl anhydride and *p*-toluenesulfonic acid. Generally, TsCl is used for the preparation of tosylates in the presence of a base (Kabalka *et al.*, 1986; Adlington *et al.*, 1981; Holand & Epsztein,1977).

Attempts to produce ester tosylate of glycerol were made with the aim to transform the hydroxyl group of glycerol into a good leaving group. The target compound was *mono*-tosylate of glycerol. The reaction was done by varying the reaction conditions such as

different solvents and amount of base used. Other than that, the protection of hydroxyl groups of glycerol was done by producing solketal, followed by the tosylation of solketal. The tosylates obtained could be used as intermediate for the preparation of glyceryl ether.

4.2 Formation of Glyceryl Tosylate

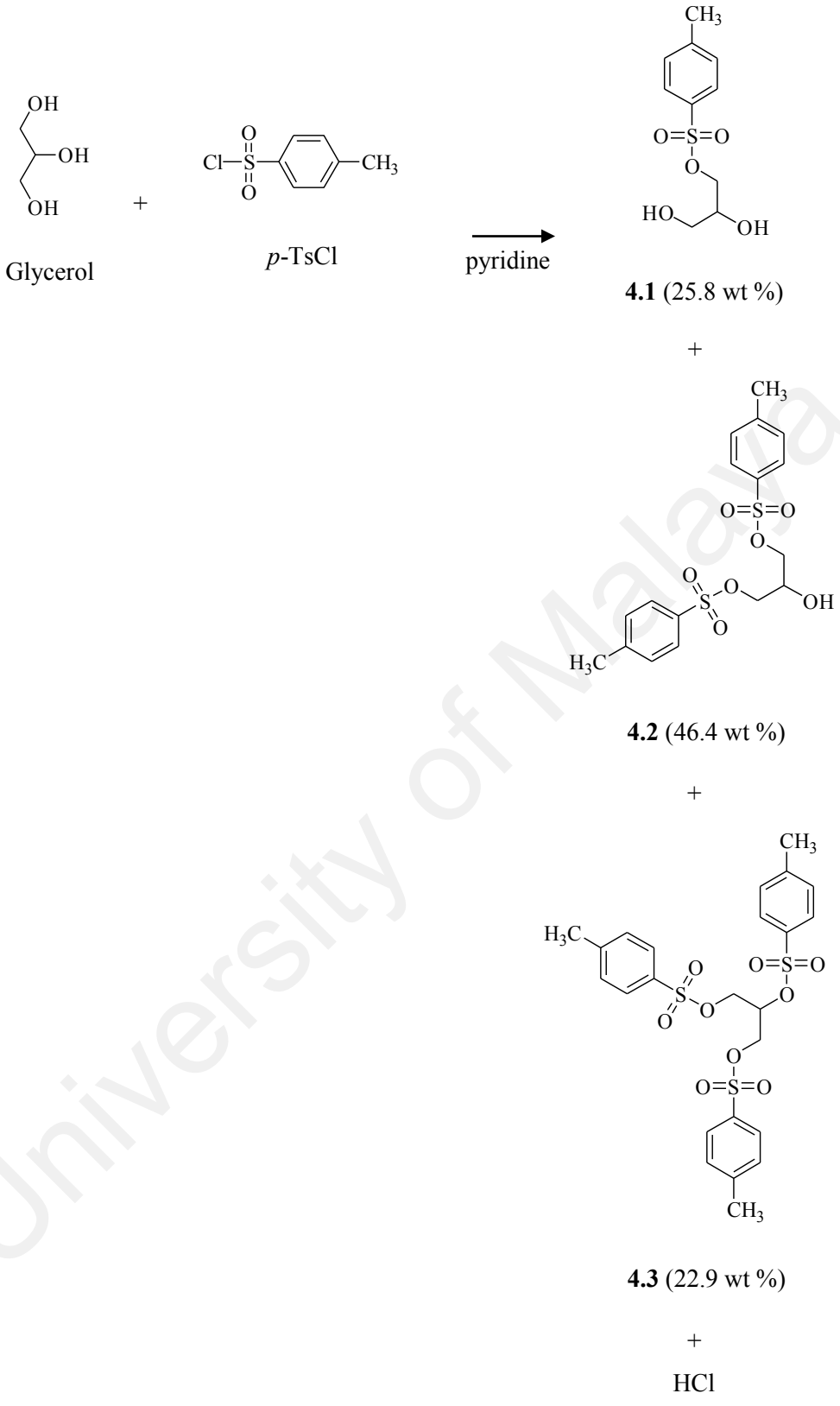
4.2.1 Reaction of Glycerol with Tosylation Agent

Reactions of glycerol and *p*-TsCl were carried out in the presence of a base. The product obtained was a mixture of *mono*-, *di*- and also *tri*-tosylate of glycerol (Scheme 4.1). A base such as pyridine was used as a solvent and also as HCl scavenger during the reaction.

The progress of the reaction was monitored using thin layer chromatography (TLC). The R_f value of each component is tabulated in Table 4.1.

Table 4.1 : R_f values of each component in the product mixture obtained from tosylation process of glycerol with *p*-TsCl

Component	R_f value
A	0.11
B	0.48
C	0.64
p-TsCl	0.91



Scheme 4.1 : Tosylation of glycerol with *p*-TsCl in the presence of pyridine

The mixture was purified by column chromatography and characterized by NMR spectroscopy. As mentioned earlier, TLC analysis showed that the reaction product consists of three different components. From ^{1}H and ^{13}C NMR spectra (Figures 4.1 to 4.6), we have identified that components A, B and C correspond to compound **4.1**, compound **4.2** and compound **4.3,** respectively.

