Production and Purification of Fatty Acid Methyl Esters from Plant Oils of Different Origin

BY

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DECLARATION

"I certify that this work has not been accepted in substance for any degree, and is not concurrently being submitted for any degree other than that of 'Doctor of Philosophy' being studied at the University of Greenwich. I also declare that this work is the result of my own investigations except where otherwise identified by references and that I have not plagiarised the work of others."

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ABSTRACT

Production and Purification of Fatty Acid Methyl Esters from Plant Oils of Different Origin

Biodiesel production from plant oils has been studied since *ca.* 1900 and is now being widely adopted as a means to reduce carbon emissions in transport applications. Its properties are similar to those of fossil diesel fuel, thus allowing its use in diesel engines both pure and in mixtures with fossil diesel fuel. Biodiesel is obtained from vegetable oils or animal fats by a transesterification reaction whereby the triglycerides, contained in the oil or fat, react with a short chain alcohol in the presence of a catalyst. The products of the reaction are fatty acid methyl esters (biodiesel) and glycerol, obtained as two separate phases. However, current reaction efficiency using a homogeneous catalyst, such as sodium hydroxide, has various drawbacks. These include non-specificity leading to saponification, difficulty in isolating the catalyst from the fatty acid methyl esters, immiscibility of the catalyst with the reactants and incomplete transesterification.

The aim of the research reported in this thesis is to provide a full description of the transesterification reaction. According to previously published methodologies, the transesterification of glycerol trioleate with sodium hydroxide should provide 97-98 % (w/w) conversion to the ester. However, the results reported, herein, indicated only 95.2% (w/w) ester content. To understand the differences in the results, the concentrations of sodium hydroxide and methanol were varied by using unrefined rapeseed oil. Results showed that the optimum reaction conditions to produce higher ester content (93.3% w/w) from unrefined rapeseed oil *i.e.* molar ratio of methanol/oil was 6:1 and 0.015 mol of sodium hydroxide at 60 min. The same results were obtained with various plants oils of different origin under similar reaction conditions, but no increase in the ester content was observed. The data suggested that the results were in accord with biodiesel specifications *i.e.* EN ISO 12937 and EN 14104. However, EN 14103 standard could not be met, perhaps due to the reversible nature of the reaction,

higher acid value in the oil and other competing reactions of the triglycerides. It was not possible to achieve the ester content (%) according to EN 14103 standard if any moisture or free fatty acid was present in the oil or during the reaction.

The failure to meet the required EN 14103 standards by using homogeneous catalytic systems paved the way for kinetics studies of the transesterification reaction. Heterogeneous catalysts offered the opportunity to study the reaction kinetics of the system because they can be separated rapidly from the reaction mixture by centrifugation. Various heterogeneous metal oxide catalysts were investigated. Strontium oxide was confirmed to be an effective catalyst but, contrary to expectations, similar catalytic activity was not observed for the other metal oxides. The experimental results obtained, by optimising reaction conditions using a heterogeneous catalyst were found to be 3% (w/w) SrO, 6:1 CH_3OH/oil molar ratio at 120 min. Therefore, subsequent reactions were planned to carry out real-time kinetic studies using SrO as a catalyst. The application of refractometry allowed real-time kinetic studies of the transesterification reaction. The ester content obtained after transesterification were determined by gas chromatography and validated the results obtained by the refractometer method. This analytical method helped to improve the reaction conditions from 120 min to 90 min using 3% (w/w) SrO and 6:1 CH_3OH/oil molar ratio to achieve 92% (w/w) ester content.

During kinetic studies, using a heterogeneous catalyst, solubility issues were observed between oil, methanol and fatty acid methyl esters (FAMEs). Therefore, a phase solubility diagram was plotted to identify the miscible region. The transesterification reaction was conducted in the miscible region of a ternary phase diagram to overcome the phase limitation problems. The ester content obtained was higher than 98% (w/w) within 25-30 min depending on the concentration (% v/v) ratio of the reactants used. These results were encouraging in terms of using a heterogeneous catalyst since its use is limited to lower ester content (%) and longer reaction time.

Samiyah Hamid [B.Sc., M.Sc. (Chemistry), M.Res. MRSC]

DEDICATION

This thesis is dedicated to my beloved parents (Ch. Hamid Mahmood and Mrs. Farida Hamid), and my husband, Mr. M. Mujahid, who are always there for me when I need them.

"If we knew what it was we were doing, it would not be called research, would it?"

Albert Einstein

CONTENTS

LIST OF FIGURES

Figure 5.6. Refractive indices and ester content (%) for experiment **2**. (Expt. **2**: 60 mL FAME: 33 mL oil: 7 mL CH_3OH, 3% (w/w) SrO at 60 °C for 90 min. Arrow represents the addition of 3% (w/w) SrO at 50 min and addition of CH_3OH at 60 min. Dotted line shows the ester content (%) with respect to time..