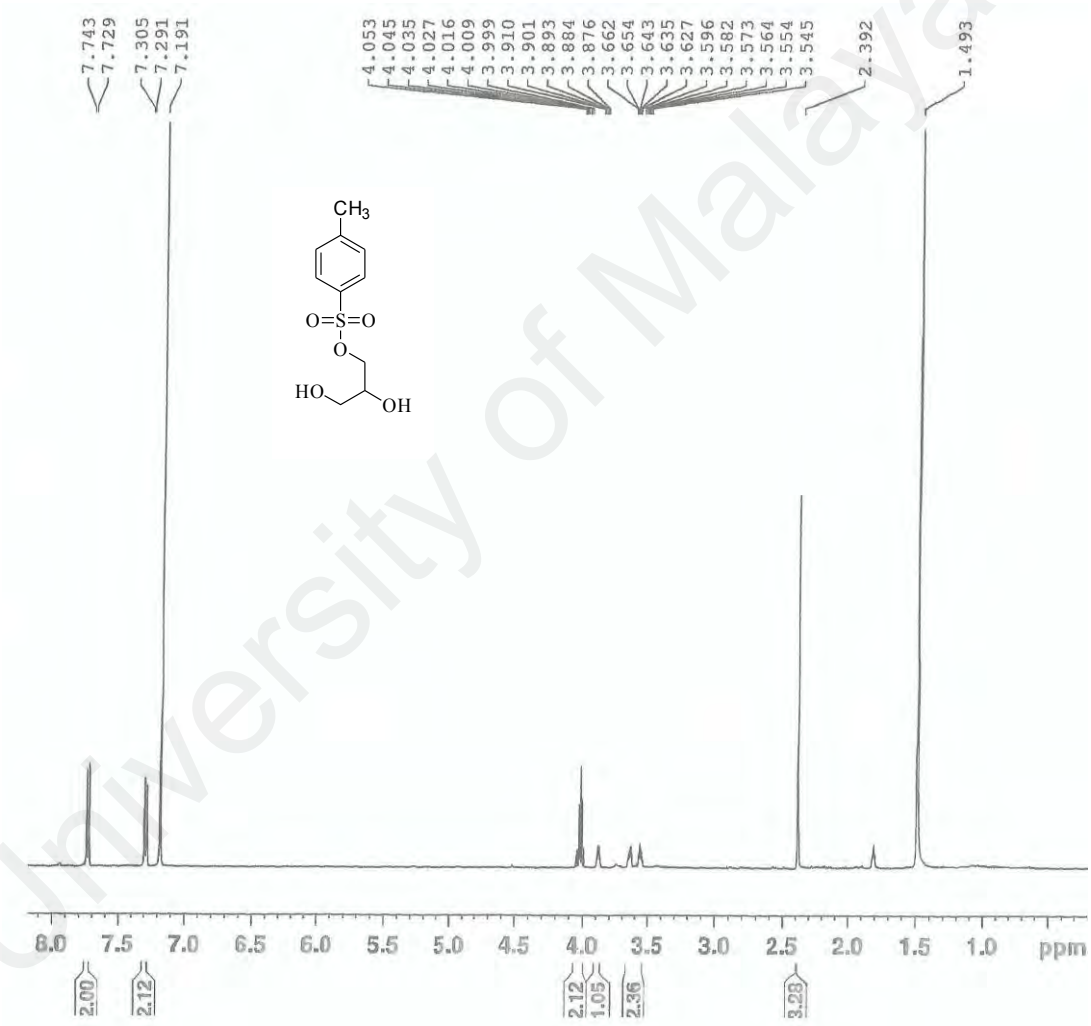


Figure 4.1 : ^{1}H NMR of compound 4.1

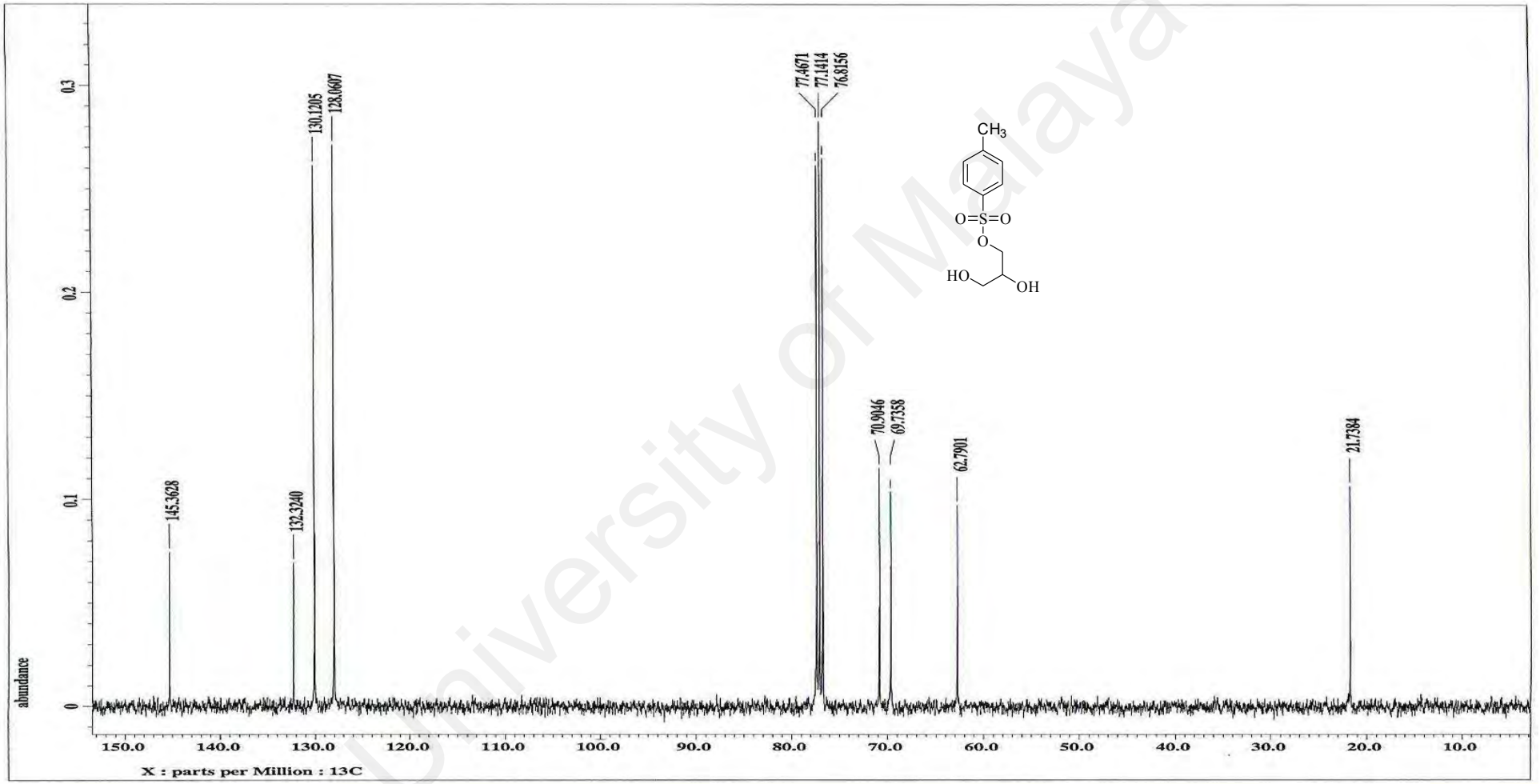


Figure 4.2 : ^{13}C NMR of compound 4.1

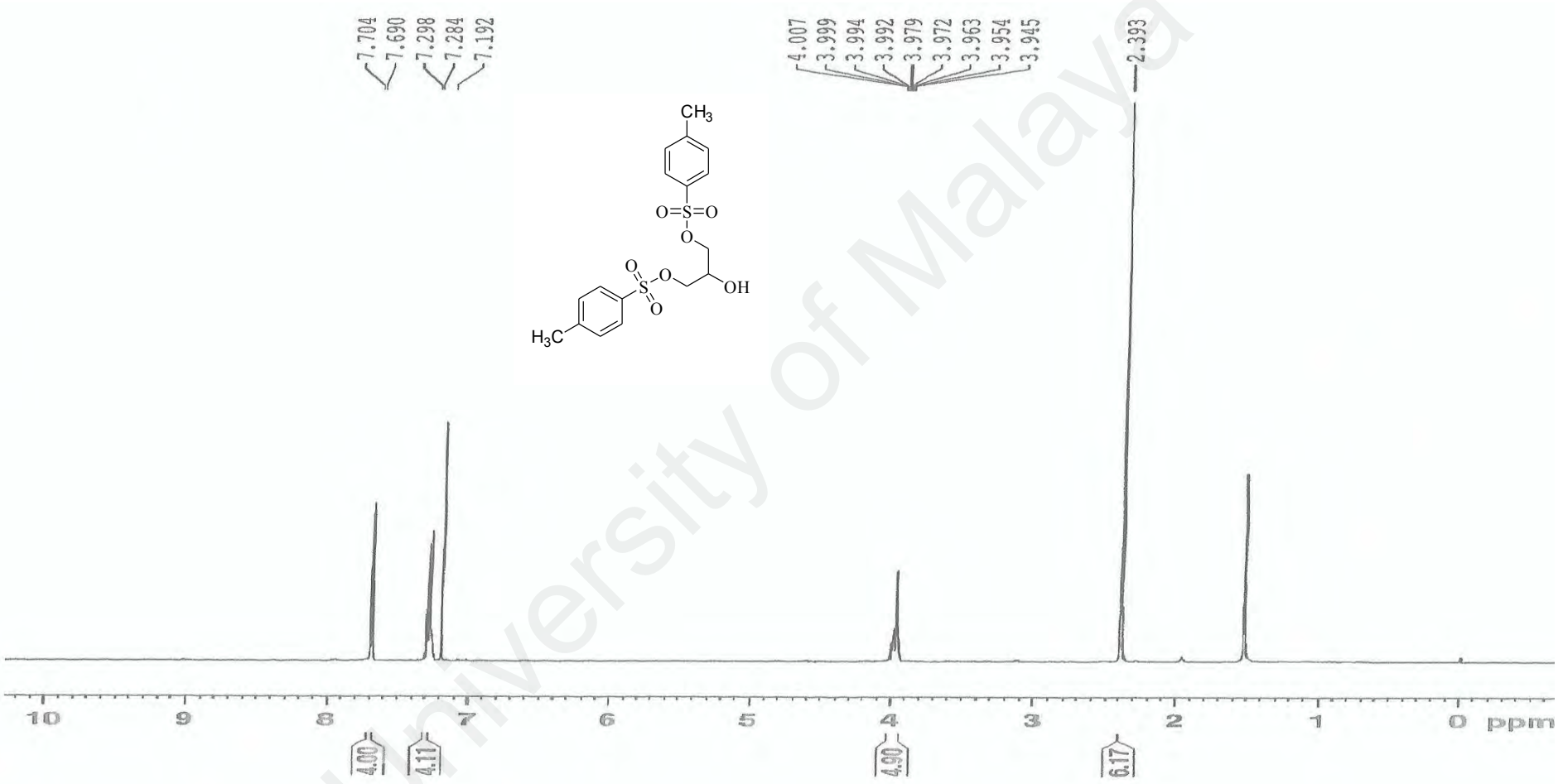


Figure 4.3 : ^{1}H NMR of compound 4.2

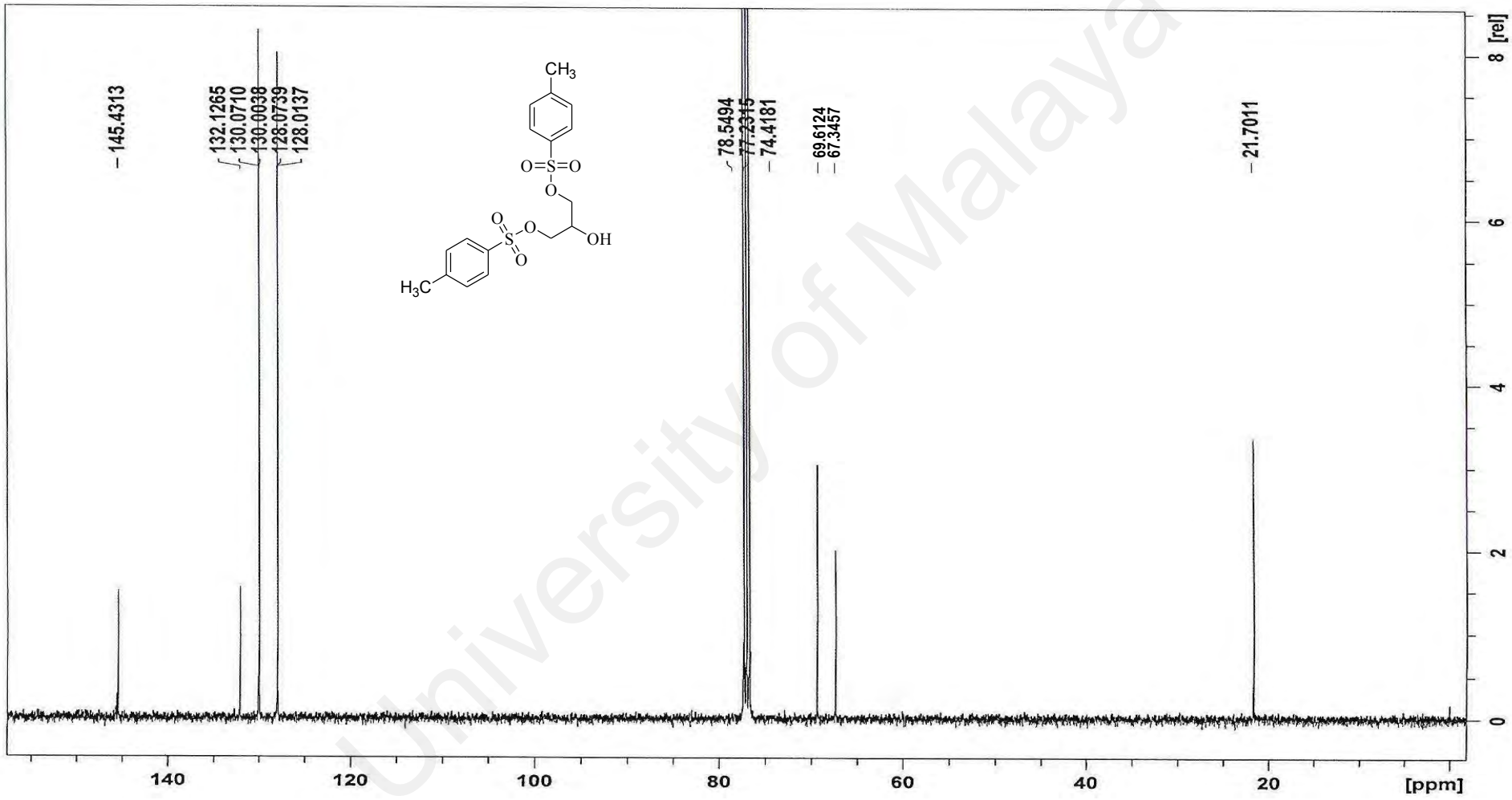


Figure 4.4 : ^{13}C NMR of compound 4.2

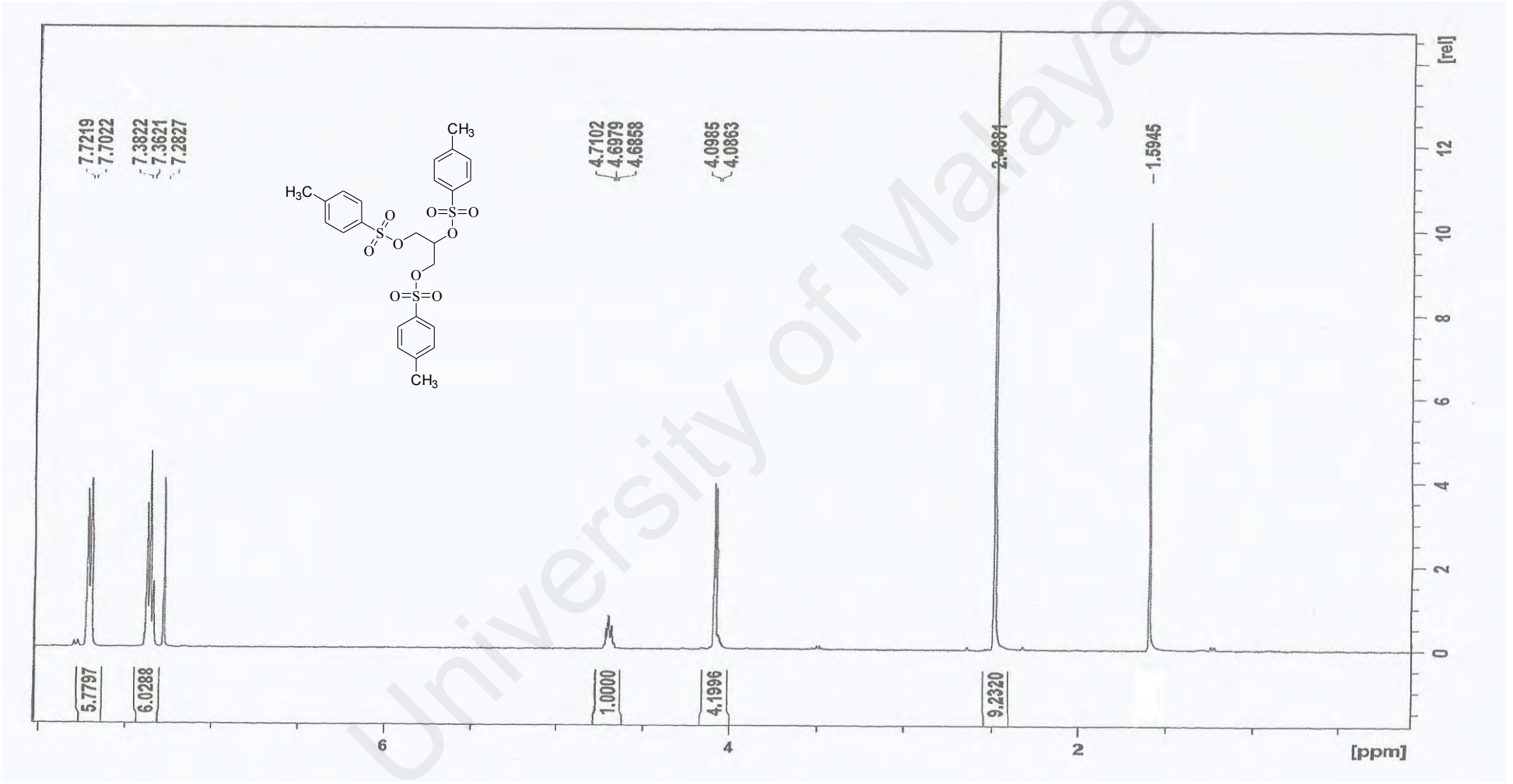


Figure 4.5 : ^{1}H NMR of compound 4.3

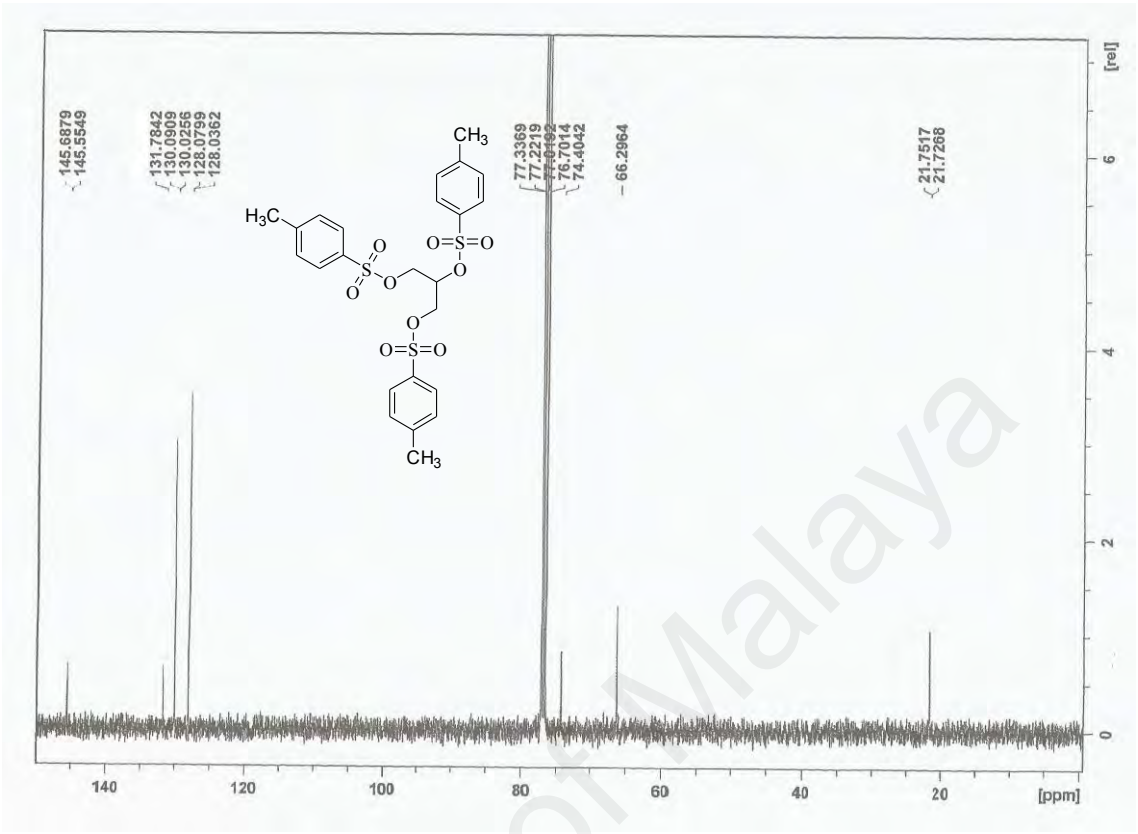
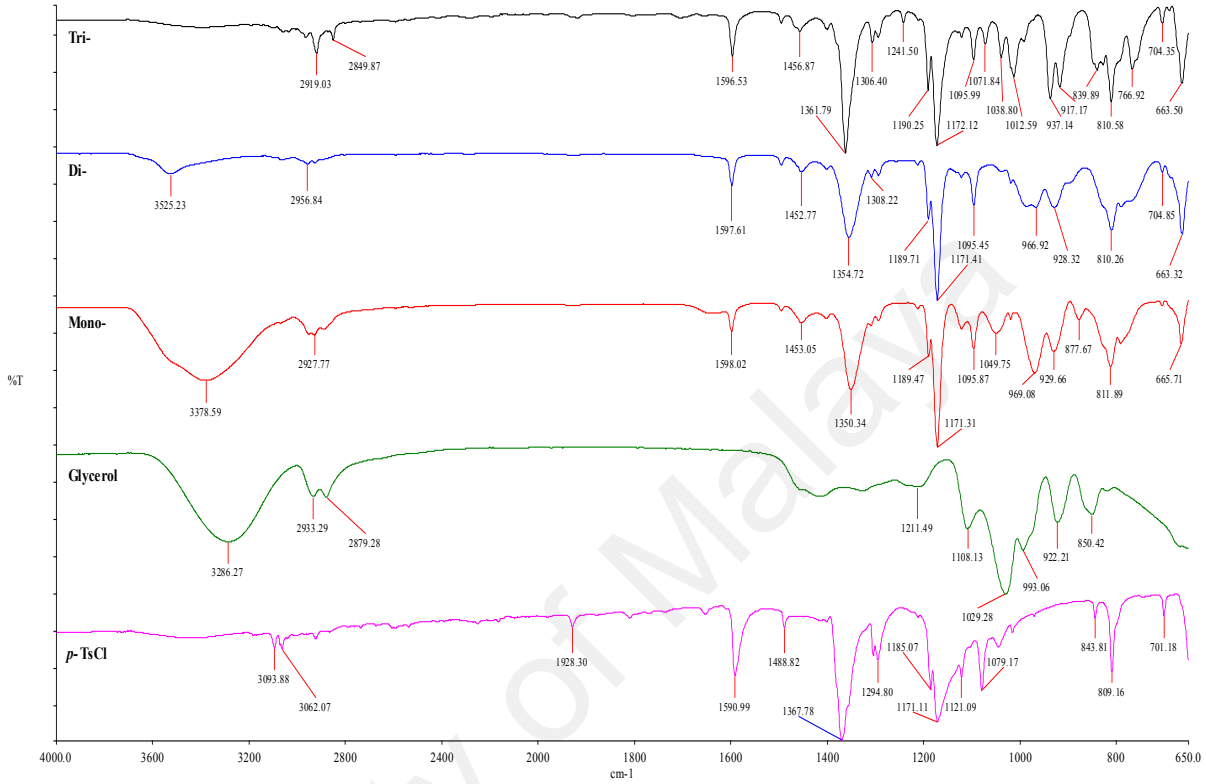


Figure 4.6 : ^{13}C NMR of compound 4.3

Those compounds were also characterized by FTIR and X-ray crystallography where possible. Figure 4.7 shows the infrared spectra of compounds **4.1-4.3** in comparison to the starting materials which were glycerol and *p*-TsCl. The –OH band in the IR spectra of compounds **4.1** and **4.2** remains. Meanwhile, the –OH band (around 3300 cm^{-1}) is apparently absent in the spectrum of compound **4.3**, indicating all three hydroxyl groups in glycerol molecule were tosylated by *p*-TsCl.

Besides the hydroxyl group, the important functional group in the compounds is the sulfonate. According to Pavia *et al.* (2001), S=O asymmetric stretch occurs at 1350 cm^{-1} and S=O symmetric stretch occurs at 1175 cm^{-1}. Meanwhile S-O stretch occurs in the

range of 1000-750 cm^{-1}. The important assignments of IR data on compounds **4.1**, **4.2** and **4.3** are tabulated in Appendix 4.1.



wherein Mono- = Compound **4.1**
Di- = Compound **4.2**
Tri- = Compound **4.3**

Figure 4.7 : IR spectra of compounds 4.1-4.3, glycerol and *p*-TsCl

4.2.2 Crystal Structure of Compound 4.3

The formation of single crystal for compound **4.3** (Figure 4.8) enabled the X-ray crystallography analysis to be carried out. Figure 4.9 shows the ORTEP diagram of the crystal. The melting point of compound **4.3**, 1,3-*Bis*{[(4-methylphenyl)sulfonyl]oxy}propan-2-yl 4-methylbenzenesulfonate is 103ºC.

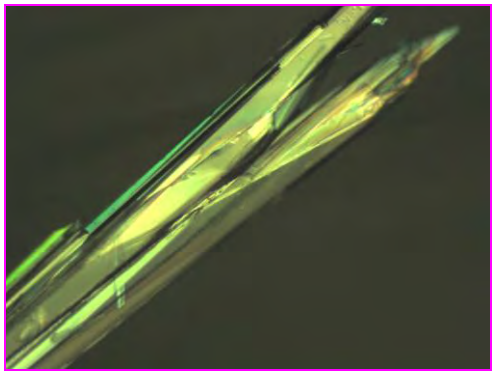


Figure 4.8 : Crystals of compound 4.3

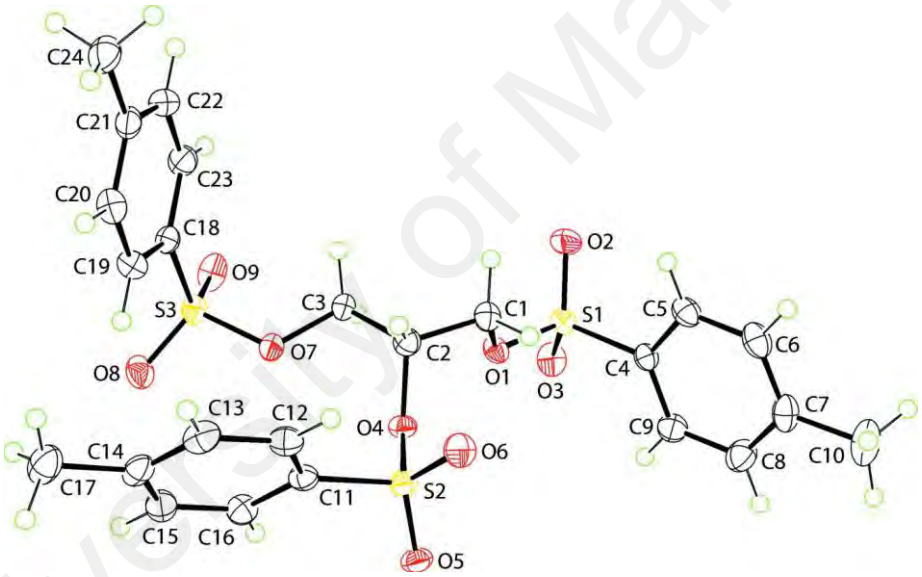


Figure 4.9: ORTEP diagram of 1,3-*Bis*{[(4-methylphenyl)sulfonyl]oxy}propan-2-yl 4-methylbenzenesulfonate

The weak hydrogen bonds occur internally in the molecule and also externally between the molecules as shown in Figure 4.10. Detailed crystal structure data was published as supplementary material in Acta Crystallography Section E (Yusof *et al.*, 2012).

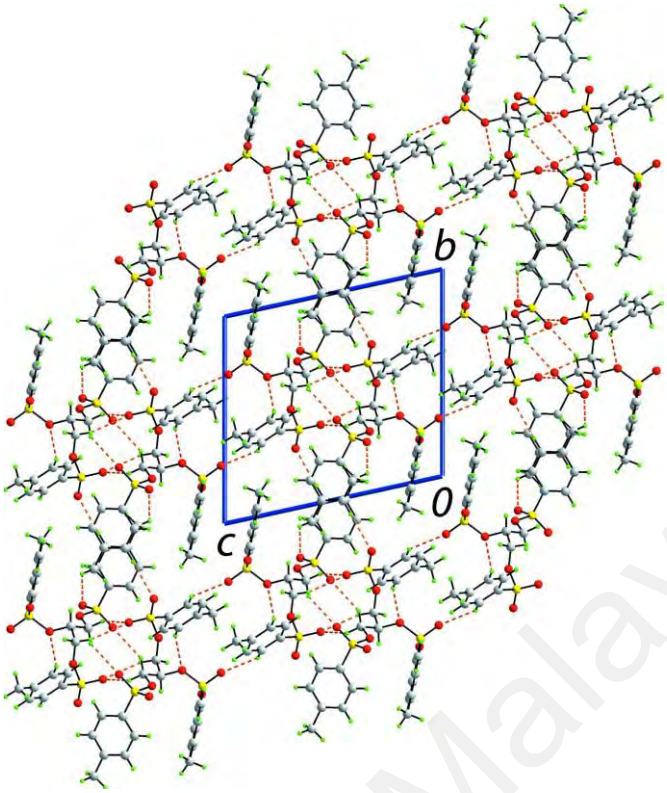


Figure 4.10 : The weak C—H· · · O interactions are shown as orange dashed lines

4.2.3 Effect of Amount of Base Used on the Tosylation of Glycerol

The effect of the amount of base used in the tosylation process was investigated where the amount of base varied between one to five mole equivalents to tosylation agent. In the tosylation process, a base plays an important role in promoting the reaction towards the formation of the desired product and also acts as HCl scavenger. An approach in optimizing the amount of the base used in obtaining a high yield of product was carried out. The base used was pyridine.

All products obtained from tosylation of glycerol with *p*-TsCl were a mixture of compounds **4.1**, **4.2** and **4.3** when the amount of pyridine varied between one to five equivalents to *p*-TsCl as shown in Figure 4.11. The mole ratio of glycerol to *p*-TsCl was

1.2. Although all reactions produced products containing the three compounds **4.1**, **4.2** and **4.3**, the increasing amount of pyridine in the reaction increased the amount of product produced.

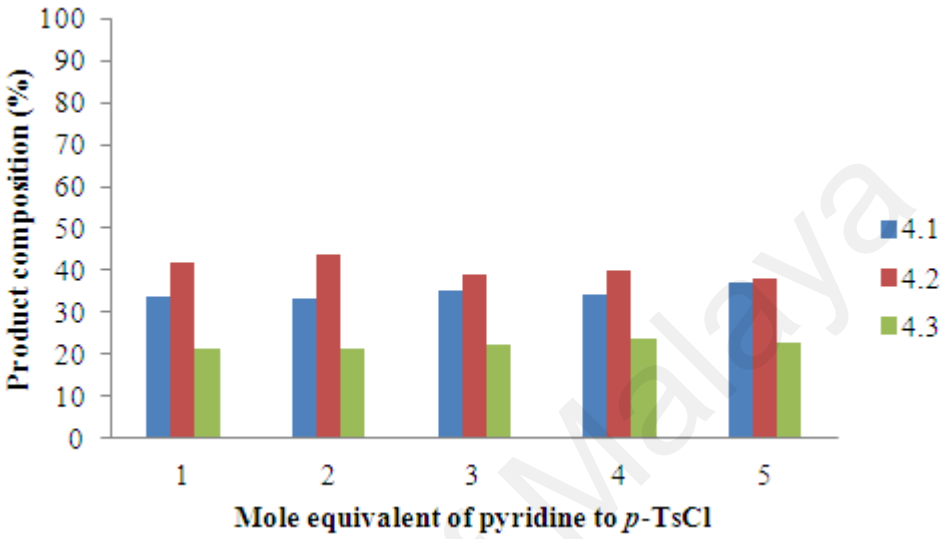


Figure 4.11 : Product composition obtained from reactions of glycerol with *p*-TsCl in the presence of different amount of pyridine

When *p*-TsCl was added last into the reaction mixture consisting of 5 mole equivalents of pyridine, DCM and glycerol, the formation of an unexpected product occurred beside the tosylation process. The product was identified by X-ray crystallography analysis since it was a single crystal. The product was a salt, 1,1'-methylenedipyridinium dichloride *mono*-hydrate. The crystal structure (Figure 4.12) of the similar salt was reported by Fu and co-workers which was obtained from different type of reaction (Fu *et al.*, 2010).

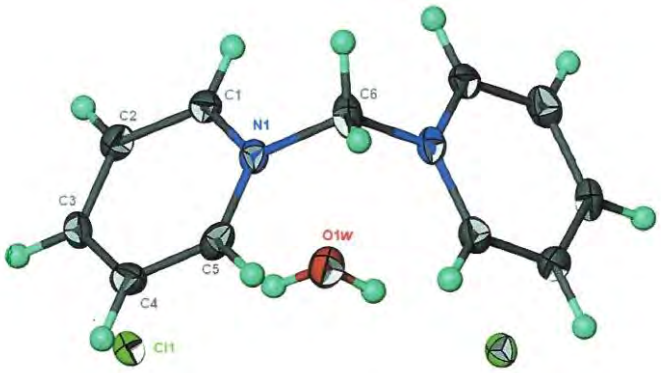
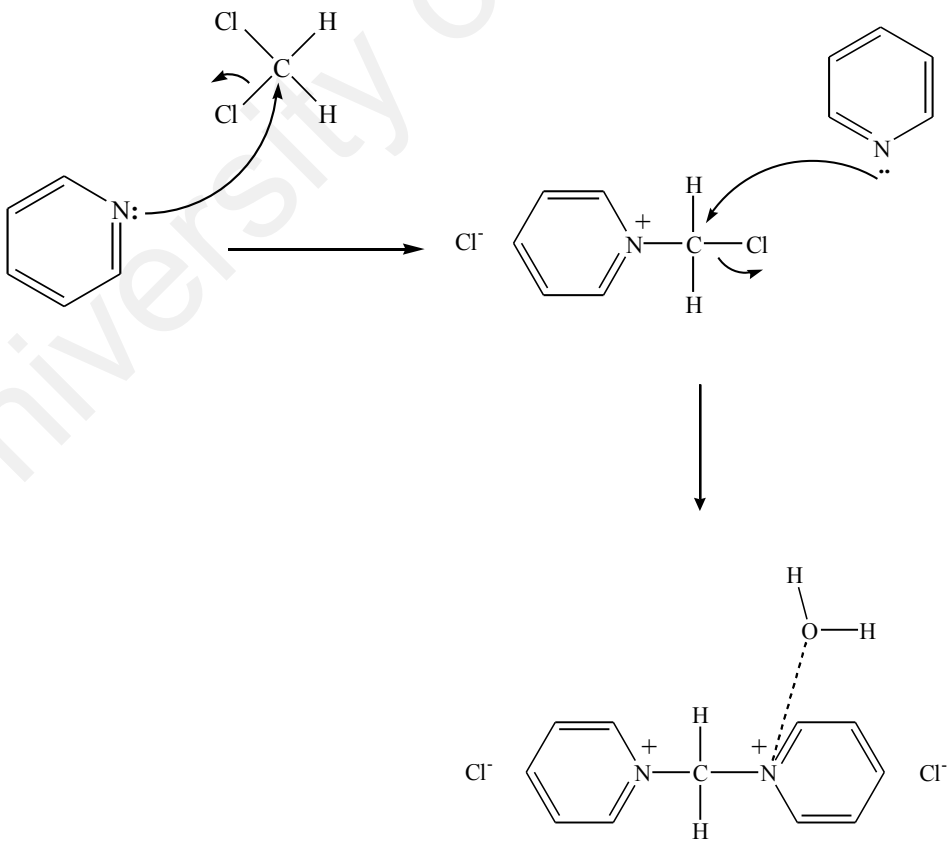


Figure 4.12 : ORTEP diagram of 1,1'-methylenedipyridinium dichloride *mono*-hydrate obtained as side product from the reaction of glycerol with *p*-TsCl in the presence of pyridine and DCM

The formation of the salt may be due to how *p*-TsCl is added into the reaction mixture. If it is added last, the reaction of pyridine and DCM may have taken place before the addition of *p*-TsCl. Scheme 4.2 shows the proposed mechanism of the salt formation from the side reaction of pyridine and DCM.



1,1'-methylenedipyridinium dichloride *mono*-hydrate

Scheme 4.2 : Salt formation from side reaction of pyridine and DCM

4.2.4 Effect of Different Solvent on the Tosylation of Glycerol

The tosylation process of glycerol was carried out in different solvents namely dichloromethane (DCM), chloroform ($CHCl_3$) and tetrahydrofuran (THF) in the presence of pyridine as a base. The reaction was done with the mole ratio of 1.2 of glycerol to *p*-TsCl with a purpose to obtain compound **4.1**. By monitoring the reaction progress through TLC analysis, the reaction is considered to completion in the absence of the spot with R_f value of 0.91, referring to *p*-TsCl.

All products obtained from tosylation of glycerol with *p*-TsCl in different solvents were a mixture of compounds **4.1**, **4.2** and **4.3** (Figure 4.13). It seems that DCM is the best solvent compared to others as the highest selectivity to compound **4.1** achieved when the reaction was carried out in DCM.

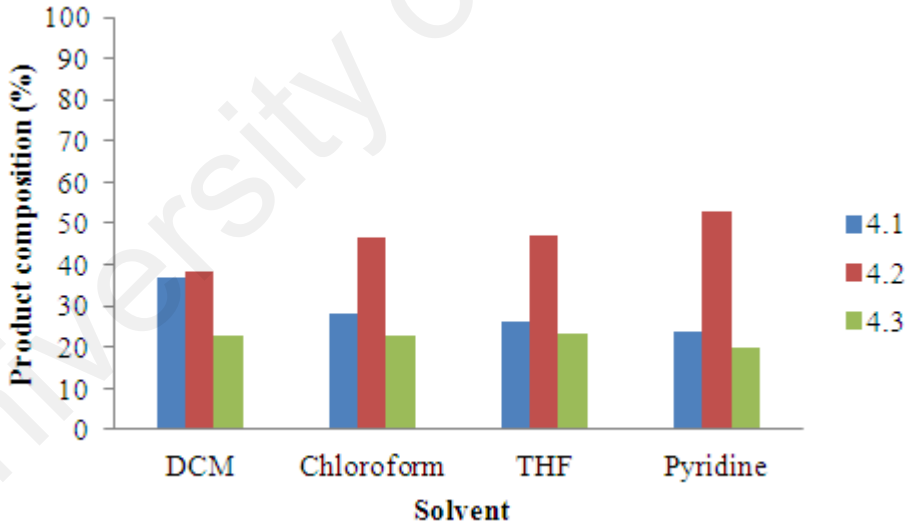


Figure 4.13 : Product composition obtained from reactions of glycerol with *p*-TsCl in the presence of pyridine and different solvent

4.2.5 Effect of Mole Ratio of Glycerol to Tosylation Agent

The effect of mole ratio of glycerol to tosylation agent was investigated in the range of 1 to 3 in DCM as a solvent. All reactions produced a mixture of compounds **4.1**, **4.2** and

4.3. The selectivity to compound **4.1** increased with the increasing ratio of glycerol/*p*-TsCl as shown in Figure 4.14. In contrast, the formation of compound **4.3** decreased with the increasing ratio of glycerol/*p*-TsCl. In fact, reaction that was conducted at the mole ratio of 3 (glycerol/*p*-TsCl) had resulted in the formation of product consisting of less than 5% compound **4.3**. The highest selectivity to compound **4.1** achieved when the reaction was carried out at the mole ratio of 3 (glycerol/*p*-TsCl).

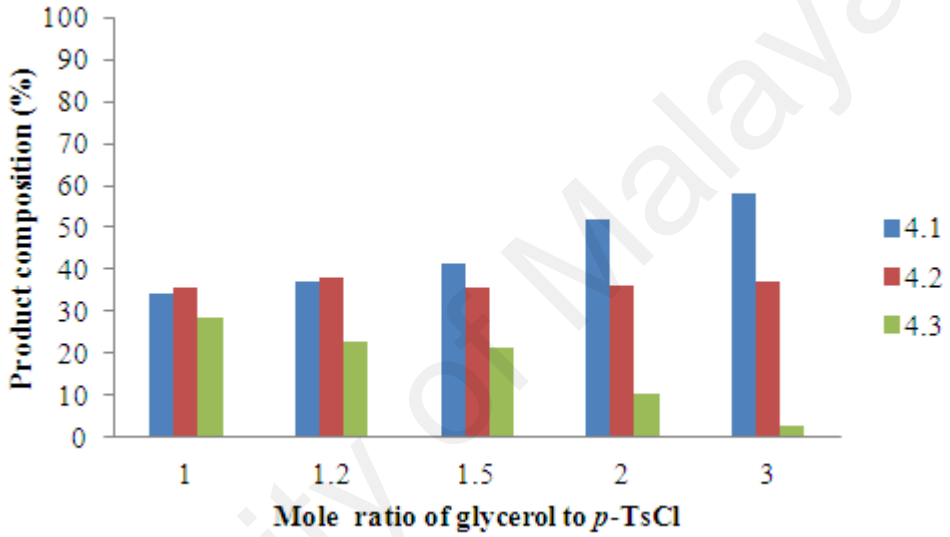


Figure 4.14 : Product composition obtained from reactions of glycerol with *p*-TsCl in the presence of pyridine at different mole ratio of reactants

4.2.6 Antibacterial Properties of Glyceryl Tosylates (Compounds 4.1, 4.2 and 4.3)

Organic molecules containing one or more carbon-sulfur bonds (organosulfur compounds), are one of the groups present in foods as natural preservatives (Cremlyn, 1996). These compounds are valued for their many important biological properties (Polshettiwar & Kaushik, 2004). The antimicrobial activity of organosulfur compounds has been reported against a wide spectrum of bacteria, fungi and viruses (Sagdic & Tornuk, 2012).

Therefore, antibacterial tests of glyceryl tosylates (compounds **4.1**, **4.2** and **4.3)** were carried out towards *Staphylococcus aureus* (gram positive) and *Pseudomonas aerugiuosa* (gram negative) bacteria. The test was done in three different concentrations (1 mg/mL, 5 mg/mL and 10 mg/mL). The results are shown in Tables 4.2 and 4.3 where all compounds **4.1**, **4.2** and **4.3** exhibited antibacterial properties against *Staphylococcus aureus* and *Pseudomonas aerugiuosa* compared to control. The zone of inhibition of different concentrations of compounds **4.1**-**4.3** was not significantly different against *Staphylococcus aureus* ($p > 0.05$). However, zone of inhibition of compounds **4.2** and **4.3** increased significantly at the concentration of 10 mg/mL compared to 1 mg/mL against *Pseudomonas aerugiuosa* ($p < 0.5$).

The disc diffusion method is known as qualitative technique since this method will only give an idea of the presence or absence of substances with antibacterial activity (Valgas *et al*., 2007). The high concentration of sample needed to exhibit the antibacterial activity indicates that compounds **4.1**-**4.3** may not be suitable to be used as antibacterial agent alone. However, it could be used in combination with other antibacterial agent for synergistic effect.

Table 4.2 : Antibacterial test of compounds 4.1, 4.2 and 4.3 against *Staphylococcus aureus* (gram positive bacteria) at different concentrations

Sample	Zone of Inhibition(mm)(Mean ± SD)		
	1 mg/mL	5 mg/mL	10 mg/mL
Compound **4.1**	6.5 ± 0.7	7.5 ± 0.7	7.5 ± 0.7
Compound **4.2**	7.0 ± 0.1	7.0 ± 1.4	8.5 ± 0.7
Compound **4.3**	6.0 ± 0.1	6.5 ± 0.7	6.5 ± 0.7
Negative control	0	0	0

Table 4.3 : Antibacterial test of compounds 4.1, 4.2 and 4.3 against *Pseudomonas aerugiuosa* (gram negative bacteria) at different concentrations

Sample	Zone of Inhibition(mm)(Mean ± SD)		
	1 mg/mL	5 mg/mL	10 mg/mL
Compound **4.1**	6.5 ± 0.7	7.5 ± 0.7	9.0 ± 1.4
Compound **4.2**	6.5 ± 0.7	7.5 ± 0.7	8.0 ± 0.1
Compound **4.3**	6.5 ± 0.7	7.5 ± 0.7	10.0 ± 1.4
Negative control	0	0	0

4.3 Acetalisation of Glycerol with Acetone

Solketal (compound **4.4**) which is also known as 1,2-*O*-isopropylidene glycerol is a well-known protected glycerol. Reaction of glycerol and acetone to produce solketal was carried out in the presence of *p*-toluenesulfonic acid (Newman and Renoll, 1945; Bruchmann *et al.*, 1999; Yu *et al.*, 2003). It can also be produced using calcium carbide as catalyst instead of *p*-toluenesulfonic acid (Maglio and Burger, 1946).

Roldán (2009) investigated the use of heterogeneous catalyst namely K10 montmorillonite for the production of solketal. With a help of zeolite membrane to remove water formed during the reaction, there was a significant improvement in glycerol conversion with a reduction in the excess acetone needed. In addition, titanium cation-exchanged montmorillonite exhibited high catalytic activity for the reaction of glycerol with ketones or aldehydes to produce cyclic acetals and the catalyst could be easily separated and reused (Takato *et al.*, 2012).

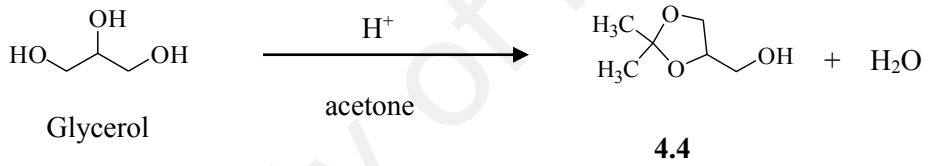
Other than that, the acetalisation of glycerol with acetone was performed over zirconia and promoted zirconia catalysts to produce solketal (Padigapati S *et al.*, 2011).

A fully continuous process for the production of solketal was also developed in the presence of heterogeneous catalyst namely Amberlyst DPT-1 (Clarkson *et al.*, 2001).

Other approach to prepare the solketal was made by Baohua *et al.* (2011) where the condensation reaction of glycerol with acetone was carried out in the presence of sulfonate-type ionic liquid catalysts. Compound **4.4** is used for an alternative route in order to synthesize only *mono*-substituted glycerol.

4.3.1 Synthesis of Solketal (Compound 4.4)

Compound **4.4** was prepared by reacting glycerol with acetone in the presence of an acidic catalyst (Scheme 4.3).



Scheme 4.3 : Preparation of compound 4.4 from glycerol and acetone

The synthesis of compound **4.4** was carried out using a traditional batch process where the reaction was catalysed by a homogeneous catalyst (*p*-TsOH) using a Dean-Stark apparatus. The set-up is used to remove water generated in the reaction. It took about eight to nine hours to complete the reaction as indicated by the volume of water trapped in the Dean-Stark trap. After neutralization of the acid used, the solvents (petroleum ether and excess acetone) were removed under reduced pressure.

Based on the gas chromatography analysis (Figure 4.15) of the reaction mixture, the conversion of glycerol to compound **4.4** was more than 90 % and the isolated yield was more than 90 %. The highest yield obtained was 91 % compound **4.4** (purity > 95 %).

This is almost similar to what was reported by Newman and Renoll (1945). The infrared spectrum of the isolated solketal (purity > 97 % by GC) is shown in Appendix 4.2.

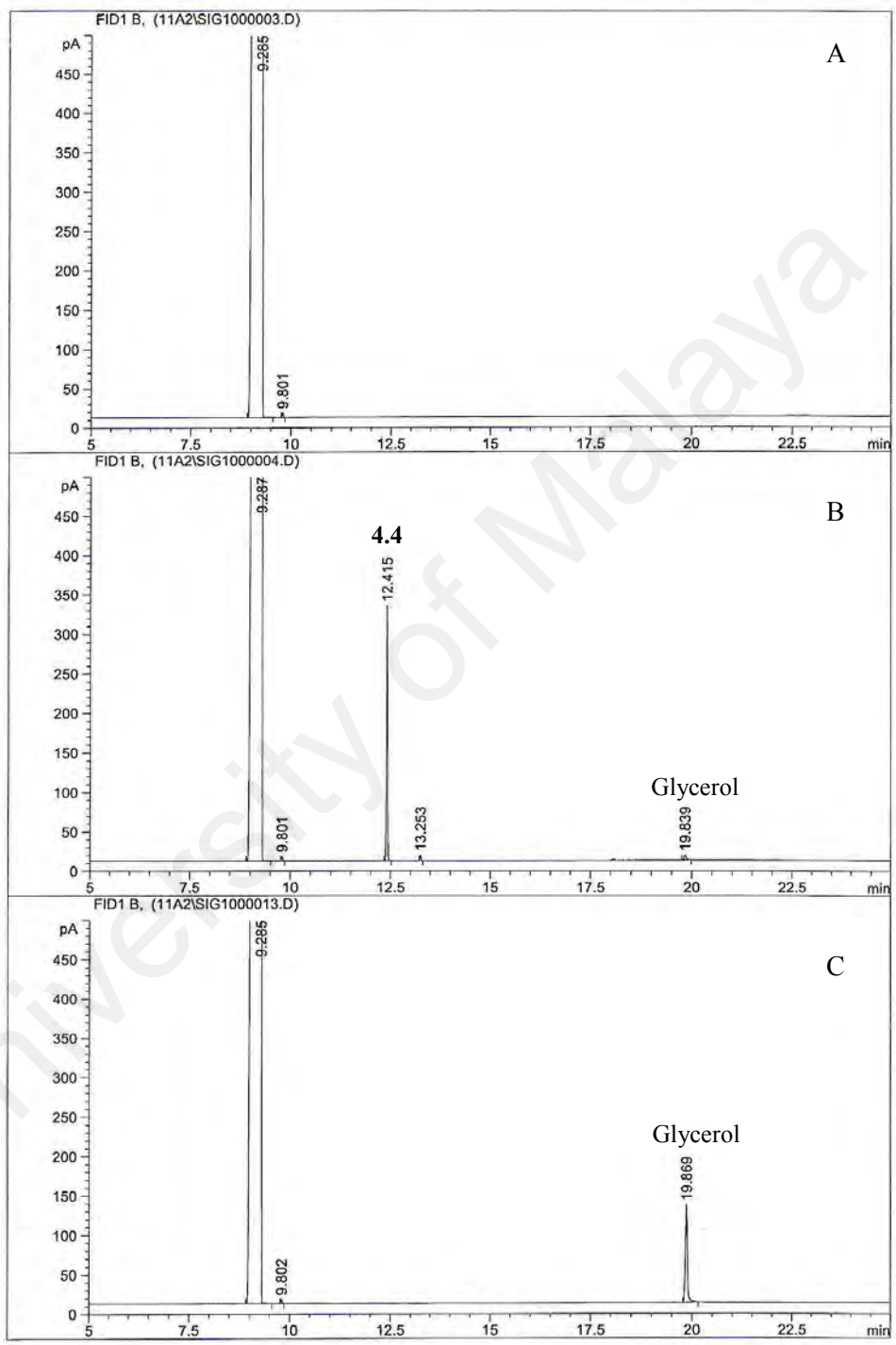


Figure 4.15 : Gas chromatogram of reaction product (B), baseline (A) and glycerol (C) for comparison

Meanwhile ^{1}H and ^{13}C NMR spectra of the isolated solketal are shown in Appendices 4.3 and 4.4, respectively. The NMR spectra confirmed the structure of compound **4.4** where the spectra show similarity to what was reported by Yu *et al.* (2003).

Besides using *p*-TsOH (homogeneous catalyst), Amberlyst 15 (heterogeneous catalyst) was used for comparison. The highest yield of compound **4.4** was 91 %. This proves that the reaction of glycerol and acetone can be done in the presence of heterogeneous catalyst and the usage of homogeneous catalyst in this reaction can be avoided due to the environmental reason. Even though the conversion of glycerol to compound **4.4** and the isolated yield is not significantly different but the later method resulted in better and easier separation of catalyst from the reaction mixture. Nowadays, the use of heterogeneous catalysts is more favorable than homogeneous catalysts because of their one major advantage, their ease of separation from the reaction product (Cole-Hamilton and Tooze, 2006).

Figure 4.16 shows the yield of compound **4.4** obtained from the condensation of glycerol with acetone in the presence of different amount of Amberlyst 15. All reactions produced more than 90 % yield of compound **4.4** even though in the presence of only 0.5 % catalyst.

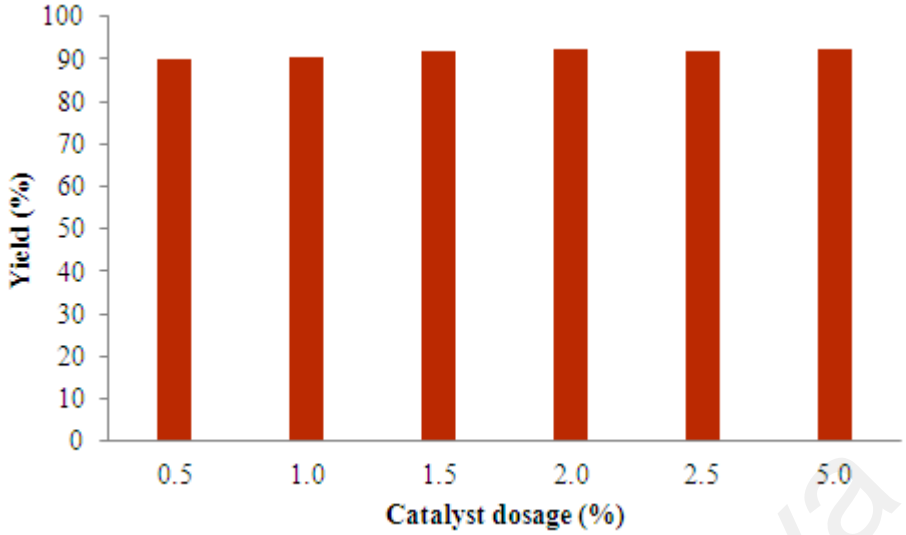
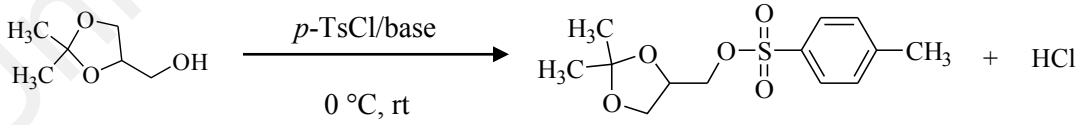


Figure 4.16 : Yield of compound 4.4 obtained from the condensation of glycerol with acetone in the presence of different amount of heterogeneous catalyst

4.4 Tosylation Process of Solketal

Compound **4.4** can be tosylated by a tosylation agent such as *p*-TsCl to form solketal tosylate (compound **4.5**). Wu and co-workers investigated the tosylation process in order to obtain compound **4.5** as an intermediate (Wu *et al.*, 2010). Attempts to produce compound **4.5** were made by reacting compound **4.4** with *p*-TsCl in the presence of pyridine (Scheme 4.4).



4.4 **4.5**

Scheme 4.4 : Synthesis of compound 4.5 from the reaction of 4.4 with *p*-TsCl in the presence of a base

Figure 4.17 shows the crystal of compound **4.5** captured under microscopic environment where it was a needle like crystals and colourless. The melting point of compound **4.5** is 47 ºC. Appendix 4.5 shows the IR spectrum of compound **4.5**.

Meanwhile ^{1}H and ^{13}C NMR spectra of compound **4.5** are shown in Appendices 4.6 and 4.7, respectively. The crystal structure of compound **4.5** was reported by Kuś *et al.* (2009).

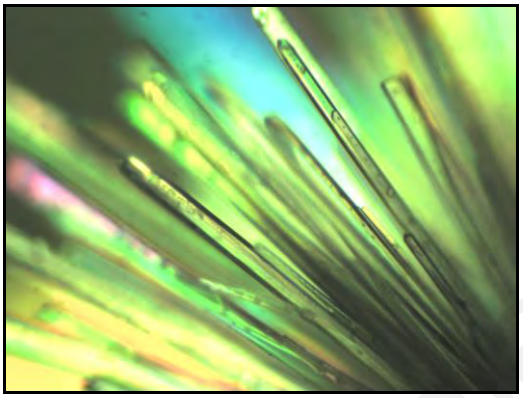


Figure 4.17 : Crystals of compound 4.5

A method for the determination of compound **4.5** using GC was developed. Gas chromatogram was recorded by gas chromatography with FID, Agilent 6890 Series, where chromatograph was connected to PC. Analyses were carried out on chromatographic capillary column HP-5 (30 m x 0.25 mm coated with film 0.25 μm) with temperature programme from 100 to 240 ºC. A suitable GC method was successfully developed for the determination of compound **4.5** in reaction mixture quantitatively. Figure 4.18 shows the example of the GC result.

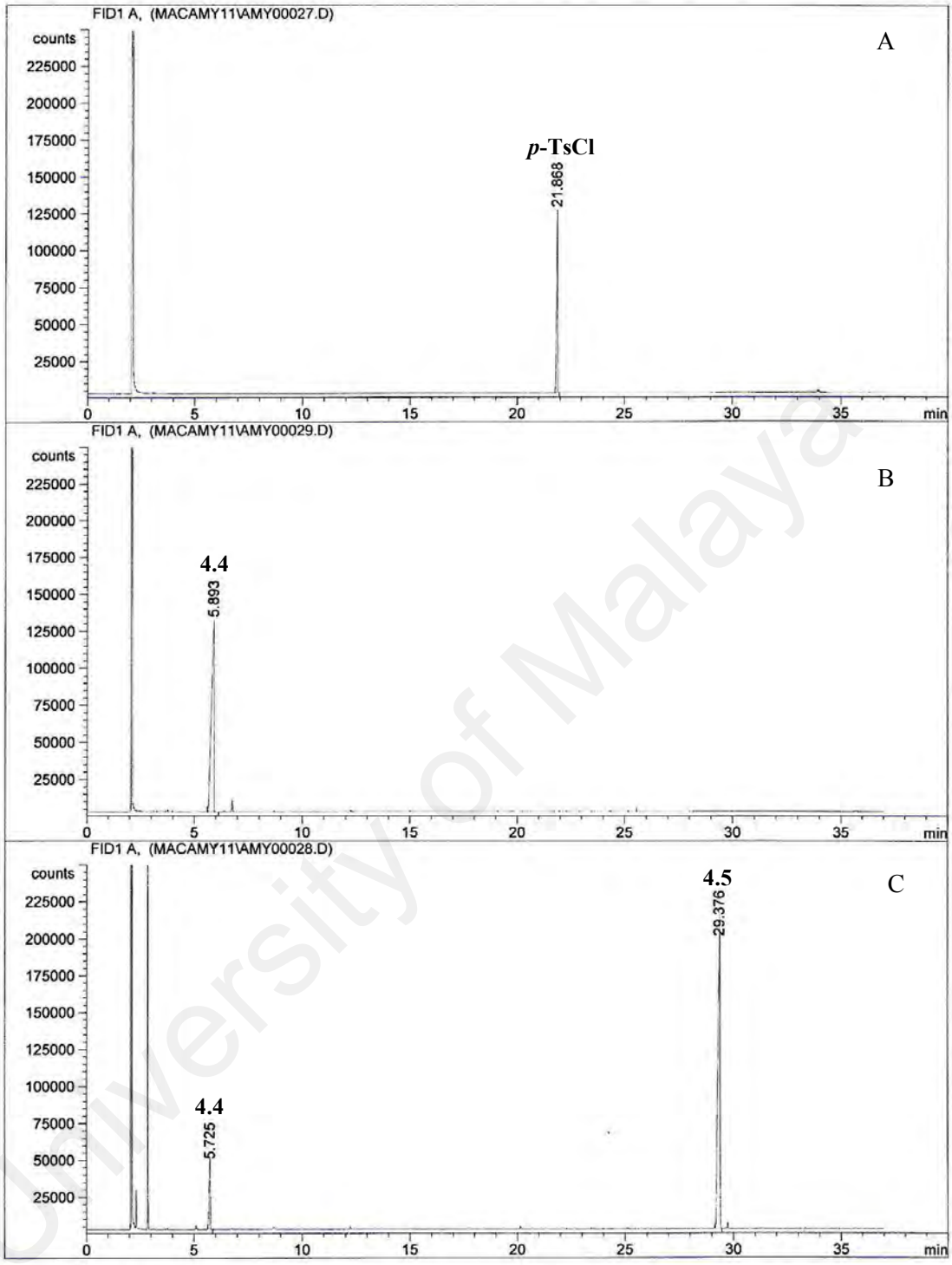


Figure 4.18: Gas chromatogram of *p*-TsCl (A), compound 4.4 (B) and product mixture of compound 4.5 (C)

High purity of compound **4.5** (> 95 %) was successfully obtained in 35 % yield from the process. Compounds **4.1**, **4.2**, **4.3** and **4.5** could be used in S_N2 nucleophilic substitution reaction for the preparation of glyceryl ether, as tosylates are known as a good leaving group.

CHAPTER 5: POTENTIAL APPLICATIONS OF GLYCERYL ETHER

5.1 Introduction

Antibacterial activity is related to compounds that locally kill bacteria or slow down their growth. There are several known methods to conduct the antibacterial test such as disc diffusion method and broth macro-dilution method (Nurmahani *et al.*, 2012). Disc diffusion test method is commonly used for screening the antibacterial activity of a compound.

Predicting the irritation potential of chemical represents an important component of the overall safety evaluation for new and existing chemical. Its safety must be substantiated prior to its use as an ingredient for consumer protection and to meet regulation requirements. A compound should not induce any irritation to the skin when it is used in product formulation especially in cosmetics products. Furthermore, it is preferable to have a product which also does not irritate the eyes. The irritation potential of a compound to the skin and eye can be assessed and predicted by using *in vitro* dermal or ocular irritection assay (Zafarizal Aldrin *et al.*, 2005; Ismail *et al.*, 2013). The dermal or ocular irritection assay is an alternative method to animal irritancy studies (Draize Test) that mimic biochemical phenomena. The *in vitro* irritection assays have been reported to correlate with *in vivo* irritancy tests (Sina *et al.*, 1995).

Emulsions are defined as disperse systems in which two or sometimes several almost insoluble liquid phases are firmly mixed. There is either oil in water (o/w) or water in oil (w/o) emulsion in the simplest case. Anyhow multiphase emulsions are also quite common such as water in oil emulsion that can be dispersed in water to obtain w/o/w emulsion for cosmetic and technical applications (Rieger and Rhein, 1997). Meanwhile

microemulsion is an optically clear, thermodynamically stable and usually a low viscous solutions (Hoar and Schulman, 1943). The uses and applications of microemulsions have been numerous such as in detergency, agrochemicals, food, pharmaceuticals, biotechnology and in a variety of chemical and industrial processes (Paul and Moulik, 2001).

In this study, the antibacterial activity of glyceryl ethers produced was evaluated using disc diffusion method besides carrying out the *in vitro* dermal irritection assay for their irritation potentials. The potential applications of compound **2.7** were further investigated in macroemulsion, microemulsion and transparent soap. The purpose was to diversify the usage of glycerol derivatives.