Figure 5.7. Refractive indices and ester content (%) for experiment **3**. (Expt. **3**: 60 mL FAME: 33 mL oil: 7 mL CH_3OH, 3% (w/w) SrO at 60 °C for 120 min. Arrow represents the addition of 3% (w/w) SrO at 60 min and addition of CH_3OH at 80 min. Dotted line shows the ester content (%) with respect to time.

Figure 5.8. Refractive indices and ester content (%) for experiment **4**. (Expt. **4**: 60 mL FAME: 33 mL oil: 7 mL CH_3OH, 3% (w/w) SrO at 60 °C for 120 min. Arrow represents the addition of CH_3OH at 60 min and addition of 3% (w/w) SrO at 100 min. Dotted line shows the ester content (%) with respect to time.

Figure 5.9. Refractive indices and ester content (%) for experiments **5** and **6**. (Expt. **5**: 100 g oil, 3% (w/w) SrO, methanol/oil molar ratio 6:1 at 60 °C for 120 min. Expt. **6**: 60 mL FAME: 33 mL oil: 7 mL CH_3OH, 3% (w/w) SrO at 60 °C for 120 min). At 60 min, methanol was added in both experiments. In Expts. **5** and **6**, 27.5 mL and 7 mL of methanol were added, respectively. Arrows represent the ester content (%) at that point. The dotted lines represent the ester content (%) noted with respect to time.

Figure 5.10. Refractive indices and ester content (%) of experiments **7** and **8**. (Expt. **7**: 100 g oil, 3% (w/w) SrO, methanol/oil molar ratio 6:1 at 60 °C for 120 min. Expt. **8**: 60 mL FAME: 33 mL oil: 7 mL CH_3OH, 3% (w/w) SrO at 60 °C for 120 min). Arrows represent the ester content (%) at that point. The dotted lines represent the ester content (%) noted with respect to time..

Figure 5.11. Refractive indices and ester content (%) for experiment **9**. (Expt. **9**: 70 mL FAME: 20 mL oil: 10 mL CH_3OH, 3% (w/w) SrO with respect to triglycerides at 60 °C for 105 min. FAME purity: 94.0% (w/w) determined

experiments, where the corresponding standard deviation was lower than 0.05 and therefore the variability among the readings was insignificant.

Figure 5.19. Refractive indices and ester content (%) for experiment **13**. (Expt.**13**: 70 mL FAME: 10 mL oil: 20 mL CH_3OH, 3% (w/w) SrO with respect to triglycerides at 60 °C for 105 min. FAME purity: 94.0% (w/w), determined by GC). Values are the mean of three replicates, error bars indicate standard deviations.

Figure 5.20. Results for the glycerol (free and total) and glycerides (mono-, di-, and tri-glycerides) in experiment **13**. The data points represents mean of three experiments, where the corresponding standard deviation was lower than 0.05 and therefore the variability among the readings was insignificant.

Figure 5.21. Refractive indices and ester content (%) for experiment **14**. (Expt. **14**: 60 mL FAME: 10 mL oil: 30 mL CH_3OH, 3% (w/w) SrO with respect to triglycerides at 60 °C for 90 min. FAME purity: 94.0% (w/w), determined by GC). Values are the mean of three replicates, error bars indicate standard deviations.

Figure 5.22. Results for the glycerol (free and total) and glycerides (mono-, di-, and tri-glycerides) in experiment **14**. The data points represents mean of three experiments, where the corresponding standard deviation was lower than 0.05 and therefore the variability among the readings was insignificant.

Figure 5.23. Refractive indices for various metal oxide catalysts. (reaction conditions: 70 mL FAME: 20 mL oil: 10 mL CH_3OH, 3% (w/w) SrO with respect to triglycerides at 60 °C for 105 min. FAME purity: 94.0% (w/w), determined by GC).

Figure 5.24. Comparison of refractive indices and ester content (%) for the transesterification reaction in the presence and absence of glucosinolate. (Glucosinolate experiment: 70 mL FAME: 20 mL oil: 10 mL CH_3OH, 3% (w/w) SrO with respect to triglycerides and 0.10 % glucosinolate at 60 °C for 105 min. FAME purity: 94.0% (w/w). Control experiment: similar conditions as for glucosinolate addition experiment except for the absence

of glucosinolate addition). Values are the mean of three replicates, error bars indicate standard deviations.

Figure 5.25. Results for the glycerol (free and total) and glycerides (MAG, DAG and TAG) in control and glucosinolate added experiments. **A:** % free glycerol content, **B:** % monoglyceride (MAG) content, **C:** % diglyceride (DAG) content, **D:** % triglyceride (TAG) content and **E:** % total glycerol.