5.2 Antibacterial Properties of Glyceryl Ethers

Glyceryl ethers were tested for their antibacterial properties by using disc diffusion test method. The test was conducted according to Hung and co-workers (Hung *et al.*, 2010). Tables 5.1 to 5.3 show the inhibition zones in mm of glyceryl ethers against *Staphylococcus aureus* (gram positive), *Pseudomonas aeruginosa* (gram negative) and *Escherichia coli* (gram negative) bacteria, respectively. The disc diffusion method is known as qualitative technique since this method will only give an idea of the presence or absence of substances with antibacterial activity (Valgas *et al*., 2007). All compounds exhibited antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* in the range of the experimental study. The zone of inhibition between compounds **2.1**-**2.7** was significantly different against *Staphylococcus aureus* ($p < 0.05$). Meanwhile there was no significant difference of zone of inhibition between compounds **2.1**-**2.7** against *Pseudomonas aeruginosa* ($p > 0.05$) and *Escherichia coli* ($p > 0.05$).

Table 5.1 : Antibacterial test of glyceryl ethers against *Staphylococcus aureus* (gram positive) bacteria

Sample	Zone of Inhibition(mm)(Mean ± SD)
Compound **2.1**	8.5 ± 0.7
Compound **2.2**	9.0 ± 1.4
Compound **2.3**	11.5 ± 0.7
Compound **2.4**	12.0 ± 1.4
Compound **2.5**	16.0 ± 1.4
Compound **2.6**	14.0 ± 1.4
Compound **2.7**	14.5 ± 0.7
Negative Control	0
Positive Control	11 ± 0.0

Table 5.2 : Antibacterial test of glyceryl ethers against *Pseudomonas aeruginosa* (gram negative) bacteria

Sample	Zone of Inhibition(mm)(Mean ± SD)
Compound **2.1**	7.5 ± 0.7
Compound **2.2**	8.5 ± 0.7
Compound **2.3**	8.5 ± 0.7
Compound **2.4**	9.0 ± 1.4
Compound **2.5**	10.5 ± 0.7
Compound **2.6**	9.5 ± 0.7
Compound **2.7**	9.0 ± 1.4
Negative control	0
Positive control	10.5 ± 0.7

Table 5.3 : Antibacterial test of glyceryl ethers against *Escherichia coli* (gram negative) bacteria

Sample	Zone of Inhibition(mm)(Mean ± SD)
Compound **2.1**	7.5 ± 0.7
Compound **2.2**	8.5 ± 0.7
Compound **2.3**	9.0 ± 1.4
Compound **2.4**	9.0 ± 0.0
Compound **2.5**	10.5 ± 0.7
Compound **2.6**	9.0 ± 1.4
Compound **2.7**	10.0 ± 0.0
Negative control	0
Positive control	9.5 ± 0.7

In addition, the zone of inhibition of compounds **2.5**, **2.6** and **2.7** was significantly larger than positive control against *Staphylococcus aureus* ($p < 0.05$). However, all compounds are not as superior as positive control as those compounds display the antibacterial activity at high concentration compared to positive control. The high concentration of sample needed to exhibit the antibacterial activity indicates that compounds **2.1**-**2.7** may not be suitable to be used as antibacterial agent alone. However, it could be used in combination with other antibacterial agent for synergistic effect.

5.3 *In vitro* Dermal Irritection Assay of Glyceryl Ethers

All compounds (**2.1**-**2.7**) were analyzed for the *in vitro* dermal irritection assay. The assay was conducted according to InVitro International (1996). This is to detect, rank and predict the dermal irritation potential of the compounds. The tests were carried out

at four different dosages (50 μL, 75 μL, 100 μL and 125 μL). Figure 5.1 shows the data obtained from the assays. A sample is classified as non-irritant when the HIE (Human Irritancy Equivalent) score is below 0.9. Table 5.4 shows the relationship of HIE score to *in vivo* irritancy classification for the dermal irritection test method (InVitro International, 1996; Zafarizal Aldrin *et al.*, 2005).

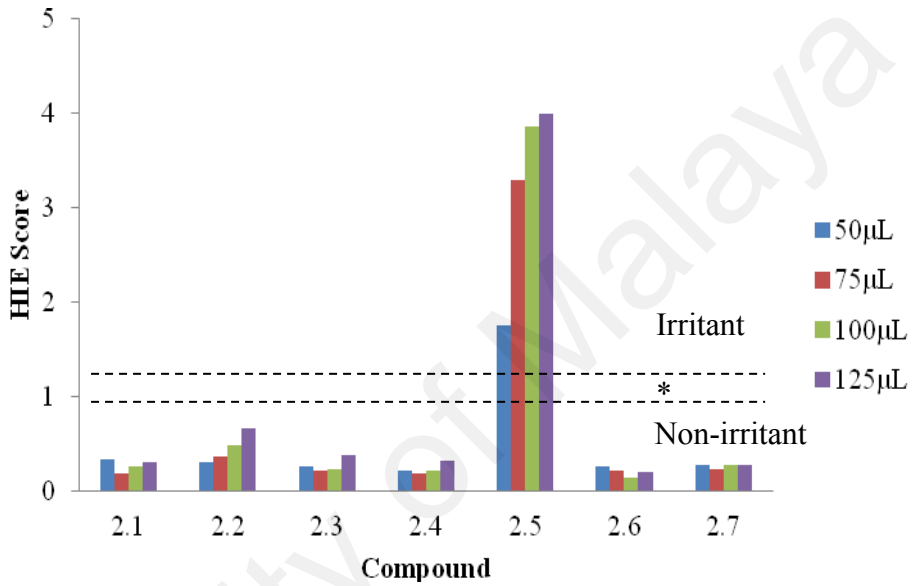


Figure 5.1 : *In vitro* dermal irritation potential of compounds 2.1-2.7

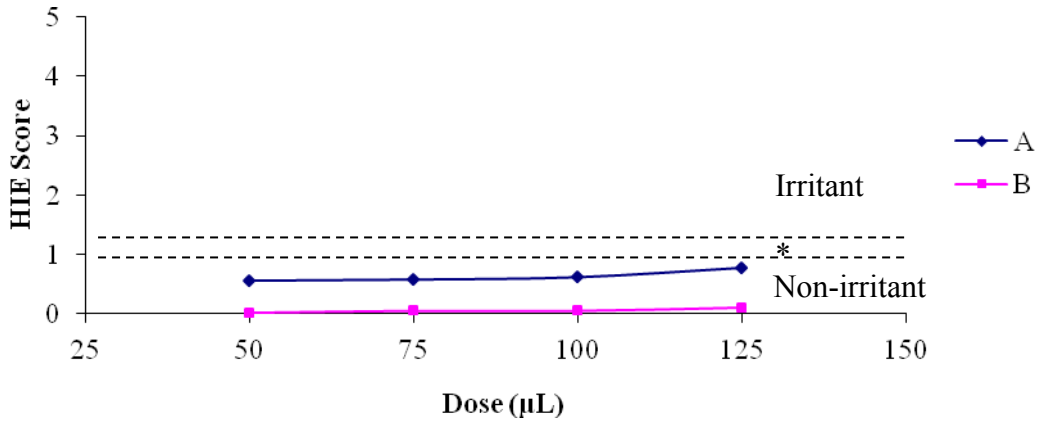
Table 5.4 : Relationship of Human Irritancy Equivalent (HIE) score to *in vivo* irritancy classification (for the dermal irritection test method)

Human Irritancy Equivalent (HIE) Score	Predicted Dermal Irritancy Classification
0.00 – 0.90	Non-Irritant
0.90 – 1.20	Non-Irritant/Irritant
1.20 – 5.00	Irritant

All compounds (**2.1-2.7**) are classified as non-irritant to the skin except compound **2.5**. It has been demonstrated that compound **2.5** increased turbidity of the reagent because

this compound promotes alterations in the structure of keratin, collagen and other dermal proteins. Additionally, as the protein denatured, they unfold and change shape, a process which termed as conformational changes. This results in disruption of the highly organized matrix structures that surround them. As this process proceeds, the proteins and matrix constituents gradually begin to form insoluble particles which gradually the clear protein solutions become cloudy, detected as an increase in the turbidity of the protein solution. Normally, as the concentration or volume of the tested irritant substance is increased, the protein reagent becomes more denatured and the amount of scattered light gradually increases until a maximal optical density is produced when all proteins have become altered. As a result, chemical irritants typically produce a linear or sigmoidal dose-response curve (InVitro International, 1996).

Meanwhile the reaction product obtained from the scaling up process that consist a mixture of compound **2.7** (65 %) as major component was further analyzed for the *in vitro* dermal irritection assay and compared to a high purity of compound **2.7** (92 %). From the test, the reaction product is classified as non-irritant (Figure 5.2). Therefore the reaction product can be used without further purification for its evaluation in potential applications.



* Non-Irritant/Irritant

Note : A = Compound **2.7**, *mono-tert*-butoxypropane diol (92 %)

B = Reaction product (compound **2.7**, 65 %)

Figure 5.2: *In vitro* dermal irritation potential of compound 2.7 with different purity

5.4 Ecotoxicity Test of *mono-tert*-butoxypropanediol (Compound 2.7)

Since compound **2.7** was used as a model compound for glyceryl ether evaluation in possible applications, the ecotoxicity of compound **2.7** was also determined using OECD (Organization for Economic Cooperation and Development) test method (OECD, 1992). The fish were exposed to different concentrations of the test substance for 96 hrs. The concentration that kills 50 % of fish (LC_{50}) was determined. Based on the ecotoxicity studies, LC_{50} of both glyceryl ether and glycerol is more than 100 mg/L which falls within the non-toxic category as classified in Table 5.5.

Table 5.5 : Classification of ecotoxicity range (based on LC_{50} by the US Fish and Wildlife Services for aquatic toxicity)

Rating	LC (mg/L)
Super toxic	< 0.01
Extremely toxic	0.01-0.1
Highly toxic	0.1-1.0
Moderately toxic	1.0-10.0
Slightly toxic	10.0-100.0
Practically non-toxic	100.0-1000.0
Relatively harmless	> 1000.0

5.5 *mono-tert*-butoxypropanediol (compound 2.7) in Macroemulsion

Potential applications of *mono-tert*-butoxypropanediol (compound **2.7**) in emulsion systems were explored. Based on hydrophilic-lipophilic balance (HLB) value of 8 to 12, emulsion systems for oil in water were developed. The concept of using the HLB value for the formation of the emulsion was applied according to Rieger and Rhein (1997). Screening for stable emulsion was carried out in the absence of glyceryl ether (compound **2.7**). The stable emulsion was used as a control in this study. Then the effects of glyceryl ether (compound **2.7**) on the emulsion system were investigated.

In order to identify the stable emulsion, two methods for stability test were conducted. The first method is the observation in phase separation of the emulsions at ambient temperature. The observation was recorded periodically. The second method is the use of accelerated test using a LUMiFuge stability analyzer. The method which is using near infra red transmission measurements during centrifugation for stability study has been reported by other researchers (Kuentz and Röthlisberger, 2003; Kanagaratnam *et al.*, 2013).

Figures 5.3 and 5.4 show the example of the results obtained from LUMiFuge stability analyzer indicating unstable and stable emulsions produced, respectively. From the graph, the data is converted into an instability index. The instability index measured is used to estimate the stability of the emulsions. The higher the value of the instability index, the more unstable the emulsion is within the estimated period of time. The highest value of the instability index is 1.

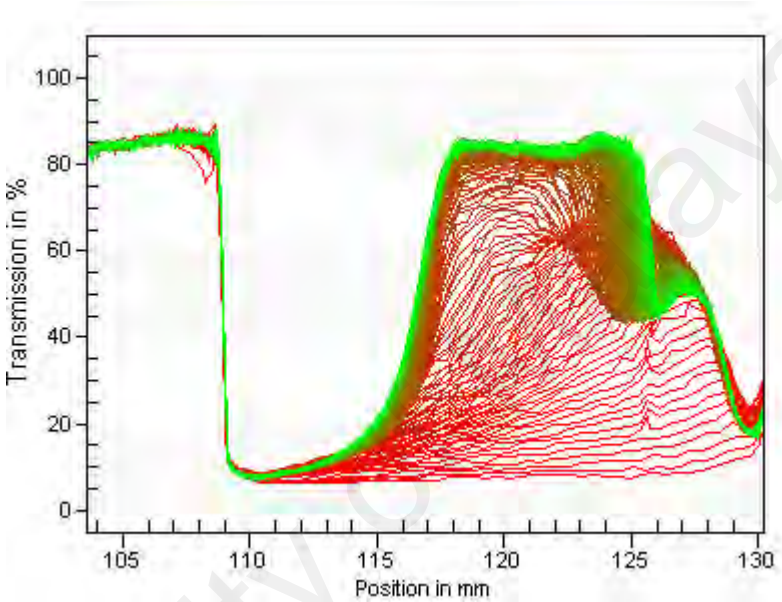


Figure 5.3 : Result obtained from LUMiFuge stability analyzer for stability test of emulsion produced shows an unstable emulsion

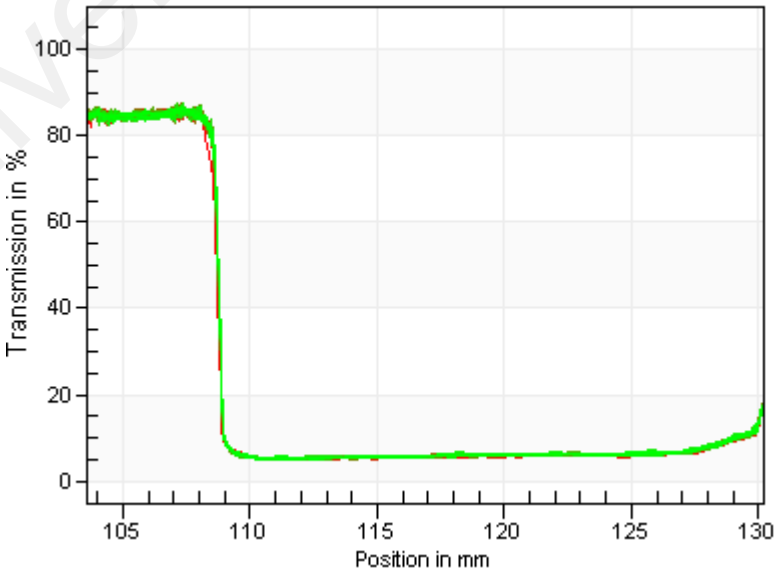


Figure 5.4 : Result obtained from LUMiFuge stability analyzer for stability test of emulsion produced shows a stable emulsion

In fact, the separation of the emulsion system can be clearly seen after the completion of the stability test using LUMiFuge stability analyzer (Figure 5.5).

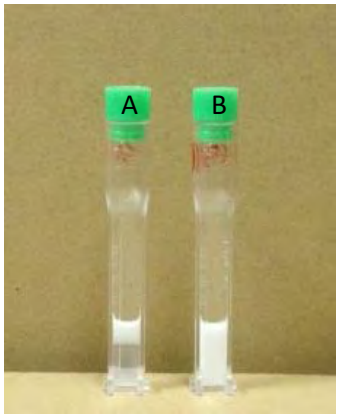
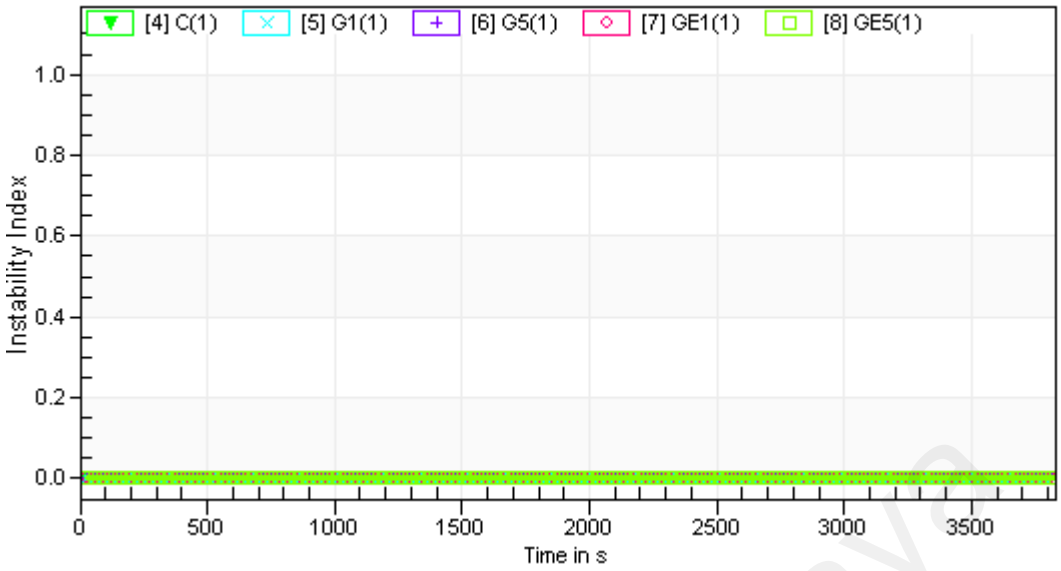


Figure 5.5 : Example of unstable (A) and stable (B) emulsion using LUMiFuge stability analyzer

Although phase separation of some emulsions did not occur when observed at ambient temperature, the LUMiFuge stability analyzer is able to show undesirable instability index. From both stability test methods, it was found that LUMiFuge stability analyzer is very useful in rapid determination of stability of the emulsions produced. A stable emulsion then was chosen for further study by incorporating glyceryl ether (compound **2.7**) in the emulsion system. The incorporation of glycerol in the emulsion system was done for comparison. The emulsions were analyzed for stability, viscosity, pH value, particle size, *in vitro* dermal irritation potential, *in vitro* ocular irritation potential and also moisturizing property. The instability index of the emulsions produced is shown in Figures 5.6 and 5.7. All emulsions are classified stable at 25 °C for 6 months and at 45 °C for 3 months based on a very low instability index.



wherein C(1) = Placebo
G1(1) = Emulsion containing 1 % glycerol
G5(1) = Emulsion containing 5 % glycerol
GE1(1) = Emulsion containing 1 % glyceryl ether
GE5(1) = Emulsion containing 5 % glyceryl ether

Figure 5.6 : The instability index of emulsions at 25 °C for 6 months based on the LUMiFuge stability analyzer

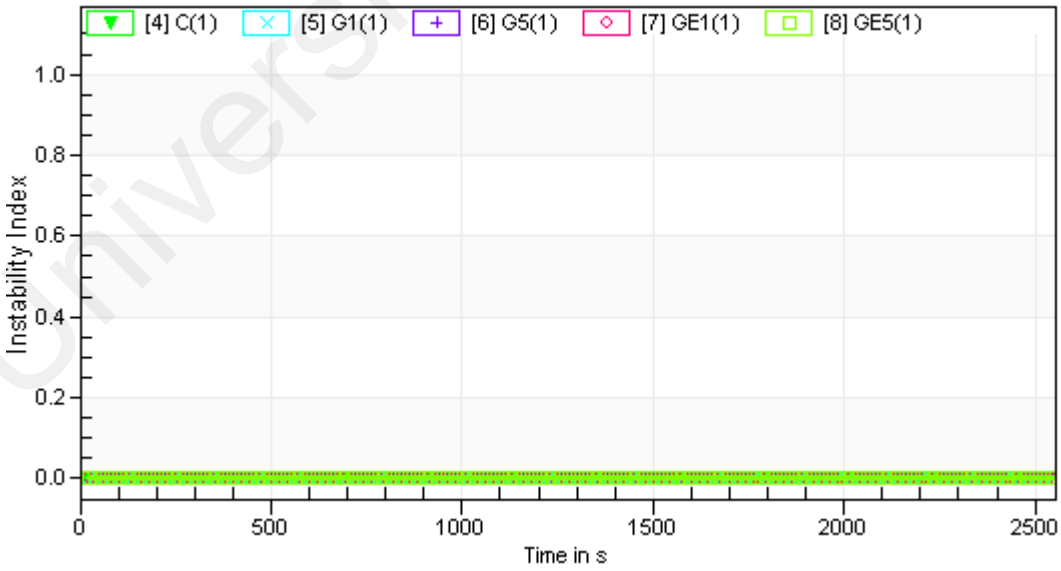
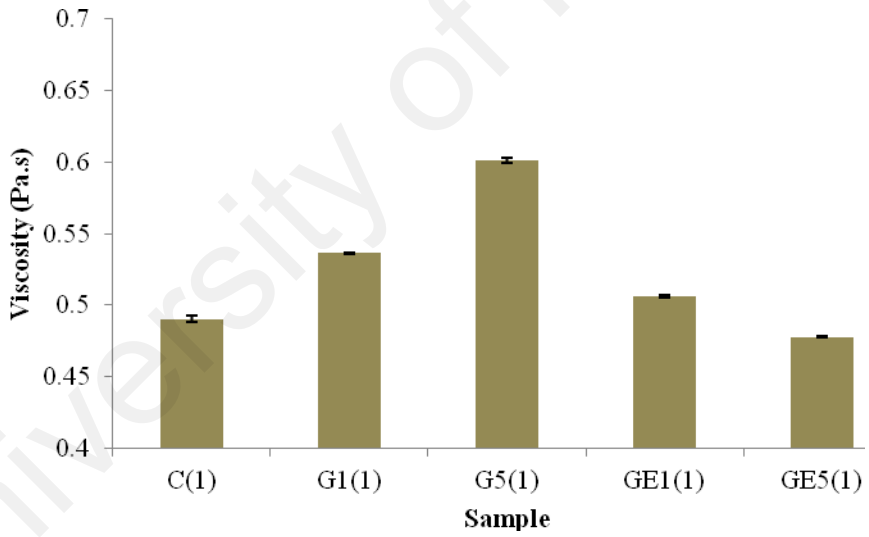


Figure 5.7 : The instability index of emulsions at 45 °C for 3 months based on the LUMiFuge stability analyzer

Compound **2.7** is not an emulsifier but it may act as a co-surfactant in the formation of emulsion. It is probably partitioned between the interface and the aqueous or oil phase. According to Resende *et al*. (2008), the presence of co-surfactant in microemulsion helps to reduce the interfacial tension of the microemulsion system.

The viscosity of the emulsions were measured and the results showed that the viscosity of the emulsions increased with the increasing of glycerol and it decreased with the increasing of glyceryl ether (compound **2.7**) as shown in Figure 5.8. According to Aghel and co-workers, glycerin is used as viscosity modifier in the formulation of a clear liquid shampoo base (Aghel *et al.*, 2007).



wherein C(1) = Placebo
G1(1) = Emulsion containing 1 % glycerol
G5(1) = Emulsion containing 5 % glycerol
GE1(1) = Emulsion containing 1 % glyceryl ether
GE5(1) = Emulsion containing 5 % glyceryl ether

Figure 5.8 : The viscosity of emulsions produced

The pH values of the emulsions produced were in the range of 6 to 6.5. The near neutral pH value correlates with low skin irritation (Baranda *et al.*, 2002). Meanwhile the

particle size of those emulsions is shown in Figure 5.9. The particle size of the emulsions produced remained unchanged even after two years of storage at ambient temperature. Thus, the emulsions produced were stable upon storage.