LIST OF SCHEMES

LIST OF TABLES

ABBREVIATIONS

Symbol	*Description*
APCI	*-atmospheric pressure chemical ionisation*
ASPO	*-Association for the Study of Peak Oil and Gas*
ASTM	*-American Society for Testing and Materials*
BuOH	*-butanol*
Ca	*-calcium*
CFCs	*-chlorofluorocarbons*
CO_2	*-carbon dioxide*
CO	*-carbon monoxide*
CH_3OK	*-potassium methoxide*
CH_3ONa	*-sodium methoxide*
DAG	*-di alkyl glyceride*
DD	*-density detection*
EN	*-European Standard*
EtOH	*-ethanol*
ETS-10	*-microporous titanosilicate*
ELSD	*-evaporative light scattering detector*
eq.	*-equivalent*
EU	*-European Union*
FAMEs	*-fatty acid methyl esters*
FAO	*-Food and Agricultural Organisation*
FFA	*-free fatty acid*
FID	*-flame ionization detector*
GHG	*-greenhouse gases*
g/mol	*-gram per mole*
HC	*-unburned hydrocarbons*
HPA	*-heteropolyacids*
HPLC	*-high performance liquid chromatography*
H_2SO_4	*-sulphuric acid*
K	*-potassium*
K_2CO_3	*-potassium carbonate*
KOH	*-potassium hydroxide*

MAG	*-mono alkyl glyceride*
MeOH	*-methanol*
MEs	*-methyl esters*
Mg	*-magnesium*
MgO	*-magnesium oxide*
mol	*-mole*
MPa	*-megapascal*
MS	*-mass spectrometry*
Na	*-sodium*
NIR	*-near infrared*
Na_2CO_3	*-sodium carbonate*
NMR	*-nuclear magnetic resonance*
NaOH	*-sodium hydroxide*
NOx	*-nitrogen oxide*
ODAC	*-oil depletion analysis centre*
P	*-phosphorus*
PBR	*-packed bed reactor*
PM	*-particulate matter*
ppm	*-part per million*
PrOH	*-propanol*
RED	*-renewable energy directive*
RME	*-rapeseed oil methyl ester*
rpm	*-revolutions per minute*
SCM	*-supercritical methanol*
SOx	*-sulphur oxide*
STO	*-sulphated tin oxide*
SZA	*-sulphated zirconia-alumina*
TAG	*-trialkyl glyceride*
TGs	*-triglycerides*
TLC	*-thin layer chromatography*
UFOs	*-used frying oils*
UV	*-ultraviolet*
VOCs	*-volatile organic compounds*
vol.	*-volume*
WCO	*-waste cooking oil*

wt.	*-weight*
WHO	*-World Health Organisation*
WZA	*-tungstated zirconia-alumina*

OVERVIEW OF THESIS

The body of the thesis is divided into six chapters, which may be summarised individually as follows.

Chapter one consists of an introduction to the subject intended to provide a historical background and an overview of modern perspectives on current and future developments in the field, as well as providing evidence of potential problems linked to this research area. The initial section is about general background on the production of biodiesel to date. The later section of this chapter is a review of literature examining past work on the uses of different type of catalysis for biodiesel production and the methods for the quantification of fatty acid methyl esters (FAMEs).

Chapter two outlines the experimental procedures and analytical techniques used to obtain and analyse the results presented in the main body of the thesis. This Chapter, therefore, covers the BS EN 14214 standard used for testing the biodiesel.

Chapter three details the results and analyses of biodiesel (FAMEs) produced by using homogeneous catalytic systems. It reports the results obtained by the transesterification of pure glycerol trioleate, optimisation of reaction conditions using unrefined rapeseed oil, and a survey on the use of refined vegetable oils for biodiesel production.

The fourth chapter is divided into two parts. In chapter 4-A, experiments are focussed on the use of heterogeneous catalysis for the production of biodiesel (FAMEs). By using strontium oxide, the reaction conditions with respect to time are reported. Chapter 4-B details the kinetics of the transesterification reaction studied by using two different analytical techniques. The both techniques are compared and contrasted.

The fifth chapter presents the ternary phase diagram that has been plotted on the basis of solubility data obtained from rapeseed oil, FAME and methanol. The transesterification reaction using heterogeneous catalysis carried out in the miscible region of a phase diagram in order to remove the phase limitation problems. The refractive index and GC measurement were carried out in parallel to study the transesterification reaction.

Finally, chapter six summarises the research work whilst outlining its main conclusions. It provides brief recommendations for future research relating to the production of biodiesel.

CHAPTER 1

INTRODUCTION

1.1. GENERAL BACKGROUND

The production of biofuels has reached unprecedented volumes over the last 10 years due to two main drivers: a) concern over future oil availability and b) global warming.

The threat of depleting reserves of fossil fuel coupled to increasing demands for diesels and uncertainty in their availability has promoted an exploration of alternative sources of energy.[1, 2] Estimates of the remaining world oil reserves and probability and timing of "Peak Oil" are uncertain. "Peak Oil" is the point in time at which the maximum global petroleum production rate is reached, after which the rate of production enters into terminal decline. It has been estimated that "Peak Oil" will occur at any time between 2007 and 2030.[3-5] Geologists in the Association for the Study of Peak Oil & Gas (ASPO), and analysts at the Oil Depletion Analysis Centre (ODAC), both predicted that Peak Oil would occur around 2010 (Figure 1.1).[6] However, a precise prediction of the peak is extremely difficult because of *e.g.* geological complexities, measurement problems, pricing variation, demand elasticity and political influences.[7] Peaking of conventional oil reserves will happen but the timing is uncertain. Oil and gas could be available from conventional or unconventional (or non-conventional) sources. Oil from conventional sources is typically the highest quality, lightest oil, which flows from underground reservoirs with comparative ease. Oil from unconventional sources is heavy and often tar-like such as heavy crude oil, oil sands, and oil shale. These sources are currently not included as a part of the oil reserves.

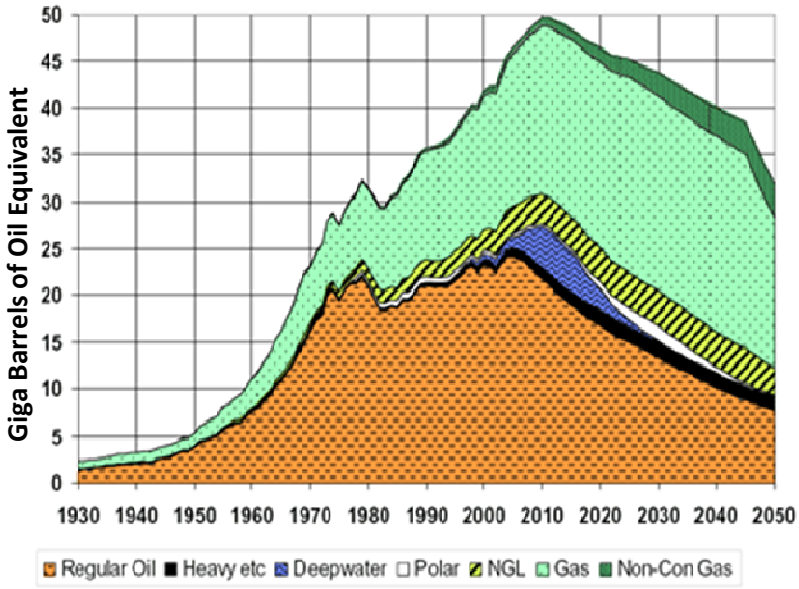


Figure 1.1. Worldwide oil production peaking at 2010.[6]

Large quantities of oil are available from non-conventional sources, but there are limitations associated with its production:

a) oil extracted from these sources typically contains contaminants such as sulphur and heavy metals and in some cases, can leave tailings - ponds containing hydrocarbon sludge.[8, 9] Due to its low quality, refinement to clean transportation fuel are more expensive than for conventional oils.

b) unconventional oils require significant amounts of energy and labour in terms of recovery and transportation compared to conventional oils, increasing production costs.

Therefore, relying on non-conventional sources of oil to meet global demand does not seem like a reasonable proposition, and hence, the interest in the potential of biofuels.

Global warming is a major concern and centres on greenhouse gases (GHG) emitted from the combustion of fuels.[10] The industrial revolution has increased the amount of GHG in the atmosphere, leading to increased change in net irradiance at

atmospheric boundaries from CO_2, methane, tropospheric ozone, CFCs and nitrous oxide. Fossil fuel burning has produced about three-quarters of the increase in CO_2 from human activity over the past 20 years. Many scientific studies reveal that overall CO_2 levels have increased 31% in the past 200 years.[11] In order to control the emissions of GHG the Kyoto Protocol was negotiated in Kyoto City, Japan in 1997 and came to effect in February 2005. In April 2010, 191 states globally signed and ratified the Kyoto Protocol. Under the protocol, 37 countries committed themselves to a reduction of four GHG (carbon dioxide, methane, nitrous oxide, sulphur hexafluoride) and two groups (hydrofluorocarbons and perfluorocarbons) of gases produced by them.[12] Countries from Europe, Canada and Japan agreed to reduce their collective GHG emissions by 5.2% from the 1990 levels.[10, 13]

Renewable energy technologies provide an excellent opportunity for mitigation of GHG emission and reducing global warming.[11] Many alternative energy sources, such as wind, solar, geothermal and biomass, fulfil the criterion of sustainability. However, few of these can fulfil the criterion of economic feasibility. Arguably, a better option, fulfilling both criteria, is that based on biofuels particularly those made from readily available biomass feedstock.[14] The combustion of biofuels releases CO_2 during burning, but this can be recycled *via* the next crop production; therefore there is no additional burden on the environment. Biomass refers to all the vegetable matter that can be obtained from photosynthesis. The great versatility of biomass as a feedstock is evident from the range of materials that can be converted into various solid, liquid and gaseous fuels using biological and thermo-chemical conversion processes. Some of the well known liquid biofuels are ethanol for gasoline engines and biodiesel for compression ignition engines or diesel engines.[15, 16]

To meet the goal of the Kyoto protocol, the European Union (EU) has agreed to attain, by 2012, a minimum penetration of 12% of renewable energy sources, with biomass contributing 7% of total consumption. This includes the use of solid, liquid or gaseous forms of biomass.[17] According to the Renewable Energy Directive (RED), the

objective is to provide 20% of the energy (electricity, heating and cooling) in the EU from renewable sources by 2020. The RED also requires all member states to ensure that 10% of total road transport fuel is obtained from renewable sources. The vast majority of this is expected from biofuels.[18] However, if biofuels are required, they should meet certain sustainability criteria. These criteria will ensure that biofuels are not produced from areas of high carbon stock or high biodiversity.