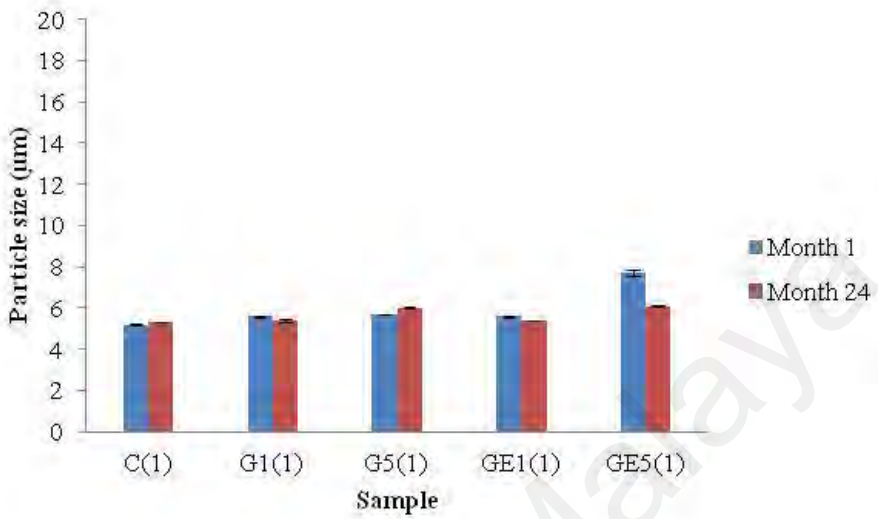
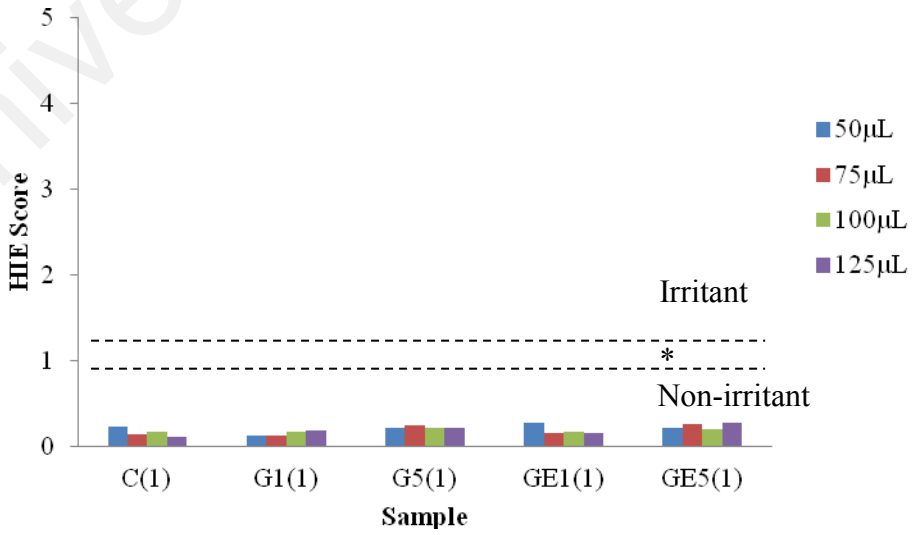


Figure 5.9 : Particle size of emulsions produced

The *in vitro* dermal irritection assay of the emulsions produced was also conducted according to InVitro International (1996). All emulsions produced are classified as non-irritant to the skin based on the HIE score (below 0.9) as shown in Figure 5.10.



* Non-Irritant/Irritant

Figure 5.10 : *In vitro* dermal irritation potential of the emulsions produced

Meanwhile the *in vitro* ocular irritection assay showed that all emulsions produced were classified as minimal irritant to the eye (Figure 5.11). Table 5.6 shows the relationship of Irritection Draize Equivalent (IDE) score to *in vivo* irritancy classification (Draize Test).

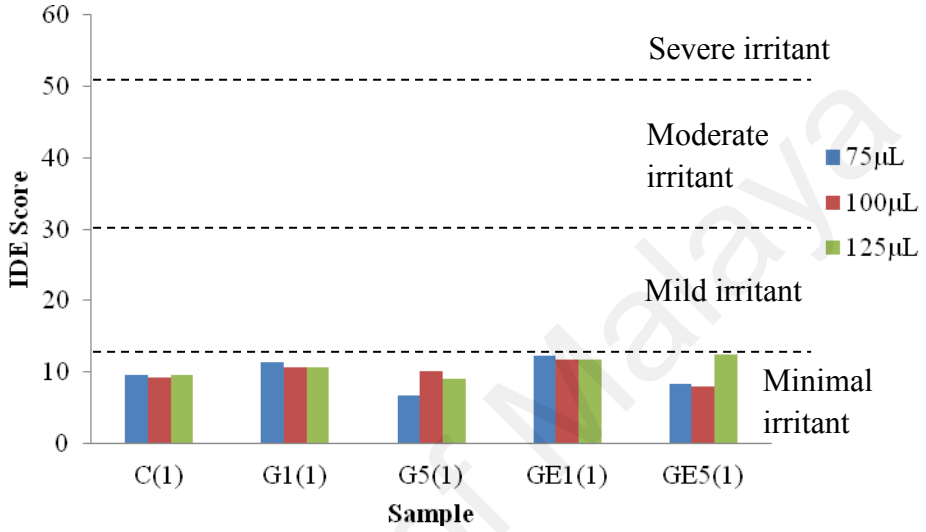


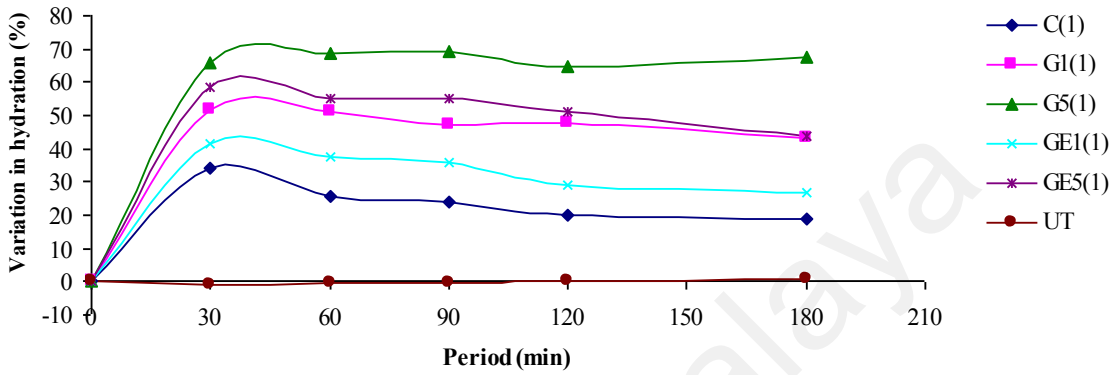
Figure 5.11 : *In vitro* ocular irritation potential of the emulsions produced

Table 5.6 : Relationship of Irritection Draize Equivalent (IDE) score to *in vivo* irritancy classification (Draize Test)

Irritection Draize Equivalent score	Predicted Ocular Irritancy Classification
0.0 – 12.5	Minimal irritant
12.5 – 30.0	Mild irritant
30.0 – 51.0	Moderate irritant
> 51.0	Severe irritant

Besides measuring the viscosity, pH value, particle size, *in vitro* dermal and ocular irritation potential of the emulsions, the moisturizing tests of the emulsions were also conducted to evaluate their moisturizing properties. The tests were done on 20 panels and the readings of hydration on skin, untreated and treated with the emulsions were

recorded every half an hour for 3 hrs. Figure 5.12 shows the percentage variation in hydration of the emulsions tested. There was no variation of skin hydration on untreated areas (UT) indicating that the test was well controlled.



wherein C(1) = Placebo
G1(1) = Emulsion containing 1 % glycerol
G5(1) = Emulsion containing 5 % glycerol
GE1(1) = Emulsion containing 1 % glyceryl ether
GE5(1) = Emulsion containing 5 % glyceryl ether
UT = Untreated

Figure 5.12 : Results of moisturizing tests of emulsions produced

Glycerol is a well known humectant. Humectant attracts water and help to keep that water bound in stratum corneum (Dobos, 2014). The presence of 1 % and 5 % glycerol in the emulsion significantly increased the hydration on the skin compared to placebo ($p < 0.05$). The presence of 1 % and 5 % glyceryl ether in the emulsions also significantly increased the percentage variation in hydration compared to placebo ($p < 0.05$). Eventhough the moisturizing property of glyceryl ether is not as superior as glycerol, but it still exhibits a good moisturizing effect to skin. A high skin hydration level of emulsion containing glycerol was reported by Alber and co-workers (Alber *et al.*, 2014). The superior moisturizing property of glycerol could be attributed to the three hydroxyl groups in glycerol molecule whereas the glyceryl ether has only two hydroxyl

groups. The presence of more hydroxyl group could bind more water, thus increase the skin hydration.

5.6 *mono-tert*-butoxypropanediol (Compound 2.7) in Microemulsion

Besides its possible use in macroemulsion, the glyceryl ether (compound **2.7**) was also incorporated in microemulsion system. A preliminary study of the glyceryl ether as a co-surfactant in microemulsion system was investigated.

A simple basic formula for oil in water microemulsion system was used for screening purpose. The study was conducted to investigate the possibility of using glyceryl ether in the formation of microemulsion system indicated by the stability of the microemulsions produced. Screening process of microemulsion formation was carried out using glyceryl ether (compound **2.7**) as a co-surfactant and 1-pentanol for comparison. 1-pentanol is a conventional co-surfactant used in microemulsion system.

A quick determination of microemulsion formation using 1-pentanol as a conventional co-surfactant (co-S) with fixed ratio of 20 % surfactant Tween 80 at 25:75 ratio of oil/water was recorded and tabulated in Table 5.7. Visual observation was done using polarized light at room temperature. Two different types of oil were used namely palm methyl ester (C_{12}-C_{18}) and medium chain triglyceride (MCT).

Table 5.7 : Visual observation with polarized light at room temperature for quick determination of microemulsions produced (1-pentanol as co-S)

S/O+W/Co-S	Palm Methyl Ester (C_{12}-C_{18})	MCT
20/75/5	Two layers	Two layers
20/70/10	Clear and low viscosity	Two layers
20/65/15	Two layers	Two layers
20/60/20	Two layers	Two layers
20/55/25	Two layers	Two layers
20/50/30	Two layers	Two layers
20/40/40	Two layers	Two layers
20/30/50	Two layers	Two layers
20/20/60	Two layers	Two layers

S = Surfactant
O + W = Oil and water
Co-S = Co-surfactant
MCT = Medium chain triglyceride

Meanwhile, quick determination of microemulsion formation using glyceryl ether (compound **2.7**) as a co-surfactant (co-S) with fixed ratio of 20 % surfactant Tween 80 at 25:75 ratio of oil/water was recorded as in Table 5.8. Addition of 40 % and above glyceryl ether (compound **2.7**) in the presence of palm methyl ester as the oil phase had led to the formation of stable microemulsions. Meanwhile the use of MCT as the oil phase required 60 % of the co-surfactant to form a stable microemulsion. For comparison, only 10 % 1-pentanol was needed to form the stable microemulsion in the presence of palm methyl ester as the oil phase and no stable microemulsions formed in the presence of MCT. In addition, phase separation was also observed when 15 % and higher amount of 1-pentanol were incorporated in the microemulsion system with palm

methyl ester. Only system 20/70/10 has the ability to form microemulsion with palm methyl ester because 10 % is the optimum amount of 1-pentanol that helps to reduce the interfacial tension between oil and water, resulting in the formation of spontaneous microemulsion. Therefore, 20/70/10 is the optimum composition of the microemulsion system to form complete microemulsion. This finding shows a good sign as bigger volume of glyceryl ether (compound **2.7**) is needed in the formation of stable microemulsion compared to 1-pentanol.

Table 5.8 : Visual observation with polarized light at room temperature for quick determination of microemulsions produced (glyceryl ether as co-S)

S/O+W/Co-S	Palm Methyl Ester (C_{12}-C_{18})	MCT
20/75/5	Two layers	Two layers
20/70/10	Two layers	Two layers
20/65/15	Two layers	Three layers
20/60/20	Two layers	Two layers
20/55/25	Two layers	Two layers
20/50/30	Two layers	Two layers
20/40/40	Clear and low viscosity	Two layers
20/30/50	Clear and low viscosity	Two layers
20/20/60	Clear and low viscosity	Clear and low viscosity

S = Surfactant
O + W = Oil and water
Co-S = Co-surfactant
MCT = Medium chain triglyceride

Different microemulsion system will need different amount of glyceryl ether in forming a stable microemulsion. Importantly, the findings showed that glyceryl ether (compound

2.7) may be used as a co-surfactant in microemulsion system. Ismail and co-workers investigated the effect of diols in microemulsions and they reported that diols specifically 1,2-hexanediol showed good potential as non-toxic co-surfactants in microemulsions because they cause very minimal irritation to the skin and eyes of end-users compared to medium-chain aliphatic alcohols such as 1-pentanol (Ismail *et al.* 2011).

5.7 *mono-tert*-butoxypropanediol (Compound 2.7) in Transparent Soap

Traditionally, glycerol is used in soaps (Pagliaro and Rossi, 2010). Specifically in this section, glycerol is used in transparent soap. In the market, transparent soaps are sold in a variety of shapes and degrees of optical clarity, and some are handcrafted by artisans. They generally contain polyols such as glycerine, propylene glycol or sorbitol (Spitz, 2009). Therefore, attempts to utilize the glyceryl ether (compound **2.7**) in soap making particularly transparent soap was done and glycerol was used as comparison. The idea is to diversify the use of glyceryl ether, thus expand the use of glycerol derivatives in possible applications including soap making. The properties of the transparent soaps produced were characterized including the colour, transparency value, pH, hardness and foam volume of the soap.

The evaluation of a soap bar colour traditionally was performed by visual comparison of fresh products. Anyhow, it is also can be done by an instrumental approach (Spitz, 2009). In this study, visual colour comparison technique was applied. In terms of visual comparison of fresh soaps colour, the transparent soap which contains glyceryl ether was yellow in colour compared to the soap that contains glycerol (Figure 5.13). At this point of time, the reason behind it still remains unknown. However, this could be an

advantage in making a yellow transparent soap without using any colouring agent in the soap formula.

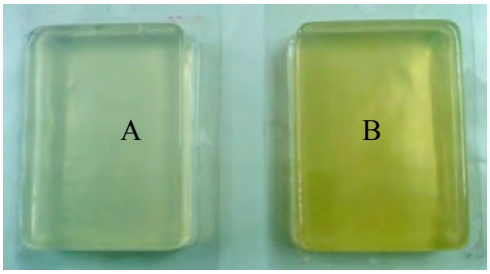


Figure 5.13 : Transparent soaps containing glycerol (A) and glyceryl ether (B)

By the definition, transparent soap should be transparent and there is a technique on how to measure the transparency value of soap. The technique has proven to be useful in quantifying the transparency of transparent soap bar. The procedure for measuring the transparency value of soap bar involves preparing the soap sample for reading by cutting a flat slice to a uniform thickness. The procedure involves calibrating the instrument, followed by measurement of the sample that is based on a light transmission through sample and then the processor in the instrument converts the data into a transparency value. The higher the transparency value, the more transparent the soap is. The highest value of the transparency is 1.

The transparency value of transparent soaps containing glyceryl ether and glycerol is 0.87 ± 0.01 and 0.83 ± 0.02, respectively. Both samples exhibited more than 0.8 transparency value. In fact, the transparency value of transparent soap containing glyceryl ether is significantly higher than the value of transparent soap containing glycerol ($p < 0.05$).

Besides the measurement of the transparency value, the pH value of the soaps was also determined. It was done based on 1 % solution of transparent soap and the results showed that the pH value of both samples was in the range of 9.97 to 10.03.

Another important property of a soap bar is the hardness. The basic consumer requirement regarding hardness is that the soap bar not be malleable when it is gripped but the bar must be malleable to some degree. The bar should meet some threshold level of hardness at room temperature. An instrumental test can be used to measure the hardness of a soap bar (Spitz, 2009).

In this study, the hardness of the soap bar was measured using a texture analyzer. A penetration depth of a 'needle' into the soap was fixed and the force or load that is needed for penetration serves as a measure of product softness. The more force or load that is needed for the penetration, the harder the soap is. Figures 5.14 and 5.15 show the results of hardness measurements of transparent soap containing glyceryl ether and the soap containing glycerol, respectively.

The maximum force (N) and the negative force area during the first compression are reported as hardness and adhesiveness, respectively (Lumor *et al.*, 2010). The force of penetration for the transparent soap containing glyceryl ether (3.30 ± 0.05 N) was less than the force of penetration for the soap containing glycerol (4.71 ± 0.03 N), indicating the soap containing glyceryl ether is softer than the soap containing glycerol. When comparing to commercial sample (3.63 ± 0.05 N), the force of penetration for the soap containing glyceryl ether was closer than the soap containing glycerol.

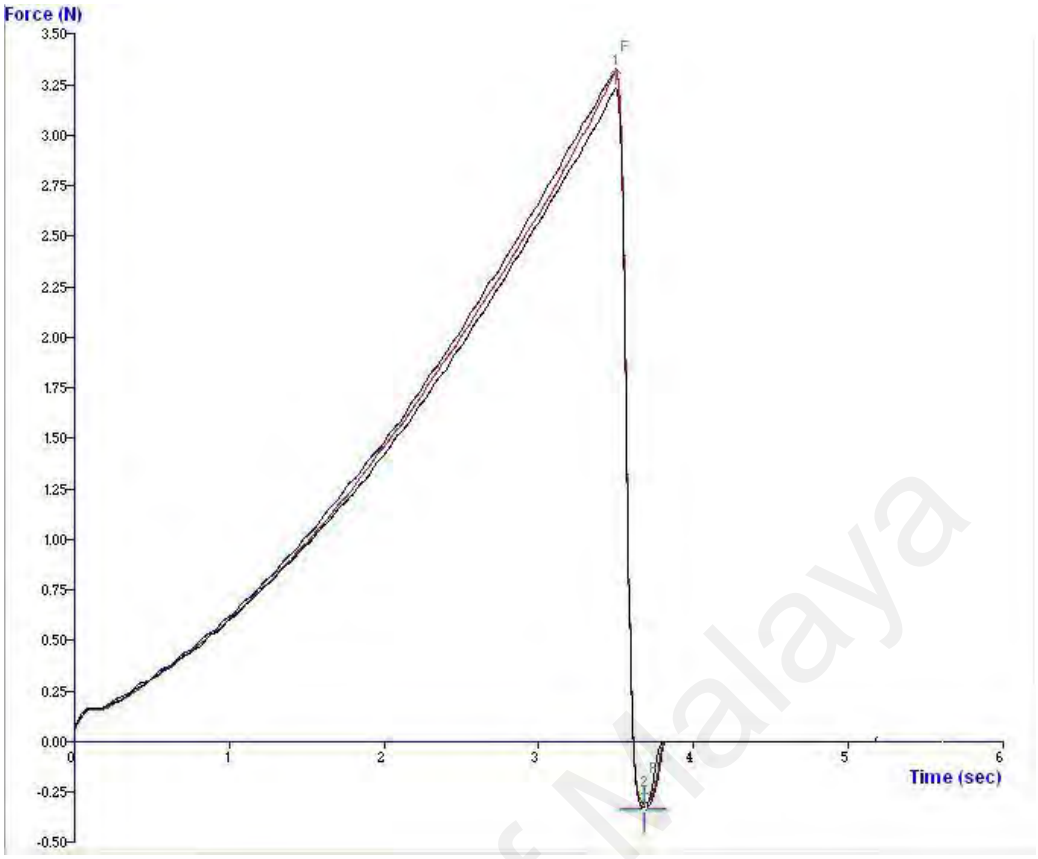


Figure 5.14 : Hardness measurement of transparent soap containing glyceryl ether

The trend of the results were also similar to adhesiveness where the force applied for the transparent soap containing glyceryl ether (-0.33 ± 0.01 N) was close to commercial sample (-0.32 ± 0.02 N) compared to the soap containing glycerol (-0.65 ± 0.04 N).

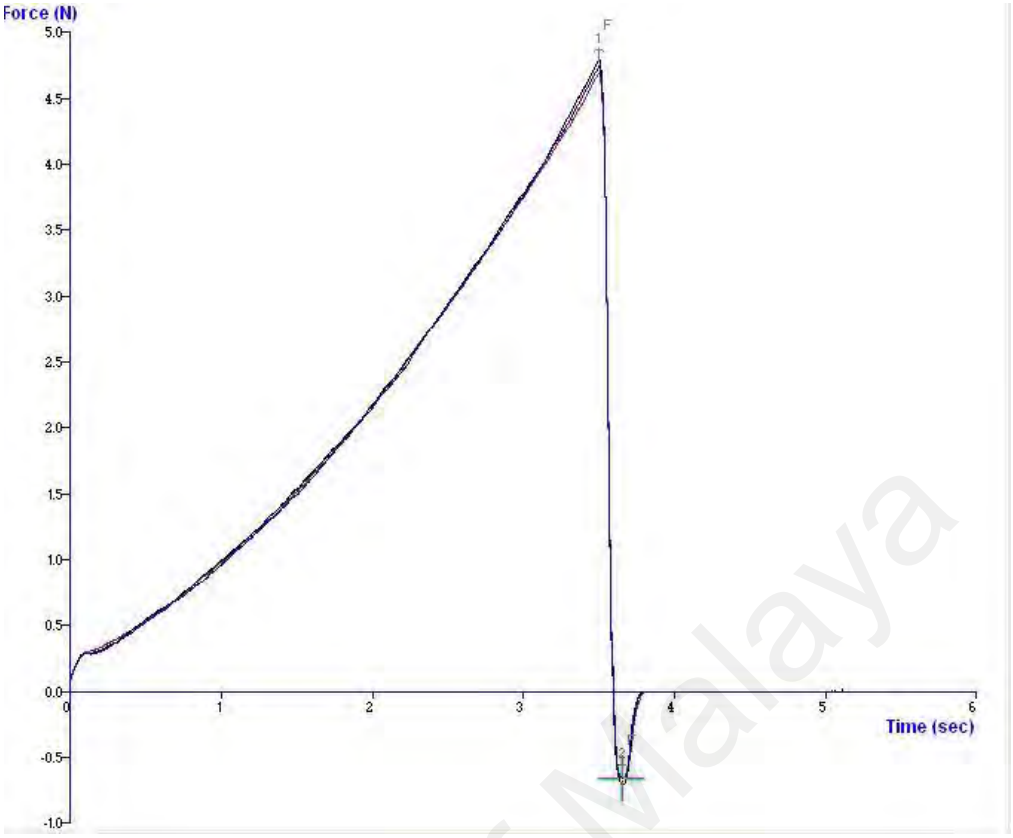
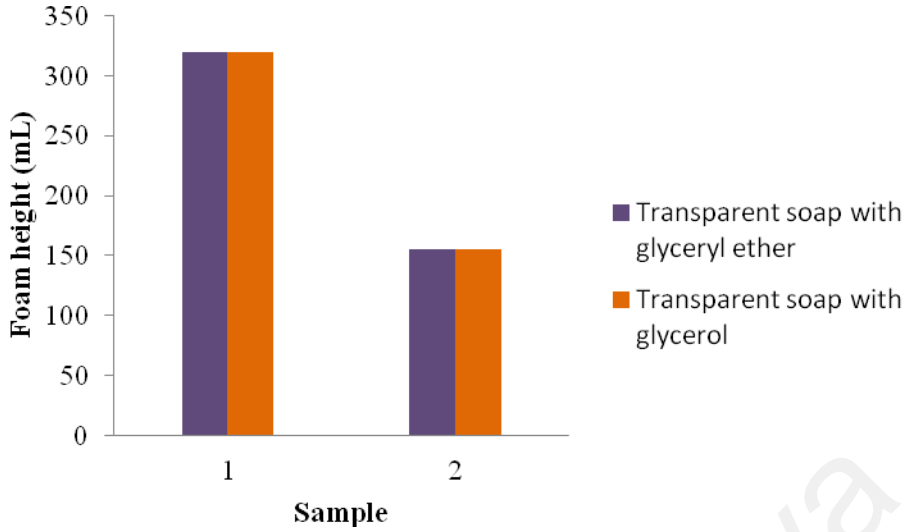