1.1.1. History of Vegetable Oils as Fuels

The concept of using biofuels originated in 1911 with the demonstration of the first diesel engine by its inventor, Rudolph Diesel.[19] He envisioned biodiesel as the only source of fuel required and far better than fossil fuels (coal, petroleum). However, a technical problem prevented the direct use of vegetable oils as an alternative to diesel fuel at that time, namely, the formation of residues, which reduced the power of the engines.[20] Experiments with vegetable oils continued from 1920 to 1940 in European nations that had tropical colonies that could produce and export vegetable oils. Vegetable oils were used as emergency fuels in South American and Asian countries, during World War II, but their use was interrupted after the war, as cheap petroleum based fuels were available again.[21]

Research on vegetable oils used for diesel engines started again after the 1970s energy crisis and was mainly conducted in Austria, South Africa and the United States. Different types of oils such as palm, soybean, sunflower, peanut and olive oils were used for diesel engines at that time. However, vegetable oils have different fuel properties when compared to diesel fuel. They have a higher density, viscosity and flash point, and lower cetane number and heating values. The direct use of vegetable oils presented engine problems after long-term use due to the oil characteristics and cold weather conditions. They caused fuel injection problems, poor fuel atomisation, incomplete combustion, deposit formation, and carbonisation of injector tips, ring sticking, lubricating oil dilution and degradation.[22, 23]

1.1.2. Biodiesel as an Alternative Fuel

Biodiesel is a fuel composed of mono-alkyl esters derived from vegetable oils or animal fats. It is usually produced by the reaction of oil or fats with an alcohol in the presence of a catalyst. Using biodiesel can help to reduce the world's dependence on fossil fuels and would have significant benefits. Some of these are technical, others are related to environmental aspects, but among them, the most important are as follows:

a) Vegetable oils are a renewable and potentially inexhaustible source of energy, with energy content close to that of diesel fuel. The lifecycle of GHG emissions of biodiesel fuel are lower than those of petro-diesel fuel, as the plants take in CO_2 while growing. In order to maintain sustainability, the plants must be fertilised and harvested, the oil pressed and biodiesel produced by reaction with methanol. Global vegetable oil production increased from 56 million tons in 1990 to 88 million tons in 2000.[24] A variety of bio-lipids can be used to produce biodiesel, which are detailed in Section 1.1.6.

b) Biodiesel can improve energy security. Expansion of the biodiesel industry creates jobs opportunities and increases the earnings of the populace, especially in local communities. When crops used to produce biodiesel are grown in the country in which the fuel is consumed, each litre of biodiesel displaces a litre of imported crude oil, reducing a country's dependence on foreign/intrinsic oil supplies.[25] Biodiesel is also produced in dedicated refineries that add to the overall domestic refining capacity, eliminating the need to import expensive finished products from other countries.

c) Biodiesel is biodegradable, reducing environmental pollution. It degrades about four times faster than petroleum diesel. The biodegradation process is faster due to the presence of oxygen content in the biofuel.[26] Makareviciene *et al*. determined that 98% of pure rapeseed oil methyl ester (RME) and 60% of pure fossil diesel fuel was biologically decomposed during a 21-day period. This means that RME fully meets the

main requirements of international standards for biological degradation (in the case of biofuels, more than 90% is degraded within 21 days).[27] Pasqualino *et al*.[28] reported that more than 98% of pure biodiesel is degraded after 28 days in comparison to 50% and 56% for diesel fuel and gasoline, respectively. The time for 50% biodegradation of diesel fuel was reduced from 28 to 22 days and from 28 to 16 days by adding 5% or 20% biodiesel, respectively in a mixture at room temperature. Therefore, the biodegradability of the diesel was reported to increase with the addition of biodiesel.