Figure 5.15 : Hardness measurement of transparent soap containing glycerol

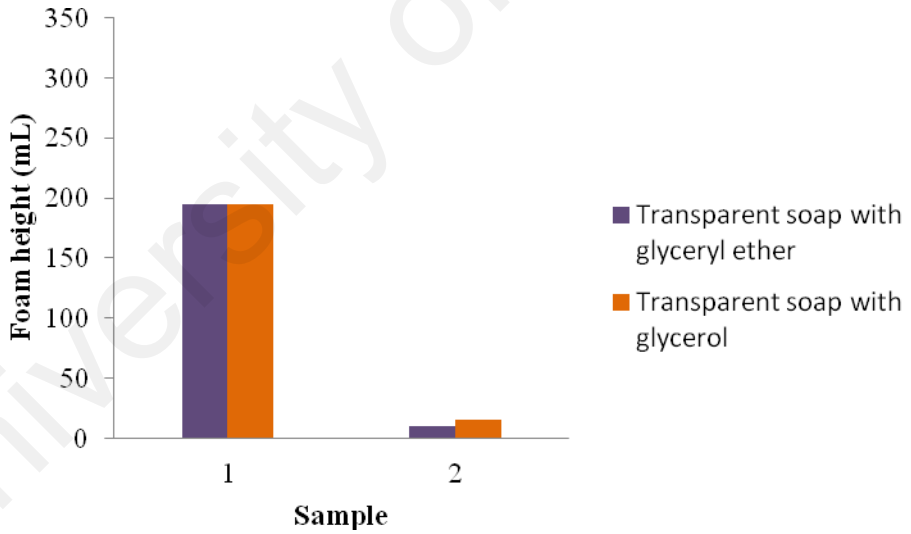
Besides the soap hardness, foam volume of soap is also important. Therefore, foaming test of the transparent soaps containing glyceryl ether and glycerol was conducted. The foaming power of soap solution was determined at different water hardness where the volume of the foam produced was read immediately and after 5 minutes. Figures 5.16 to 5.18 show the results of foaming test of the transparent soaps at different water hardness. There was no difference in foaming power or foam stability for both transparent soaps indicating the glyceryl ether is suitable for transparent soap making.



wherein 1 = Foaming power

2 = Foam stability

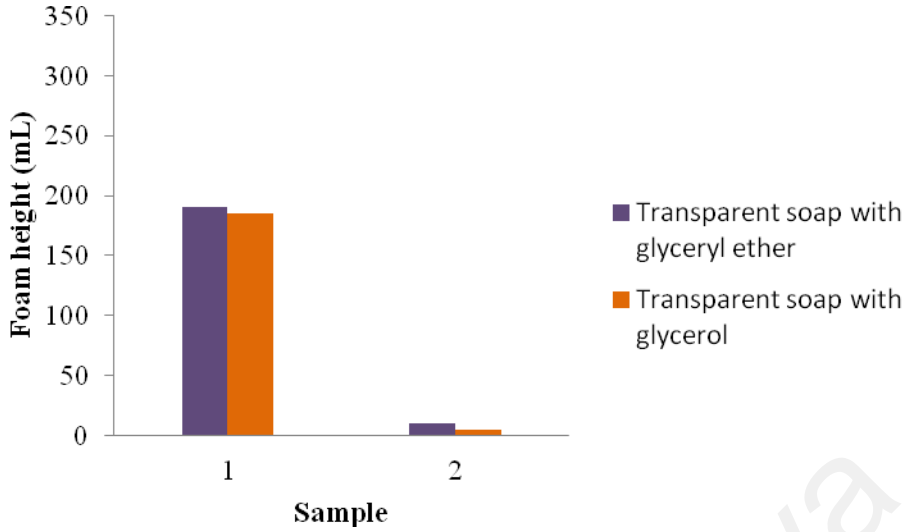
Figure 5.16 : Foaming test of transparent soaps in RO water



wherein 1 = Foaming power

2 = Foam stability

Figure 5.17 : Foaming test of transparent soaps in 50 ppm water hardness



wherein 1 = Foaming power

2 = Foam stability

Figure 5.18 : Foaming test of transparent soaps in 350 ppm water hardness

The stability test of the soap was also conducted at room temperature. After 6 months of storage, some crystals formed in the soap containing glycerol while no formation of crystal observed in the soap containing glyceryl ether. The formation of crystal is undesirable in transparent soap although it may not affect the performance of the soap. This will give an advantage to formulator when using glyceryl ether in the transparent soap making instead of glycerol.

CHAPTER 6: EXPERIMENTAL DETAILS

6.1 General Procedures

Infrared (IR) spectra were recorded using a Nicolet/Magna-IR 550, Spectrometer Series II or Spectrum 100 FT-IR Spectrometer, USA. All NMR spectra were recorded on a Bruker AVN 400 or Bruker AVN 600 Spectrometer, Switzerland. Gas chromatogram was recorded by gas chromatography with FID, Agilent 6890 Series, USA where chromatograph was connected to PC. Analyses were carried out on chromatographic capillary column HP INNOWAX (30 m x 0.32 mm coated with film 0.5 μm) with temperature programme from 40 to 220 ºC or column HP-5 (30 m x 0.25 mm coated with film 0.25 μm). Mass spectra were recorded on GC-MS Agilent Technologies, 7890A GC System, USA connected to 5975C insert MSD with Triple-Axis Detector. Crystal structure data was collected using Agilent SuperNova Dual diffractometer with an Atlas detector, USA.

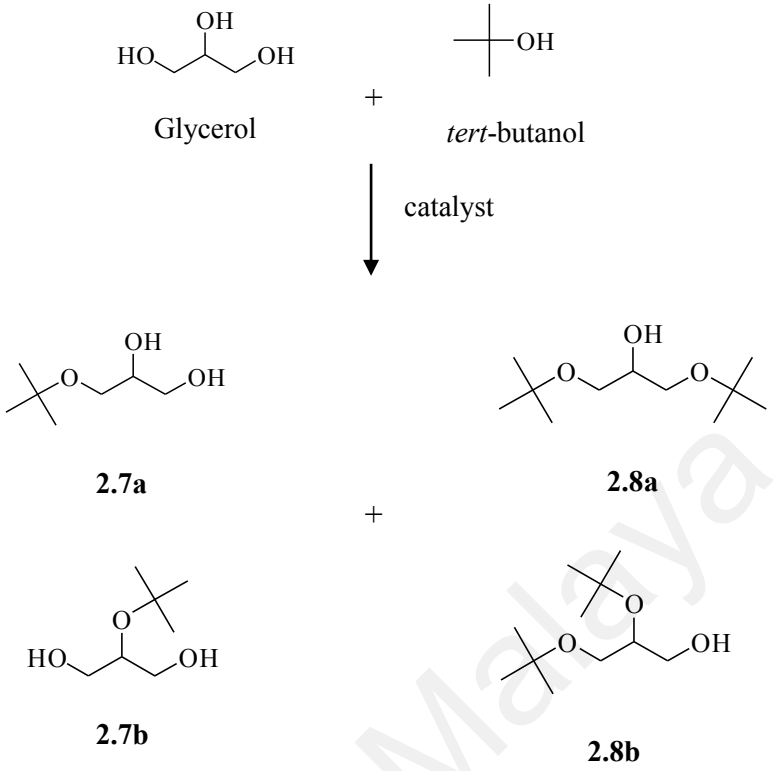
Column chromatography was performed using silica gel 60. Analytical TLC was performed using silica gel 60 precoated glass plates, Merck, Germany which were viewed under ultra violet light or iodine vapour.

Glycerol anhydrous was purchased from J.T. Baker, USA and *p*-TsCl was purchased from Fluka, South Korea. Sodium metal was purchased from Merck, Germany. Amberlyst 15, Amberlyst 36 and Montmorillonite K10 were purchased from Aldrich, USA. Sulphuric acid (purity, 96.3 %) was purchased from J.T.Baker, USA while *p*-TsOH was purchased from Merck, Germany. All other reagents used were of analytical grade and used as received unless stated otherwise.

6.2 Experimental Details for Chapter 2

6.2.1 Direct Etherification of Glycerol with *tert*-Butanol

In a typical experiment, glycerol was reacted with *tert*-butanol at atmospheric pressure in the presence of acidic catalyst. The effects of temperature, duration, mole ratio of reactants, the type and the amount of catalyst used were investigated. The conversion of glycerol was obtained based on the unreacted glycerol left in the reaction product which was determined by using gas chromatography. The product mixture was subjected to preparative TLC (hexane/diethyl ether: 60/40) in order to separate the components. The major component of the product mixture was isolated and subjected to ^{1}H and ^{13}C NMR analyses. The NMR spectrum of the isolated component confirmed that the compound was compound **2.7a** (Appendix 2.1). ^{1}H NMR ($CDCl_3$) δ 1.15 (9H,s,CH_3), 3.14 (1H,t,OH), 3.31 (1H,d,OH), 3.40 (2H,m), 3.57 (1H,m), 3.65 (1H,m), 3.75 (1H,m). ^{13}C NMR ($CDCl_3$) δ 27.5, 63.5, 64.5, 71.0, 73.5. The second isolated compound from the product mixture was also subjected to proton and ^{13}C NMR analyses. The component is compound **2.8a** as expected (Appendix 2.2). ^{1}H NMR ($CDCl_3$) δ 1.22 (18H,s), 2.61 (1H,s,OH), 3.41 (4H,m), 3.80 (1H,m). ^{13}C NMR ($CDCl_3$) δ 27.4, 62.9, 70.2, 73.0. The product mixture was also subjected to GC-MS. From the mass spectra (Appendixes 2.6-2.9), the possible compounds were determined. Compound **2.8a**, *m/z* = 204 (M), *m/z* = 57 (100 %); compound **2.8b**, *m/z* = 204 (M), *m/z* = 57 (100 %); compound **2.7a**, *m/z* = 148 (M), *m/z* = 57 (100 %) and compound **2.7b**, *m/z* = 148 (M), *m/z* = 57 (100 %).



6.2.1.1 Effect of Catalyst : Type and Dosage

Glycerol (18.4 g, 0.2 mole) was charged into a 250 mL 3-neck-round bottom flask and then *tert*-butanol (59.3 g, 0.8 mole) was charged into the same flask together with *p*-TsOH (0.92 g, 5 % from the weight of glycerol). The mixture was heated to 80-85 °C for 7 hrs. The glycerol dissolved in *tert*-butanol during the heating process and etherification occurred in homogenous phase. The progress of the reaction was monitored *via* TLC and GC. The cooled reaction product was evaporated to remove water and unreacted *tert*-butanol under reduced pressure. The crude product was subjected to GC analysis in order to determine the conversion of glycerol. Reactions of glycerol with *tert*-butanol were also carried out in the presence of other catalysts namely Amberlyst 15, Amberlite IR-120, Amberlyst 36, Montmorillonite K10 and sulphuric acid in the presence of different catalyst dosage. The catalyst dosage varied from 2.5 % to 10 %. Reactions in the presence of less than 2.5 % catalyst were also conducted but the glycerol conversion was lower than the glycerol conversion using 2.5

% catalyst. Therefore, the catalyst dosage of 2.5 % and above was used for further investigation in this research work.

6.2.1.2 Effect of Reaction Temperature at Different Catalyst Dosage

Glycerol (18.4 g, 0.2 mole) was charged into a 250 mL 3-neck-round bottom flask and then *tert*-butanol (59.3 g, 0.8 mole) was charged into the same flask together with Amberlite IR-120 (0.46 g, 2.5 % from the weight of glycerol). The mixture was heated to 40-45 ºC for 5 hours. The progress of the reaction was monitored *via* TLC and GC. The cooled reaction product was filtered to separate the catalyst. Then the filtrate (product) was evaporated to remove water and unreacted *tert*-butanol under reduced pressure. The crude product was subjected to GC analysis in order to determine the conversion of glycerol. Reactions of glycerol with *tert*-butanol were also carried out at 60-65 ºC and 80-85 ºC in the presence of different catalyst dosage (2.5 % - 10 %).

6.2.1.3 Effect of Mole Ratio of Glycerol to *tert*-Butanol

Glycerol (18.4 g, 0.2 mole) was charged into a 250 mL 3-neck-round bottom flask and then *tert*-butanol (59.3 g, 0.8 mole) was charged into the same flask together with Amberlite IR-120 (0.46 g, 2.5% from the weight of glycerol). The mixture was heated to 80-85 ºC for 5 hours. The progress of the reaction was monitored *via* TLC and GC. The cooled reaction product was filtered to separate the catalyst. Then the filtrate (product) was evaporated to remove water and unreacted *tert*-butanol under reduced pressure. The crude product was subjected to GC analysis in order to determine the conversion of glycerol. Reactions of glycerol with *tert*-butanol were also carried out at different mole ratio of glycerol to *tert*-butanol (1:1 - 1:8) and at different temperature (40-85 ºC) in the presence of different catalyst dosage (2.5 % - 5 %).

6.2.1.4 Scale Up Production of *mono-tert*-butoxypropanediol (Compound 2.7)

The production of glyceryl ether was done at larger scale, up to 5 L reactor capacity. The reactions were carried out using 1 L, 2 L and 5 L reactor capacity.

Experiment 1

Glycerol (155.32 g, 1.69 mole) was charged into a 1 L 3-neck-round bottom flask and then *tert*-butanol (500 g, 6.75 mole) was charged into the same flask together with Amberlite IR-120 (7.76 g, 5 % from the weight of glycerol). The mixture was heated to 80-85 °C for 5 hrs. The cooled reaction product then was filtered to separate the catalyst. Then the filtrate (product) was evaporated to remove water and unreacted *tert*-butanol under reduced pressure. The crude glyceryl alkyl ether was subjected to GC analysis in order to determine the conversion of glycerol and the products composition. Product = 173 g – 179 g.

Experiment 2

Glycerol (368.4 g, 4 mole) was charged into a 2 L 3-neck-round bottom flask and then *tert*-butanol (1186 g, 16 mole) was charged into the same flask together with Amberlite IR-120 (18.42 g, 5 % from the weight of glycerol). The mixture was heated to 80-85 °C for 5 hrs. The cooled reaction product then was filtered to separate the catalyst. Then the filtrate (product) was evaporated to remove water and unreacted *tert*-butanol under reduced pressure. The crude glyceryl alkyl ether was subjected to GC analysis in order to determine the conversion of glycerol and the products composition. Product = 450 g – 461 g.

Experiment 3

Glycerol (621.3 g, 6.75 mole) was charged into a 5 L flask and then *tert*-butanol (2000.05 g, 26.99 mole) was charged into the same flask together with Amberlite IR-120 (15.53 g, 2.5 % or 31.07 g, 5 % from the weight of glycerol). The mixture was heated to 80-85 °C for 5-7 hrs. The cooled reaction product then was filtered to separate the catalyst. Then the filtrate (product) was evaporated to remove water and unreacted *tert*-butanol under reduced pressure. The crude glyceryl alkyl ether was subjected to GC analysis in order to determine the conversion of glycerol and the products composition. Product = 780 g – 798 g. Separation and purification of the product mixture were carried out using simple vacuum distillation, fractional vacuum distillation, short path distillation and liquid-liquid extraction.

Simple Vacuum Distillation

Product mixture was charged into a flask equipped with a condenser and vacuum system. The distillate was collected in 1-neck round bottom flask. The capacity of the flask depends on the volume of the sample used.

Fractional Vacuum Distillation

The set up for this system is quite similar to simple vacuum distillation. The difference is only the Vigreux column that has to be inserted in the fractional vacuum distillation set-up.

Short Path Distillation

The short path distillation method was used to isolate product from glycerol through gentle thermal separation method. The distillation process was carried out by varying the internal condenser and evaporator temperatures. The capacity of the distiller unit is

1 L to 2 L. The aim was to obtain the glyceryl ether containing more than 60 % *mono-tert*-butoxypropanediol with less than 5 % glycerol.

Liquid-liquid Extraction

Sample was extracted with organic solvent where the volume of solvent used depends on the amount of sample.

6.3 Experimental Details for Chapter 3

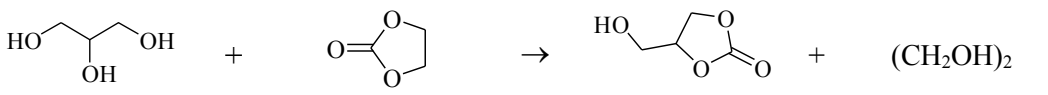
6.3.1 Preparation of Glycidol (compound 3.2)

Preparation of glycidol (compound **3.2**) involved two-step reaction. The first step was to synthesize 4-hydroxymethyl-1,3-dioxolan-2-one (compound **3.1**) and then compound **3.1** was further subjected to heating process to produce compound **3.2**.

6.3.1.1 Preparation of 4-hydroxymethyl-1,3-dioxolan-2-one (Compound 3.1)

In a typical experiment, glycerol was reacted with 1,3-dioxolan-2-one or dimethyl ester under reduced pressure or at atmospheric pressure in the presence or absence of catalyst. The conversion of glycerol was obtained based on the unreacted glycerol left in the product which was determined by using gas chromatography.

Experiment 1

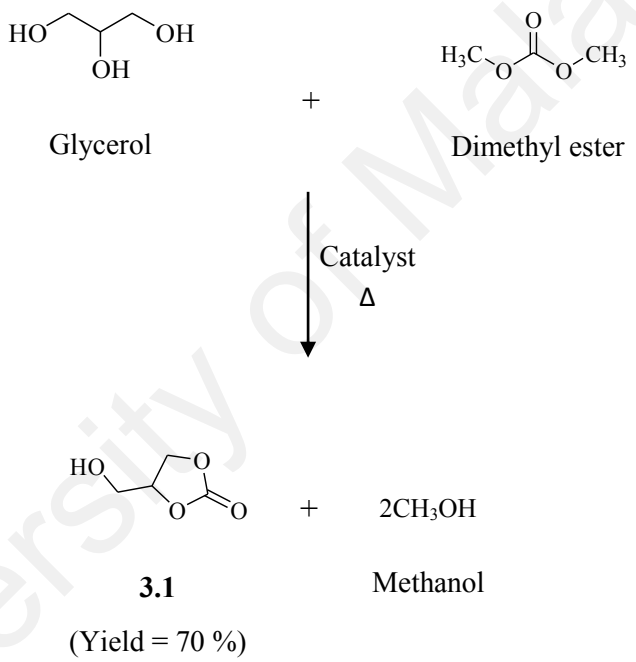


Glycerol | 1,3-dioxolan-2-one | **3.1** (Yield = 86 %) | Ethylene glycol

Glycerol (92.00 g, 1 mole) was charged into a 500 mL 3-neck-round bottom flask and then 1,3-dioxolan-2-one (95.01 g, 1.08 mole) was charged into the same flask. The

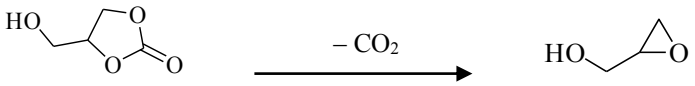
mixture was stirred with a magnetic stirrer and was heated up to 150 °C under reduced pressure. The 1,3-dioxolan-2-one dissolved in glycerol during the heating process and the reaction occurred in homogenous phase. Ethylene glycol formed as a by-product and was removed during the reaction by vacuum distillation. The product was analyzed using GC and standard chemical was used as a reference in order to determine the yield of the product obtained.

Experiment 2



Glycerol (80.10 g, 0.8698 mole) was charged into a 500 mL 3-neck-round bottom flask and then dimethyl ester (234.90 g, 2.6077 mole) was charged into the same flask together with potassium carbonate. The mixture was stirred with a magnetic stirrer and was heated. The mixture became homogeneous as the temperature raised and it was refluxed at 70-75 ºC for 3 hrs. The product was analyzed using GC and standard chemical was used as a reference in order to determine the yield of the product obtained.

6.3.1.2 Preparation of Glycidol (compound 3.2) from Compound 3.1



3.1 **3.2**
(Yield = 50 %)

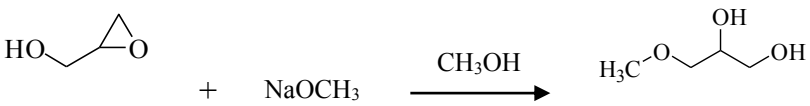
Product obtained from section 6.3.1.1 was charged into a flask together with sodium sulfate, equipped with condenser and vacuum system. Then, the mixture was heated under reduced pressure and the glycidol (compound **3.2**) formed was distilled off into a collector. The product was analyzed using GC and standard chemical was used as a reference in order to determine the yield of the product obtained. Compound **3.2**, *m/z* = 74 (M), *m/z* = 44(100 %), 31, 15.

6.3.2 Preparation of Glyceryl Ethers from Glycidol (compound 3.2)

6.3.2.1 Glyceryl Ethers Formation by Ring Opening of Glycidol (compound 3.2) under Basic Medium

Glycidol was reacted with alkoxide ion to produce glyceryl ether (compounds **2.1a**, **2.2a** and **2.7a**).

Experiment 1

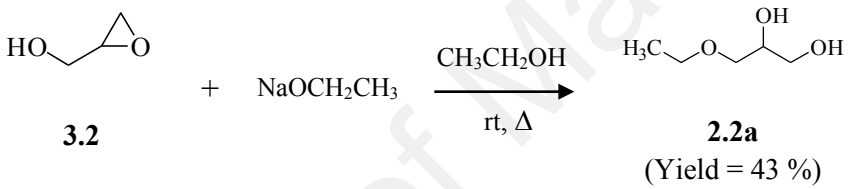


3.2 **2.1a**
(Yield = 45 %)

Glycidol (0.7408 g, 0.01 mole) and sodium methoxide, 30 % in methanol (1.8007 g, 0.01 mole) were charged into reaction flask. The reaction was done under an inert atmosphere and reaction temperature varied from room temperature, 50 ºC to reflux

condition. The reaction period was in the range of 15 minutes to 60 minutes in order to investigate suitable reaction condition to produce compound **2.1a**. The product was extracted into diethyl ether and the ether was removed under reduced pressure. The product then was purified by using preparative TLC. The NMR spectra confirmed that the structure is compound **2.1a**. ^{1}H NMR ($CDCl_3$) δ 3.40 (3H,s,-C<u>H</u>$_3$), 3.61 (2H,m,-C<u>H</u>$_2$OH), 3.63 (1H,dd,J=7.7,3.9Hz,-C<u>H</u>$_2$-O-CH$_3$), 3.71 (1H,dd,J=7.6,2.5Hz,-C<u>H</u>$_2$-O-CH$_3$), 3.89 (1H,m,-C<u>H</u>-CH$_2$-OH). ^{13}C NMR ($CDCl_3$) δ 59.2, 64.0, 70.6, 74.2.