d) Use of biodiesel instead of conventional diesel fuel would significantly reduce exhaust emissions such as the overall life cycle of carbon dioxide (CO_2), particulate matter (PM), carbon monoxide (CO), sulphur oxides (SO_x), volatile organic compounds (VOCs), and unburned hydrocarbons (HC). Balat *et al*. reported that 100% biodiesel emits lower "tail pipe" exhaust emissions compared to diesel fuel; nearly 50% fewer PM emissions, nearly 50% fewer CO emissions and approximately 68% fewer HC emissions. Furthermore, since biodiesel can be considered a sulphur-free fuel, combustion produces less SO_x emissions than are observed for diesel fuel.[29]

e) Biodiesel is safer to handle than petroleum fuel because of its low volatility. There is always a danger of accidental ignition when the fuel is being stored, transported, or transferred due to the high-energy content of all liquid fuels.[30] The possibility of having an accidental ignition is related, in part, to the temperature at which the fuel will ignite when exposed to a flame or a spark known as the flash point temperature. This is the lowest temperature at which it can vaporise to form an ignitable mixture in air. The lower the flash point of a fuel, the lower the temperature required for the fuel to form a combustible mixture. For example, gasoline has a flash point of -43 °C, which means that gasoline can form a combustible mixture at temperatures as low as -43 °C. On the other hand, biodiesel has a flash point of >130 °C, meaning it can not form a combustible mixture until it is heated well above the boiling point of water. For this reason, it is not a hazardous material and its handling is not subject to

operational safety rules. This is a significant advantage over mineral oil diesel in terms of storage and handling.

f) Biodiesel has good lubricant properties compared to petroleum diesel oil. Recently, with the introduction of low sulphur and ultra-low sulphur diesel fuel, many of the compounds that previously provided lubricating properties to petrodiesel fuel have been removed. Lubrication properties of biodiesel help in improving the amount of wear or scarring that occurs between two metal parts in fuel injectors and fuel pump. Biodiesel reduced long-term engine wear in test diesel engines to less than half of that observed in engines running on current low sulphur diesel fuel. The lubricity of ultra-low sulphur diesel can be dramatically improved by blending biodiesel in amounts as little as 5%, and this can also extend the life of an engine's fuel injection system.[31]

g) Biodiesel can be blended with petroleum diesel, allowing biodiesel to be introduced gradually to build up the industry. Blends of biodiesel and petroleum diesel are recognised with the symbol B-XX.[32] The XX is replaced by the volume percentage of biodiesel in a blend. An example of a common blend is 20 percent biodiesel with 80 percent petroleum, which would be labelled as B-20. Pure or neat biodiesel is often referred to as B-100.

However, there are still some limitations of using biodiesel that have to be solved and they are as follows:

a) The oxidation and polymerisation of biodiesel fuel during combustion and storage. Oxidation and polymerisation reactions are triggered due to the presence of unsaturated fatty acid chains and the double bond in the parent molecule (carbonyl group of triglyceride), which immediately reacts with oxygen when exposed to air. The greater the level of unsaturation, the more susceptible the oil becomes to oxidation.[33] Vegetable oil with a high percentage of monounsaturated fatty acids will typically oxidise only at high temperatures, whereas those oils with a higher amount of

polyunsaturates (*e.g.* linoleic and linolenic acid) readily autoxidise at room temperature.[23]

b) Poor low-temperature flow properties (high cloud point). The cloud point is the temperature at which a liquid fatty material becomes cloudy due to the formation of crystals and solidification of saturates. Saturated fatty acids have higher melting points than unsaturated fatty acids and therefore saturated fatty acids crystallise at higher temperature than the unsaturated fatty acids. Methyl and ethyl esters will crystallise and separate from diesel during winter time operations. This can cause problems with fuel lines and filters associated with pumping of fuel and engine operations. One way to get rid of this problem is to remove high melting saturated esters by inducing crystallisation at low temperature, a process known as "winterization". This process depresses the cloud point of esters by equilibrating them at temperatures below the cloud point, over an extended period of time, then filtering away the solids.[22]

c) A slight increase in NO_x emission when using biodiesel and/or its blends. The increase in NO_x emission is due to the presence of unconverted triglycerides present in biofuel mixtures that may be associated with an increased oxygen content in biofuels.[34] Invariably, all biodiesels have some level of oxygen bonded to it chemical structures. Being an oxygenated fuel, the unconverted methyl esters also supply oxygen to air inducted into the combustion chamber and this may aid the formation of NO_x. Schonborn *et al*. have shown that NO_x emission increases with a decrease in carbon chain length and increases with unsaturation.[35] A change in any of these properties may change the NO_x emission.

1.1.3. Development of the Biodiesel Industry

The biodiesel industry has experienced a dramatic growth in many parts of the world. More than 25 countries in Europe are now using pure biodiesel or mixtures with

diesel. In 2010, the main biodiesel producers in Europe were Germany, Spain, France, Italy and the Netherlands (Table 1.1).

Table 1.1. Biodiesel production in Europe from 2005 to 2010.[36]

Biodiesel Production (in thousands of tons)						
COUNTRY	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
Germany	169	2662	2890	2819	2539	4933
Spain	73	99	168	207	859	4100
France	492	743	872	1815	1959	2505
Italy	396	447	363	595	737	2375
The	0	18	85	101	323	1036
Portugal	1	91	175	268	250	468
Belgium	1	25	166	277	416	670
UK	51	192	150	192	137	609
Greece	3	42	100	107	77	662
Austria	85	123	267	213	310	560
Denmark	71	80	85	231	233	250
Poland	100	116	80	275	332	710
Sweden	1	13	63	231	233	212
Czech Rep	133	107	61	104	164	427
Slovakia	78	82	46	146	101	156
Finland	0	0	39	85	220	340
Romania	0	10	36	65	29	307
Lithuania	7	10	26	66	98	147
Slovenia	8	11	11	9	9	105
Bulgaria	0	4	9	11	25	425
Latvia	5	7	9	30	44	156
Hungary	0	0	7	105	133	158
Ireland	0	4	3	24	17	76
Cyprus	1	1	1	9	9	20
Malta	2	2	1	1	1	5
Estonia	7	1	0	0	24	135
Total	**3,184**	**4,890**	**5,713**	**7,755**	**9,046**	**21,904**

1.1.4. Fatty Acids and Oils

The feedstock for biodiesel is composed of varying types of lipids. The latter are a class of hydrocarbon compounds that are water-insoluble, yet soluble in non-polar organic solvents. Typically, fats are produced by animals and oils by plants, but both are mainly made of triglyceride molecules. Triglyceride is composed of one to three fatty acids attached to a glycerol backbone by ester linkages (Figure 1.2). Other glyceride

species, such as diglycerides and monoglycerides, are obtained from triglycerides (TGs) by the substitution of one or two acid moieties, respectively, with hydroxyl groups (Figure 1.2).

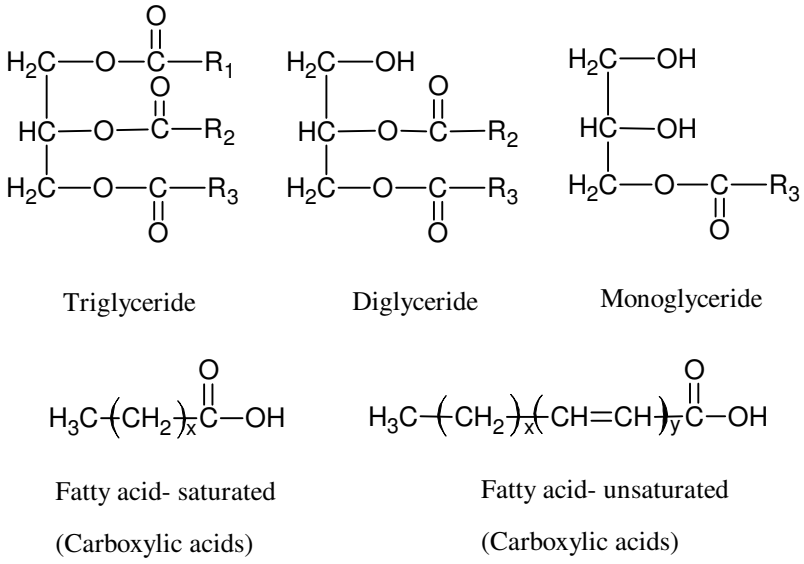


Figure 1.2. Chemical structures of vegetable oils and animal fats (R_1, R_2, R_3 = alkyl groups, where x= 4-24 and y= 1-3).

Apart from triglycerides, vegetable oils contain free fatty acids (generally 1–5% w/w), phospholipids (2.1-2.8% w/w), phosphatides (0.0012-0.022% w/w), carotenes (0.013-0.025% w/w), tocopherols (0.27-0.77% w/w), and traces of sulphur and water.[37] Fatty acids are the primary component of all biodiesel feedstock. They are long-chain mono-carboxylic acids with the general structure of $CH_3(CH_2)_XCOOH$ (Figure 1.2). The number of carbon atoms in the chain is usually between 12 and 24. Individual fatty acids are typically associated by the symbol C followed by YY:X where the YY stands for the number of carbon atoms and X is the number of double bonds. An example of this notation is the unsaturated fatty acid oleic acid denoted by C18:1 *i.e.* there are 18 carbons with one double bond. The positions of any double bonds are specified by superscript numbers following Δ (delta); a 18-carbon fatty acid with one double bond between C-9 and C-10 (C-1 being the carboxyl carbon) and another bond between C-12 and C-13 is designated as 18:2 ($\Delta^{9, 12}$). The data in Table 1.2 summarises the chemical structure of common fatty acid found in vegetable oils.[38-40] The various vegetable oils and esters are distinguished by their fatty acid compositions. The fatty

acids which are commonly found in vegetable oils are stearic, palmitic, oleic, linoleic and linolenic. Fatty acids differ in relation to the chain length, degree of unsaturation or presence of other chemical functionalities.[19]

Table 1.2. Chemical structure of common fatty acids in vegetable oils.