Experiment 2

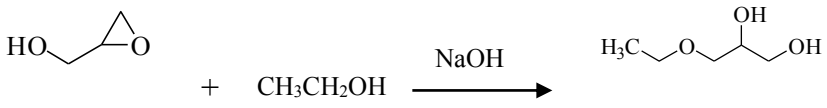


3.2 **2.2a**

(Yield = 43 %)

Sodium metal (0.23 g, 0.01 mole) was dissolved in absolute ethanol to produce sodium ethoxide. Then, glycidol (0.7408 g, 0.01 mole) was reacted with the previous sodium ethoxide solution in ethanol in reaction flask under an inert atmosphere. The reaction temperature was kept between 55-60 °C. The reaction period was 60 minutes. The product was extracted into diethyl ether and the ether was removed under reduced pressure. The product then was purified by using preparative TLC. The NMR spectra confirmed that the structure was compound **2.2a**. ^{1}H NMR ($CDCl_3$) δ 1.24 (3H,t,J=7.5Hz,C<u>H</u>$_3$CH$_2$-), 3.55 (4H,m,-C<u>H</u>$_2$-CH$_3$;-C<u>H</u>$_2$-OH), 3.64 (1H,dd,J=7.6,3.6Hz,-C<u>H</u>$_2$-O-CH$_2$CH$_3$), 3.73 (1H,dd,J=7.6,2.6Hz,-C<u>H</u>$_2$-O-CH$_2$CH$_3$), 3.89 (1H,m,-C<u>H</u>-CH$_2$-OH). ^{13}C NMR ($CDCl_3$) δ 15.1, 64.2, 67.0, 70.5, 72.2.

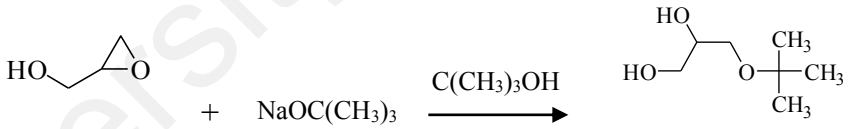
Experiment 3



3.2 **2.2a**
(Yield = 24 %)

Sodium hydroxide (0.40 g, 0.01 mole) was mixed with ethanol to produce sodium ethoxide. Then, glycidol (0.7408 g, 0.01 mole) was added into the mixture in reaction flask under an inert atmosphere. The reaction temperature was kept between 55-60 ºC. The reaction period was 60 minutes. The product was extracted into diethyl ether and the ether was removed under reduced pressure. The product then was analyzed using GC where compound **2.2a** obtained from previous experiment was used as a reference in order to determine the yield of the product obtained.

Experiment 4



3.2 **2.7a**
(Yield = 5-10 %)

Sodium hydroxide (0.3001 g, 0.0075 mole) was mixed with *tert*-butanol (15 mL). Then, glycidol (0.5556 g, 0.0075 mole) was added into the mixture in reaction flask under an inert atmosphere. The reaction temperature was kept between 55-60 ºC. The reaction period was 60 minutes. The product was extracted into diethyl ether and the ether was removed under reduced pressure. The product then was analyzed using GC where compound **2.7a** obtained from Chapter 2 was used as a reference in order to determine the yield of the product obtained.

6.3.2.2 Glyceryl Ethers Formation by Ring Opening of Glycidol (compound 3.2) under Acidic Medium

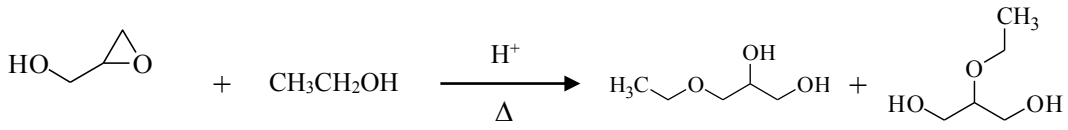
Glycidol was reacted with seven alcohols namely methanol, ethanol, propanol, butanol, *iso*-propanol, *iso*-butanol and *tert*-butanol in the presence of acidic catalyst to produce *mono*-methoxypropanediol (compound **2.1**), *mono*-ethoxypropanediol (compound **2.2**), *mono*-propoxypropanediol (compound **2.3**), *mono*-butoxypropanediol (compound **2.4**), *mono-iso*-propoxypropanediol (compound **2.5**), *mono-iso*-butoxypropanediol (compound **2.6**) and *mono-tert*-butoxypropanediol (compound **2.7**), respectively.

Experiment 1

3.2 **2.1a** (77 %) **2.1b** (23 %)

Glycidol (0.7408 g, 0.01 mole), methanol (4.05 mL) and 0.0037 g acidic catalyst (Amberlyst 15 or Montmorillonite K10) were charged into a reaction flask. The reaction temperature varied from room temperature to 60 ºC. The reaction period was in the range of 15 minutes to 300 minutes in order to investigate suitable reaction condition to produce compound **2.1**. The reaction product was filtered and excess methanol was removed under reduced pressure. The product was extracted into ether and the ether was removed under reduced pressure. The product then was analyzed by using GC and GC-MS. Mass spectra of both isomers of the product are shown in Appendix 3.5. Compound **2.1a**, *m/z* = 106 (M), *m/z* = 75 (50 %), 45 (100 %), 43 (52 %), 31 (22 %); compound **2.1b**, *m/z* = 106 (M), *m/z* = 75 (100 %), 45 (44 %), 43 (41 %), 31 (18 %). The catalyst dosage was varied from 0.5 % to 7.5 % in order to increase the yield of the product. The highest product yield was 60 %.

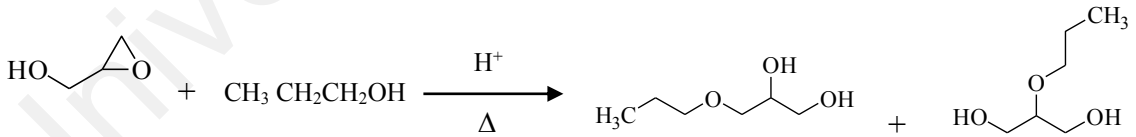
Experiment 2



3.2 **2.2a** (86 %) **2.2b** (14 %)

Glycidol (0.7408 g, 0.01 mole), ethanol (5.84 mL) and Montmorillonite K10 (0.037g) were charged into one-neck-round bottom flask. The reaction temperature was kept between 55-60 ºC for three hrs to produce compound **2.2**. The reaction product was filtered and excess ethanol was removed under reduced pressure. The product was extracted into ether and the ether was removed under reduced pressure. The product then was analyzed by using GC and GC-MS. Mass spectra of both isomers of the product are shown in Appendix 3.6. Compound **2.2a**, *m/z* = 120 (M), *m/z* = 89 (33 %), 61 (93 %), 59 (100 %), 45 (25 %), 43 (59 %), 31 (59 %), 29 (38 %); compound **2.2b**, *m/z* = 120 (M), *m/z* = 89 (100 %), 61 (97 %), 45 (19 %), 43 (87 %), 31 (35 %), 29 (33 %). Product yield = 49 %.

Experiment 3

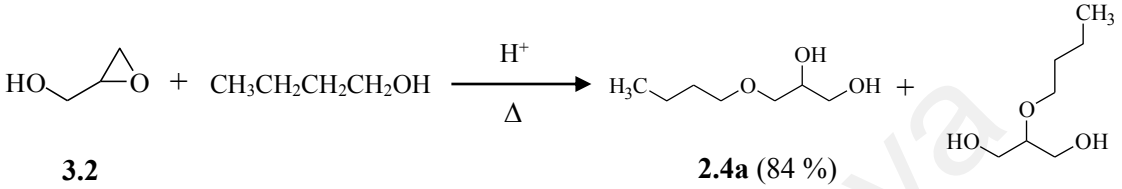


3.2 **2.3a** (79 %) **2.3b** (21 %)

Glycidol (0.7408 g, 0.01 mole), propanol (7.48 mL) and Montmorillonite K10 (0.037g) were charged into one-neck-round bottom flask. The reaction temperature was kept between 55-60 ºC for three hrs to produce compound **2.3**. The reaction product was filtered and excess propanol was removed under reduced pressure. The product was extracted into ether and the ether was removed under reduced pressure. The product then was analyzed by using GC and GC-MS. Mass spectra of both isomers of the

product are shown in Appendix 3.7. Compound **2.3a**, *m/z* = 134 (M), *m/z* = 103 (10 %), 73 (36 %), 61 (55 %), 43 (100 %), 31 (16 %); compound **2.3b**, *m/z* = 134 (M), *m/z* = 103 (25 %), 61 (100 %), 43 (59 %), 31 (15 %). Product yield = 24 %.

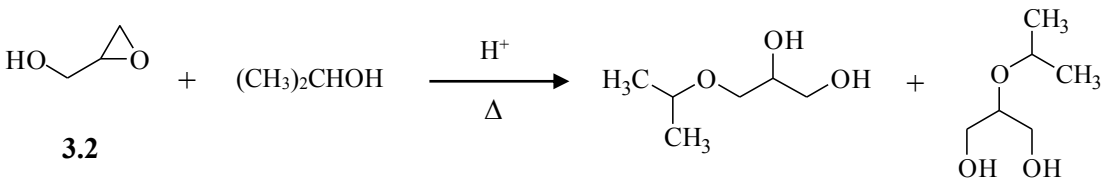
Experiment 4



3.2 **2.4a** (84 %) **2.4b** (16 %)

Glycidol (0.7408 g, 0.01 mole), butanol (9.15 mL) and Montmorillonite K10 (0.037g) were charged into one-neck-round bottom flask. The reaction temperature was kept between 55-60 ºC for three hrs to produce compound **2.4**. The reaction product was filtered and excess butanol was removed under reduced pressure. The product was extracted into ether and the ether was removed under reduced pressure. The product then was analyzed by using GC and GC-MS. Mass spectra of both isomers of the product are shown in Appendix 3.8. Compound **2.4a**, *m/z* = 148 (M), *m/z* = 117 (17 %), 87 (60 %), 75 (15 %), 73 (8 %), 61 (75 %), 57 (100 %); compound **2.4b**, *m/z* = 148 (M), *m/z* = 117 (28 %), 75 (5 %), 73 (14 %), 61 (90 %), 57 (100 %). Product yield = 15 %.

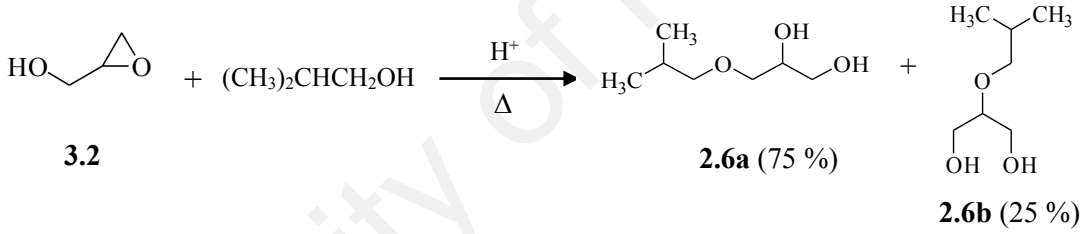
Experiment 5



3.2 **2.5a** (77 %) **2.5b** (23 %)

Glycidol (0.7408 g, 0.01 mole), *iso*-propanol (7.66 mL) and Montmorillonite K10 (0.037g) were charged into one-neck-round bottom flask. The reaction temperature was kept between 55-60 ºC for three hrs to produce compound **2.5**. The reaction product was filtered and excess *iso*-propanol was removed under reduced pressure. The product was extracted into ether and the ether was removed under reduced pressure. The product then was analyzed by using GC and GC-MS. Mass spectra of both isomers of the product are shown in Appendix 3.9. Compound **2.5a**, *m/z* = 134 (M), *m/z* = 103 (8 %), 73 (48 %), 61 (75 %), 43 (100 %), 31 (16 %); compound **2.5b**, *m/z* = 134 (M), *m/z* = 103 (24 %), 61 (100 %), 43 (56 %), 31 (12 %). Product yield = 45 %.

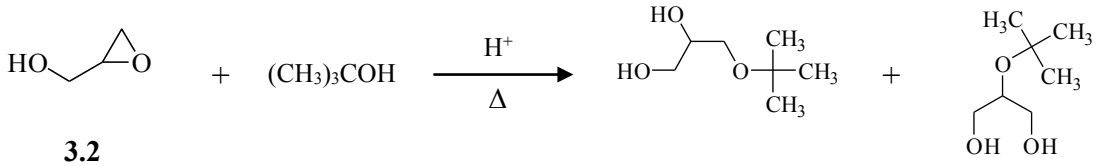
Experiment 6



3.2 **2.6a** (75 %) **2.6b** (25 %)

Glycidol (0.7408 g, 0.01 mole), *iso*-butanol (9.23 mL) and Montmorillonite K10 (0.037g) were charged into one-neck-round bottom flask. The reaction temperature was kept between 55-60 ºC for three hrs to produce compound **2.6**. The reaction product was filtered and excess *iso*-butanol was removed under reduced pressure. The product was extracted into ether and the ether was removed under reduced pressure. The product then was analyzed by using GC and GC-MS. Mass spectra of both isomers of the product are shown in Appendix 3.10. Compound **2.6a** *m/z* = 148 (M), *m/z* = 117 (12 %), 87 (36 %), 75 (19 %), 61 (42 %), 57 (100 %); compound **2.6b**, *m/z* = 148 (M), *m/z* = 117 (19 %), 75 (4 %), 61 (40 %), 57 (100 %). Product yield = 21 %.

Experiment 7



3.2

2.7a (81 %)

2.7b (19 %)

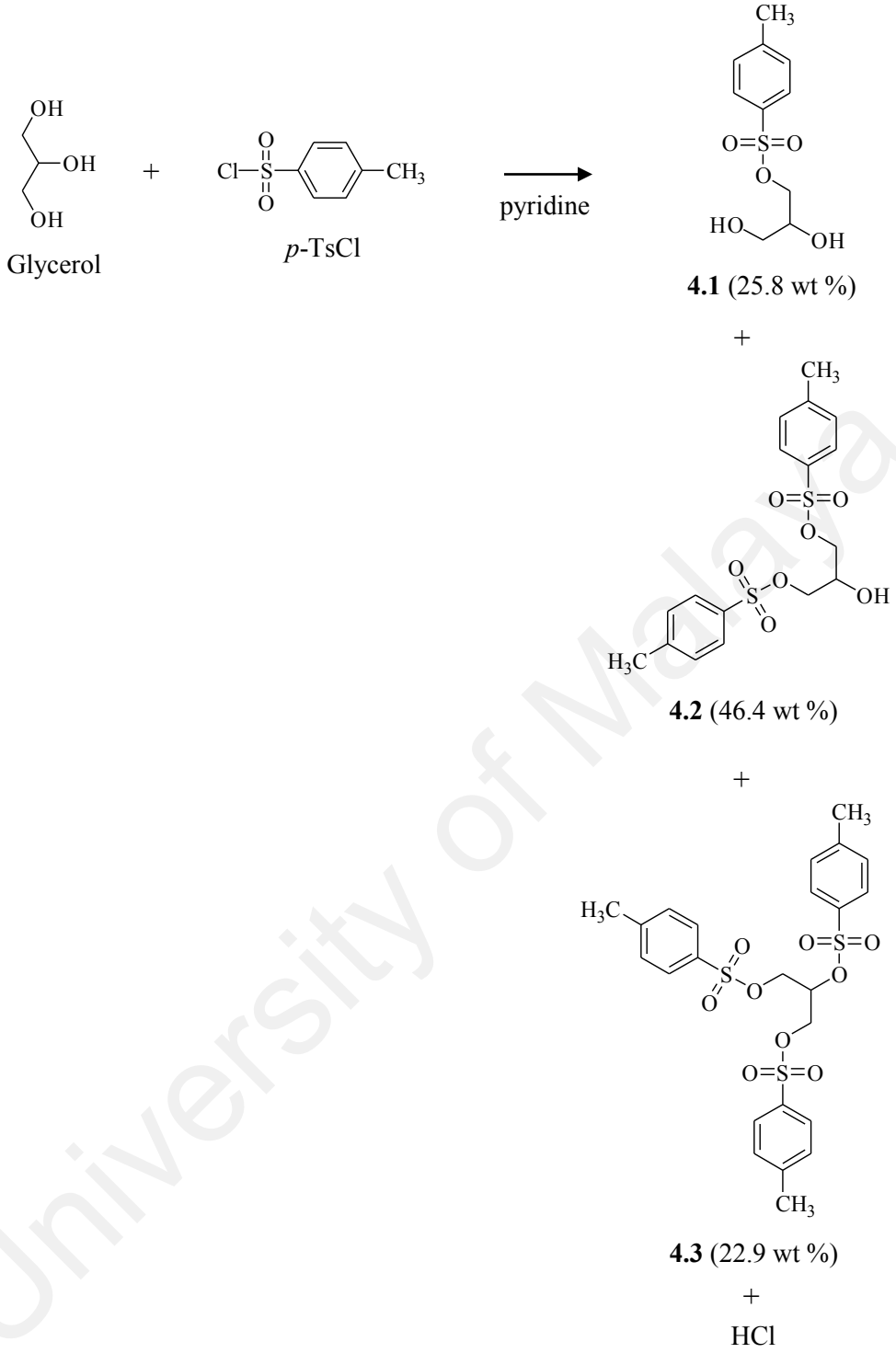
Glycidol (0.7408 g, 0.01 mole), *tert*-butanol (9.43 mL) and Montmorillonite K10 (0.037g) were charged into one-neck-round bottom flask. The reaction temperature was kept between 55-60 ºC for three hrs to produce compound **2.7**. The reaction product was filtered and excess *tert*-butanol was removed under reduced pressure. The product was extracted into ether and the ether was removed under reduced pressure. The product then was analyzed by using GC and GC-MS. Mass spectra of both isomers of the product are shown in Appendix 3.11. Compound **2.7a**, *m/z* = 148 (M), *m/z* = 117 (6 %), 87 (9 %), 75 (15 %), 61 (17 %), 57 (100 %); compound **2.7b**, *m/z* = 148 (M), *m/z* = 117 (17 %), 75 (2 %), 61 (5 %), 57 (100 %). Product yield = 31 %.

6.4 Experimental Details for Chapter 4

6.4.1 Preparation of Glyceryl Tosylate

A mixture of glycerol (5.53 g, 0.06 mole), pyridine (4 mL, excess) and *p*-TsCl (9.53 g, 0.05 mole) was stirred in 50 mL dichloromethane and the progress of the reaction was monitored by thin layer chromatography. On completion of the reaction, the reaction mixture was washed free from pyridine with 1N hydrochloric acid, and finally water. The organic layer was separated from the aqueous layer. Sodium sulphate anhydrous then was added into the organic layer, filtered and the solvent then was evaporated to give crude tosylated product of glycerol. Based on thin layer chromatography analysis, the crude product mixture (10.5 g) was then chromatographed on a silica gel column

eluting with hexane-Et_2O (v/v) = 5:5 to give three individual components (Compound **4.1**, **4.2** and **4.3** refer to *mono*-, *di*- and *tri*-tosylate of glycerol, respectively) and analyzed by infrared and nucleus magnetic resonance spectroscopy besides x-ray crystallography where possible. Compound **4.1** : ^{1}H NMR ($CDCl_3$, 600 MHz) : δ 2.39 (s, 3H), 3.57 (m, 1H), 3.64 (m, 1H), 3.89 (m, 1H), 4.03 (m, 2H), 7.29 (d, J=8.4, 2H), 7.73 (d, J=8.4, 2H). ^{13}C NMR ($CDCl_3$, 600 MHz) : δ 21.7, 62.8, 69.7, 70.9, 128.1, 130.1, 132.3, 145.4. Compound **4.2** : ^{1}H NMR ($CDCl_3$, 600 MHz) : δ 2.39 (s, 6H), 3.98 (m, 5H), 7.28 (d, J=8.4, 4H), 7.69 (d, J=8.4, 4H). ^{13}C NMR ($CDCl_3$, 600 MHz) : δ 21.7, 67.3, 69.6, 128.0, 130.0, 132.1, 145.4. Compound **4.3** : ^{1}H NMR ($CDCl_3$, 400 MHz) : δ 2.49 (s, 9H), 4.08 (m, 4H), 4.69 (m, 1H), 7.36 (d, J=8.0, 6H), 7.70 (d, J=8.0, 6H). ^{13}C NMR ($CDCl_3$, 400 MHz) : δ 21.7, 66.3, 74.4, 128.0, 130.0, 131.8, 145.6. Compound **4.3** was crystallized and recrystallized to obtain a single crystal which was suitable for X-ray crystallography. The solvents used including n-hexane, diethyl ether, benzene or chloroform. *Crystal data* for compound **4.3** : $C_{24}H_{26}O_9S_3$, T = 100 K, triclinic, a = 7.6887 (3) Å, b = 12.9635 (5) Å, c = 13.6887 (5) Å, α = 98.943 (3)° and β = 100.292 (3)°. V = 1265.91 (8) Å^3 (Appendix).



6.4.1.1 Effect of Amount of Base Used on the Tosylation of Glycerol

p-TsCl (0.6001 g, 0.0031 mole) was added into 10 mL dichloromethane. Glycerol (0.3478 g, 0.0038 mole) was mixed with pyridine (0.25 mL, 0.0031 mole) in a round bottom flask. Then, the *p*-TsCl solution was added gradually into glycerol solution

while maintaining the temperature of the reaction mixture to 0 °C. The mixture was then kept at room temperature until the completion of reaction. The reaction mixture was washed free from pyridine with 1N hydrochloric acid, and finally water. The organic layer was separated from the aqueous layer. Sodium sulphate anhydrous then was added into the organic layer, filter and the solvent then was evaporated to give crude product (glyceryl tosylate). The crude product mixture was then chromatographed on a silica gel column eluting with hexane-Et_2O (v/v) = 5:5. The reaction was repeated in the presence of different amount of base ranging from 1-5 mole equivalent of pyridine to *p*-TsCl. The weight of crude product obtained from each reaction is tabulated in Table 6.1.

Table 6.1 : Weight of crude product (g) obtained from the tosylation of glycerol with *p*-TsCl in the presence of different amount of base

Entry	Mole equivalent of pyridine to *p*-TsCl	Weight of crude product (g)
1	1	0.18
2	2	0.27
3	3	0.35
4	4	0.41
5	5	0.58

6.4.1.2 Effect of Different Solvent on the Tosylation of Glycerol

p-TsCl (0.6002 g, 0.0031 mole) was added into 10 mL dichloromethane. Glycerol (0.3478 g, 0.0038 mole) was mixed with pyridine (1.268 mL, 0.0157 mole) in a round bottom flask. Then, the *p*-TsCl solution was added gradually into glycerol solution while maintaining the temperature of the reaction mixture to 0 °C. The mixture was then kept at room temperature until the completion of reaction. The reaction mixture

was washed free from pyridine with 1N hydrochloric acid, and finally water. The organic layer was separated from the aqueous layer. Sodium sulphate anhydrous then was added into the organic layer, filter and the solvent then was evaporated to give crude product (glyceryl tosylate). The crude product mixture was then chromatographed on a silica gel column eluting with hexane-Et_2O (v/v) = 5:5. The reaction was repeated in the presence of different solvent namely chloroform, THF and also pyridine. The weight of crude product obtained from each reaction is tabulated in Table 6.2.

Table 6.2 : Weight of crude product (g) obtained from the tosylation of glycerol with *p*-TsCl in the presence of different solvent

Entry	Solvent	Weight of crude product (g)
1	DCM	0.58
2	Chloroform	0.45
3	THF	0.23
4	Pyridine	0.41

6.4.1.3 Effect of Mole Ratio of Glycerol to Tosylation Agent

p-TsCl (0.6001 g, 0.0031 mole) was added into 10 mL dichloromethane. Glycerol (0.2898 g, 0.0031 mole) was mixed with pyridine (1.268 mL, 0.0157 mole) in a round bottom flask. Then, the *p*-TsCl solution was added gradually into glycerol solution while maintaining the temperature of the reaction mixture to 0 °C. The mixture was then kept at room temperature until the completion of reaction. The reaction mixture was washed free from pyridine with 1N hydrochloric acid, and finally water. The organic layer was separated from the aqueous layer. Sodium sulphate anhydrous then was added into the organic layer, filter and the solvent then was evaporated to give

crude product (glyceryl tosylate). The crude product mixture was then chromatographed on a silica gel column eluting with hexane-Et_2O (v/v) = 5:5. The reaction was repeated at different mole ratio of glycerol to *p*-TsCl. The weight of crude product obtained from each reaction is tabulated in Table 6.3.

Table 6.3 : Weight of crude product (g) obtained from the tosylation of glycerol with *p*-TsCl at different mole ratio of glycerol to *p*-TsCl

Entry	Mole ratio of glycerol to *p*-TsCl	Weight of crude product (g)
1	1.0	0.60
2	1.2	0.58
3	1.5	0.42
4	2.0	0.32
5	3.0	0.21

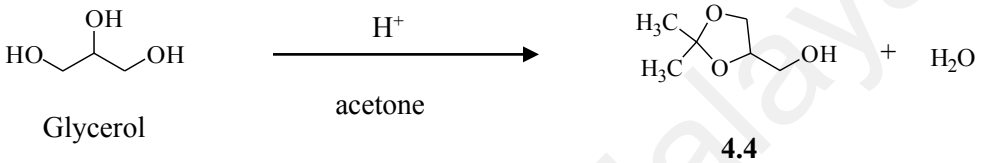
6.4.1.4 Antibacterial Test of Glyceryl Tosylates (Disc Diffusion Test Method)

Staphylococcus aureus and *Pseudomonas aeruginosa* were grown for 24 hrs on trypton soy agar. Suspensions of these tested bacteria were prepared in autoclaved water. The concentrations of the bacteria were adjusted to 1.0 x 10^6 cfu. A sterile swab was submerged in the suspensions containing bacteria and then swiped on the entire surface of the agar. Tryptone soy agar was inoculated by bacteria concentrations. Samples with different concentrations were prepared in DMSO as solvent. A sterile filter paper disc was dipped in the selected sample concentration and placed on the agar with sterilized forceps. Disc dipped into solvent was applied as negative control. The agar plates (in an inverted position) containing the filter paper disc of all concentrations were incubated in an oven at 35 °C for 24 hrs. After incubation, the diameter of each zone (in mm) was

measured with a ruler. Studies were performed in duplicates. The data collected were analyzed and statistically compared between samples (ANOVA and t-test). Differences in any groups of data were considered significant for a probability to value ≤ 0.05 (i.e. at 95 % confident level).

6.4.2 Preparation of Solketal (Compound 4.4)

Experiment 1



Glycerol

4.4
(Yield = 91 %)

In a round bottom flask, glycerol (23.03 g, 0.25 mole), *p*-TsOH (0.58 g), acetone (73.5 mL) and 100 mL petroleum ether were heated and stirred. The reaction mixture was refluxed until no more water collected in a Dean-Stark trap. Then the mixture was cooled to room temperature. Sodium acetate was added to the mixture. The product mixture then was rotary evaporated to remove an excess acetone and petroleum ether. The product then was subjected to fractional distillation process to yield a high purity of solketal (compound **4.4**). ^{1}H NMR ($CDCl_3$) δ 1.29 (3H, s, CH_3), 1.35 (3H, s, CH_3), 2.89 (1H, -OH), 3.49-4.19 (5H, m). ^{13}C NMR ($CDCl_3$) δ 25.3, 26.7, 63.0, 65.8, 76.3, 109.4.

Experiment 2

In a round bottom flask, glycerol (23.03 g, 0.25 mole), Amberlyst 15 (0.58 g), acetone (73.5 mL) and 100 mL petroleum ether were heated and stirred. The reaction mixture was refluxed until no more water collected in a Dean-Stark trap. Then the mixture was cooled to room temperature and the reaction mixture was filtered to remove heterogeneous acidic catalyst. The product mixture then was rotary evaporated to

remove an excess acetone and petroleum ether. The product then was subjected to fractional distillation process to yield a high purity of solketal (compound **4.4**).

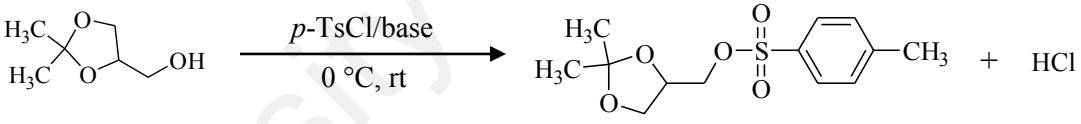
Experiment 3

Similar reaction as experiment 2 (section 6.4.2) was repeated but the reaction was done in the presence of different amount of catalyst.

6.4.3 Preparation of Solketal Tosylate (Compound 4.5)

Solketal tosylate was prepared by reacting solketal with *p*-TsCl in the presence of a base. In this study, the base used was pyridine.

Experiment 1



4.4

4.5

(Yield = 35 %)

p-TsCl (1.95 g, 0.0102 mole) was added into 20 mL dichloromethane. Compound **4.4** (0.91 g, 0.0068 mole) was mixed with pyridine (2.74 mL, 0.034 mole). Then, the *p*-TsCl solution was added gradually into the mixture. The mixture was kept at room temperature for 7 hrs. The reaction mixture was washed free from pyridine with 1N hydrochloric acid, and finally water. The solvent then was evaporated to give crude product. Recrystallization yielded a high purity of compound **4.5**. ^{1}H NMR ($CDCl_3$) δ 1.22 (6H, s, CH_3), 2.39 (3H, s, CH_3), 3.69-4.22 (5H, m), 7.31 (2H, d), 7.75 (2H, d). ^{13}C NMR ($CDCl_3$) δ 20.6, 24.1, 25.6, 65.2, 68.4, 71.9, 109.0, 127.0, 129.0, 144.1.

6.5 Experimental Details for Chapter 5

6.5.1 Antibacterial Test of Glyceryl Ethers (Disc Diffusion Test Method)

Staphylococcus aureus, *Pseudomonas aeruginosa* and *Escherichia coli* were grown for 24 hrs on trypton soy agar. Suspensions of these tested bacteria were prepared in autoclaved water. The concentrations of the bacteria were adjusted to 1.0 x 10^6 cfu. A sterile swab was submerged in the suspensions containing bacteria and then swiped on the entire surface of the agar. Tryptone soy agar was inoculated by bacteria concentrations. Samples were prepared in sterile water as solvent (1.0 g/mL). A sterile filter paper disc was dipped in the sample and placed on the agar with sterilized forceps. Disc dipped into water was applied as negative control. Meanwhile streptomycin (10 μg) was applied as positive control. The agar plates (in an inverted position) containing the filter paper disc of all samples were incubated in an oven at 35 °C for 24 hrs. After incubation, the diameter of each zone (in mm) was measured with a ruler. Studies were performed in duplicates. The data collected were analyzed and statistically compared between samples (ANOVA and t-test). Differences in any groups of data were considered significant for a probability to value ≤ 0.05 (i.e. at 95 % confident level).

6.5.2 *In vitro* Dermal Irritection Assay of Glyceryl Ethers

The dermal irritection assay is quantitative *in vitro* test methods that mimic acute dermal irritation test. To perform the dermal irritection standardized assay, the test sample is applied to a synthetic biobarrier that is coated with a dye-containing keratin-collagen matrix. Following application, the sample is absorbed by and permeates through this synthetic biobarrier to gradually come into contact with a proprietary solution containing highly ordered globulins and glycoproteins. Reaction of the test sample with these proteins and macromolecular complexes promotes conformational changes that may be readily detected as an increase in the turbidity of the protein

solution. With the dermal irritection test, turbidity as well as the dye that has been dissociated from the biobarrier during transit of the applied sample may be detected at a wavelength of 450 nm.

The dermal irritancy potential of a test sample is expressed as a Human Irritancy Equivalent (HIE). These scores are defined by comparing the increase in optical density (OD_{450}) produced by the test material to a standard curve that is constructed by measuring the increase in OD produced by a set of calibration substances. These calibrators have been selected for use in these tests because their irritancy potential has been previously documented in a series of *in vivo* investigations.

All data are calculated and analyzed *via* a computer program, which determines assay result acceptance based upon qualification parameters defined in the program. In general, the program has been designed to accept sample data as qualified if the following criteria are met: the OD values of Calibrators and internal Quality Control samples fall within specified ranges.

6.5.3 Ecotoxicity Test

The ecotoxicity of the product was determined using OECD 203, Fish, Acute Toxicity Test.

6.5.4 *mono-tert*-butoxypropanediol (Compound 2.7) in Macroemulsion

The first step was to develop a stable emulsion so-called placebo [C(1)] in the absence of glycerol or glyceryl ether by varying the amount of surfactant mixture. Glycerol or glyceryl ether was added into the formulation when a stable emulsion was obtained. Ingredients for emulsions (expressed as percentage) are shown in Table 6.4.

Table 6.4 : Ingredients for emulsions

Phase A (water phase)				Phase B (oil phase)	
Water (%)	**Brij721 (%)**	**Glycerol (%)**	**Glyceryl ether (%)**	**MCT (%)**	**Brij72 (%)**
To 100	0.3 – 2.4	0 – 5	0 – 5	5 – 10	0.3 – 3.6

Phase A was heated at 70 ^{o}C with stirring. In another beaker, phase B was heated at 70 ^{o}C. The phase A was homogenized at 6000 rpm. Then the phase B was added to phase A and homogenized at 10,000 rpm. Finally, the homogenized mixture was stirred until ambient temperature was reached. Glyceryl ether or glycerol was mixed in the phase A in order to investigate the effect of the compound in emulsion system.

6.5.4.1 Stability Study of Macroemulsions

i) Normal Observation

Each sample was divided into three sample bottles and stored at 3 different conditions:

a) Ambient temperature (24 months)

b) Freeze/Thaw (6 days)

   Sample was stored for 24 hrs at room temperature and then stored at 5 °C for 24 hrs. The cycle was repeated for 3 cycles.

c) 45 °C (3 months)

ii) Instrumental Method

The stability of prepared emulsions was measured using LUMiFuge stability analyzer. The samples were filled into rectangular polycarbonate cells (PC10 mm with a PP stopper). The loaded cells were placed horizontally in the

centrifuge. This system measures near infra red (NIR) transmission profiles continuously during centrifugation resulting in 256 measurements. LUMiFuge software calculated the integral of every transmission curve over a chosen length (the sample length). A graphical representation of transmission as a function of position presents the transmission profile (Kanagaratnam et al, 2013). From data obtained, the software also calculates the instability index of the samples.

6.5.4.2 pH

The pH value was determined using HANNA Instruments pH 211, Microprocessor pH Meter.

6.5.4.3 Viscosity

A rheometer, MCR 300, PAAR PHYSICA was used to determine the viscosity of prepared emulsions.

6.5.4.4 Particle Size

Particle size analysis of prepared emulsions was carried out at the 1st and 24th months by a laser diffraction particle analyser, the Malvern Hydro 2000S (Worcestershire, England). The particle size of the emulsions was described by the cumulants mean diameter according to Stanley-Wood and Lines, 1992 (as cited in Loo *et al.*, 2014).

6.5.4.5 *In vitro* Dermal Irritection Assay of Macroemulsions

The dermal irritection assay is quantitative *in vitro* test methods that mimic acute dermal irritation test. To perform the dermal irritection standardized assay, the test sample is applied to a synthetic biobarrier that is coated with a dye-containing keratin-collagen matrix. Following application, the sample is absorbed by and permeates

through this synthetic biobarrier to gradually come into contact with a proprietary solution containing highly ordered globulins and glycoproteins. Reaction of the test sample with these proteins and macromolecular complexes promotes conformational changes that may be readily detected as an increase in the turbidity of the protein solution. With the dermal irritection test, turbidity as well as the dye that has been dissociated from the biobarrier during transit of the applied sample may be detected at a wavelength of 450 nm.

The dermal irritancy potential of a test sample is expressed as a Human Irritancy Equivalent (HIE). These scores are defined by comparing the increase in optical density (OD_{450}) produced by the test material to a standard curve that is constructed by measuring the increase in OD produced by a set of calibration substances. These calibrators have been selected for use in these tests because their irritancy potential has been previously documented in a series of *in vivo* investigations.

All data are calculated and analyzed *via* a computer program, which determines assay result acceptance based upon qualification parameters defined in the program. In general, the program has been designed to accept sample data as qualified if the following criteria are met: the OD values of Calibrators and internal Quality Control samples fall within specified ranges.

6.5.4.6 *In vitro* Ocular Irritection Assay of Macroemulsions

The ocular irritection assay is quantitative in vitro test methods that mimic acute ocular irritation test. To perform the ocular irritection standardized assay, the test sample is applied to a synthetic biobarrier composed of a semi-permeable membrane. Following application, the sample is absorbed by and permeates through this synthetic biobarrier

to gradually come into contact with a proprietary solution containing highly ordered globulins and glycoproteins. Reaction of the test sample with these proteins and macromolecular complexes promotes conformational changes that may be readily detected as an increase in the turbidity of the protein solution. With the Ocular Irritection test, turbidity may be detected at a wavelength of 405 nm.

The ocular irritancy potential of a test sample is expressed as an Irritection Draize Equivalent (IDE). These scores are defined by comparing the increase in optical density (OD_{405}) produced by the test material to a standard curve that is constructed by measuring the increase in OD produced by a set of calibration substances. These calibrators have been selected for use in these tests because their irritancy potential has been previously documented in a series of *in vivo* investigations.

All data are calculated and analyzed *via* a computer program, which determines assay result acceptance based upon qualification parameters defined in the program. In general, the program has been designed to accept sample data as qualified if the following criteria are met: the OD values of Calibrators and internal Quality Control samples fall within specified ranges.

6.5.4.7 Acute Moisturizing Test of Macroemulsions

A study on acute moisturizing effect of prepared emulsions was conducted on 20 subjects. For the study, the skin hydration was measured before and after product application. The product was applied on pre-marked test areas while the control was untreated skin. The measurement was taken before and at 30, 60, 90, 120 and 180 minutes after product application. All measurements were carried out by using the Corneometer CM 825 (Courage and Khazaka, Germany), which measures the skin

moisture. The measuring principle of the instrument is based on the capacitance measurement of a dielectric medium. The dielectric constant of the skin changes with the water content. The changes in water content of the stratum corneum are converted to arbitrary units of hydration (Berardesca *et al.*, 1997). The data collected were analyzed and statistically compared between treated and controlled area (ANOVA and t-test). Differences in any groups of data were considered significant for a probability to value ≤ 0.05 (i.e. at 95 % confident level).

6.5.5 *mono-tert*-butoxypropanediol (Compound 2.7) in Microemulsion

The basic formula for microemulsion system was applied where the system contains water, oil, surfactant and co-surfactant. The ratio of oil to water was fixed to 25:75 by weight percent in which the system is for oil in water microemulsion. The amount of surfactant which is Tween 80 was fixed to 20 % by weight and the co-surfactant which is glycerol derivatives in this case varied from 0 % to 60 % by weight. Conventional co-surfactant namely 1-pentanol was used for comparison. Two types of oils namely palm oil methyl ester C_{12}-C_{18} (POME C_{12}-C_{18}) and medium chain triglycerides (MCT) were used in this study. Table 6.5 shows the details of the formula (total amount = 8 g) with the products code. GT(POME)X and P(POME)X refers to product containing glyceryl ether and 1-pentanol respectively, using POME C_{12}-C_{18} as the oil. GT(MCT)X and P(MCT)X refers to product containing glyceryl ether and 1-pentanol respectively, using MCT as the oil.

Table 6.5 : Formulations of microemulsion

Product code	Surfactant (%)	Oil + Water (25:75) (%)	Co-S (%)
GT(POME)5 P(POME)5 GT(MCT)5 P(MCT)5	20	75	5
GT(POME)10 P(POME)10 GT(MCT)10 P(MCT)10	20	70	10
GT(POME)15 P(POME)15 GT(MCT)15 P(MCT)15	20	65	15
GT(POME)20 P(POME)20 GT(MCT)20 P(MCT)20	20	60	20
GT(POME)25 P(POME)25 GT(MCT)25 P(MCT)25	20	55	25
GT(POME)30 P(POME)30 GT(MCT)30 P(MCT)30	20	50	30
GT(POME)40 P(POME)40 GT(MCT)40 P(MCT)40	20	40	40
GT(POME)50 P(POME)50 GT(MCT)50 P(MCT)50	20	30	50

Table 6.5 continue

Product code	Surfactant (%)	Oil + Water (25:75) (%)	Co-S (%)
GT(POME)60 P(POME)60 GT(MCT)60 P(MCT)60	20	20	60

6.5.5.1 Stability Study of Microemulsion

The stability study was performed at room temperature for one or two days and continued at 45 ºC for a month. The observation was done using polarized light by recording whether the microemulsion is stable (no separation) or not stable (separate into two layers or multilayers).

6.5.6 *mono-tert*-butoxypropanediol (Compound 2.7) in Transparent Soap

The incorporation of glyceryl ethers produced in transparent soap was done based on the formula in Table 6.6. Transparent soap containing glycerol was also prepared for comparison.

Table 6.6 : Ingredients for transparent soap bar

Ingredients (%)	Soap containing glycerol	Soap containing glyceryl ether
Stearic Acid	8	8
Myristic Acid	8	8
EDTA	0.1	0.1
TEA	11	11
NaOH	4	4
Sucrose	15	15
Texapon 70 (SLES)	5	5
Dehyton K (betaine)	3	3
Deionized water	35	35
Glycerol	10	-
Glyceryl ether	-	10
pH adjuster	1-5	1-5

Fatty acids were heated to 70-80 °C in a beaker and sodium hydroxide was added into the melted fatty acids to form soap. In another beaker, the rest of other ingredients excluding pH adjuster were mixed and heated to 75-85 °C. Then the soap was added into the mixture and stirred until clear solution. Finally, the pH of the transparent soap solution was adjusted to 9-10 before pouring into the mould and left to cool. The transparent soap was analyzed for hardness, transparency value, pH value and foaming power.

6.5.6.1 Hardness of Transparent Soap Bar

The hardness of the soap bar was measured using TA.XT.plus Texture Analyzer.

6.5.6.2 Transparency Value of Transparent Soap Bar

Transparency value was determined using a Transparency Meter FTTM002. The data collected were analyzed and statistically compared between samples (t-test). Differences were considered significant for a probability to value ≤ 0.05 (i.e. at 95 % confident level).

6.5.6.3 pH Value of Transparent Soap Bar

pH measurement was done based on 1 % solution of transparent soap in deionized water.

6.5.6.4 Foaming Test

Foaming power and stability test was done using in-house method at different water hardness. 1 % solution of soap in water was prepared. 500 mL measuring cylinder was used. Foam height (mL) was recorded immediately after stroke (initial) and after 5 mins.

CHAPTER 7: CONCLUSION

The main objective of this study was to produce glyceryl ether and other related compounds that could be used as an intermediate in producing glyceryl ether. It was also an object of this study to evaluate the antibacterial activity and dermal irritation potential of the compounds produced, and to conduct a study on the applications of glyceryl ether in macroemulsion, microemulsion and transparent soap making.

One-pot etherification reaction of glycerol with *tert*-butanol in the presence of acidic catalyst was successfully conducted. The effect of the following variables on the glycerol conversion was investigated: i) type and amount of catalyst, ii) reaction temperature, iii) reaction period and iv) mole ratio of reactants. The type and amount of catalysts used affect the conversion of glycerol. In addition, reaction temperature, mole ratio of reactants and reaction period were also important factors that influence the percentage of glycerol conversion. The highest glycerol conversion achieved was 80 % when the reaction was carried out at 80-85 °C for 5 hours with 1:4 mole ratio of glycerol to *tert*-butanol in the presence of Amberlite IR-120 as catalyst.

The product obtained is a liquid and water-soluble which consists of compound **2.7** as the major component. The formation of compound **2.8** was also detected in the reaction product. The *in vitro* dermal irritection assay showed that the product is classified as non-irritant as a mixture or single component. From the ecotoxicity test, the product was found to be non-toxic.

The scale up process for the production of compound **2.7** was done up to 5 L reactor capacity in order to provide sufficient amount of product for its application evaluations.

The glycerol conversion was in the range of 50 % to 58 % based on gas chromatography analysis. This conversion was slightly low compared to small scale reaction conducted at similar reaction parameters where the glycerol conversion was about 75-80 %. Glyceryl ether consists of more than 60 % compound **2.7** with less than 5 % glycerol is required for its application as a co-surfactant in microemulsion system and this product composition has been used as a basis to investigate its potential in other applications. Therefore, several separation methods were applied namely simple vacuum distillation, fractional vacuum distillation, liquid-liquid extraction and short path distillation in order to obtain glyceryl ether with more than 60 % compound **2.7** and less than 5 % glycerol. All separation methods successfully yielded the desired product composition.

Besides one-pot synthesis, two-step reaction for producing glyceryl ether was also conducted. The first step was the formation of intermediates namely compound **3.1** and **3.2**. Then the ring opening of compound **3.2** was done in basic and acidic media. In a basic condition, compounds **2.1a**, **2.2a** and **2.7a** were successfully produced, agreeable theoretically. In contrast, compound **2.1a** was detected as the major product instead of compound **2.1b** in acidic medium. This shows that in acidic medium, there is a possibility of having two isomers. Therefore, compounds **2.1a** can also be produced through the ring opening of compound **3.2** in acidic condition besides in basic medium. Other compounds (compounds **2.2a-2.7a)** show a similar pattern where the formation of both isomers was detected in acidic condition.

In addition, attempts to protect and tosylate the hydroxyl group in glycerol molecule were made. The purpose was to produce other glycerol derivatives that could be used as an intermediate in producing glyceryl ether. Firstly, the tosylation of glycerol was done

without any protection of the hydroxyl groups of glycerol. However, the product obtained was a mixture of compounds **4.1**-**4.3** eventhough the reaction was done at 1:1 mole ratio of glycerol to *p*-TsCl. The reaction was not selective to the target compound **4.1**. After purification process, these compounds **4.1**-**4.3** were successfully isolated and characterized. They were also tested for their antibacterial properties. All three compounds exhibited antibacterial activity against *Staphylococcus aureus* (gram positive bacteria) and *Pseudomonas aerugiuosa* (gram negative bacteria) compared to control.

Secondly, two of the hydroxyl groups of glycerol were protected by transforming glycerol into compound **4.4** before the tosylation process. Then, compound **4.4** was converted to **4.5** selectively and GC method was developed in order to ease the determination of the product in reaction medium. Compounds **4.1**, **4.2**, **4.3** and **4.5** could be used as intermediate in S_N2 nucleophilic substitution reaction for the preparation of glyceryl ether, as tosylates are known as a good leaving group.

All synthesized compounds (compounds **2.1**-**2.7**) were evaluated for antibacterial and *in vitro* irritation potential besides the ecotoxicity test of compound **2.7**. Furthermore, potential applications of glyceryl ether obtained from scaling up process (compound **2.7**) in macroemulsions, microemulsions and soap making were successfully investigated. Macroemulsions with glyceryl ether (compound **2.7**) showed lower viscosity values than macroemulsions with glycerol. Furthermore, the macroemulsions also exhibited moisturizing property compared to control macroemulsion. Glyceryl ether could be an alternative for glycerol when one wants to formulate a product with lower viscosity yet moisturize the skin.

Recommendations for further study are as follow;-

1) Scaling up process for the production of compound **2.7** (*mono-tert*-butoxypropanediol) may be done with improved product yield if one can design a suitable reactor for that particular reaction. A suitable reactor design is very important especially for pre-commercialization and commercialization purposes with a good balance in cost.
2) Based on the findings on potential applications of glyceryl ether in macroemulsions, microemulsions and transparent soap making, more study on the application of glycerol derivatives should be conducted in order to diversify the usage of glycerol and its derivatives.

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