Name of fatty acids	Chemical name of fatty acids	Structure	Molecular Formula
Lauric	Dodecanoic	12:0	$C_{12}H_{24}O_2$
Myristic	Tetradecanoic	14:0	$C_{14}H_{28}O_2$
Palmitic	Hexadecanoic	16:0	$C_{16}H_{32}O_2$
Stearic	Octadecanoic	18:0	$C_{18}H_{36}O_2$
Oleic	Octadecenoic	18:1	$C_{18}H_{34}O_2$
Linoleic	Octadecadienoic	18:2	$C_{18}H_{32}O_2$
Linolenic	Octadecatrienoic	18:3	$C_{18}H_{30}O_2$
Arachidic	Eicosanoic	20:0	$C_{20}H_{40}O_2$
Gadoleic	Eicosenoic	20:1	$C_{20}H_{38}O_2$
Behenic	Docosanoic	22:0	$C_{22}H_{44}O_2$
Erucic	Docosenoic	22:1	$C_{22}H_{42}O_2$
Lignoceric	Tetracosanoic	24:0	$C_{24}H_{48}O_2$
Nervonic	Tetracosenoic	24:1	$C_{24}H_{46}O_2$

Oils from different sources have different fatty acid compositions (Table 1.3). Saturated acids of molecular weight greater than stearic acid are the major component in a few uncommon seed oils. Arachidic, behenic and lignoceric are minor components of groundnut, rapeseed, and cottonseed oils. Biodiesel properties are strongly influenced by the properties of the individual fatty esters. Esters prepared using long chain fatty acids or saturated fatty acids show a higher cetane number. Cetane numbers are a measure of the ease of ignition and smoothness of combustion. Ignition properties are better with a higher cetane number, which also results in higher combustion efficiency.

Esters prepared with a highly unsaturated fatty acid show a low cetane number and oxidise easily. Generally, cetane number, heat of combustion, melting point and viscosity of neat fatty compounds increase with increasing chain length and decrease with increasing unsaturation.[41] It therefore appears reasonable to enrich the biodiesel with saturated fatty esters, in order to improve the properties of the whole fuel.[42, 43]

Table 1.3. Typical fatty acid compositions of vegetable oils and animal fats.[44, 45]

	Composition by weight (%)							
	Myristic C14:0	**Palmitic C16:0**	**Palmitoleic C16:1**	**Stearic C18:0**	**Oleic C18:1**	**Linoleic C18:2**	**Linolenic C18:3**	**Sat.**
Rapeseed oil		3.5		0.9	64.4	22.3	8.2	4.4
Olive oil		9.2	0.8	3.4	80.4	4.5	0.6	12.6
Sunflower oil		6.1		3.3	16.9	73.7		9.4
Safflower oil		8.6		1.9	11.6	77.9		10.5
Soybean	0.1	10.6		4.8	22.5	52.3	8.2	15.5
Palm oil		35.0		7.0	44.0	14.0		42.0
Corn oil		11.6		1.8	25.1	60.6	0.4	13.6
Cottonseed oil		28.3		0.8	13.2	57.5		29.1
Peanut oil		11.3		2.3	48.2	31.9	0.9	18.8
Poultry fat		22.2	8.4	5.1	42.3	19.3	1.0	35.7
lard	1.7	17.3	1.9	15.6	42.5	9.2	0.4	34.6
Tallow	4.8	28.4		14.8	44.6	2.7		52.0
Yellow grease	2.4	23.2	3.8	13.0	44.3	7.0	0.7	38.6
Brown grease	1.7	22.8	3.1	12.5	42.4	12.1	0.8	37.0

1.1.5. Biodiesel Feedstock

Biodiesel can be produced from oils and fat that is sourced from:

- oleaginous plants: African palm (*Elaeis guineensis*),[46] castor (*Ricinus communis*),[47] soybean (*Glycine max*),[48, 49] cotton seed (*Gossypium hirsutum*),[50-52] rapeseed (*Brassica napus*),[53-55] sunflower (*Helianthus annuus*),[56, 57] physic nut (*Jatropha curcas L*),[58] jojoba (*Simmondsia chinensis*),[59] winter rape (*Brassica rapa*).[60]
- used vegetable oils: from restaurants and hotel industries and households; [61-64]
- animal fats: from slaughterhouses.[1]

The raw materials used in the production of biodiesel have gained increased importance, not only because of their influence on the properties of the resulting biodiesel but also because of their cost.[45, 65, 66] The sources for biodiesel production are chosen according to availability, in each region or country. Biodiesel is mainly manufactured from rapeseed oil in Europe and soybean oil in the United States.[67] The cost of biodiesel has become high in comparison to conventional diesel due to uncertainty (food crisis, climate change) in the availability of raw feedstock and growing demands for biodiesel production. In order to decrease the price of biodiesel, attention has been focussed on:

- the use of non-edible oils such as used frying oils (UFOs) and microalgae oil, which are also renewable.
- blending of biodiesel with diesel fuel.[68]

1.1.6. Rapeseed Oil

Rape, mustard (*Brassica alba, Brassica nigra*) and crambe (*Crambe abyssinica*) seed oils all belong to the same family, the *Brassicaceae*. The oil content usually lies within the range of 40-60% weight of the seed. Rapeseed, which is also termed canola in United States, produces 1,190 to 1,500 litres of oil per hectare giving it the highest yield of any conventional oil seed field crop.[69] After oil extraction, the residual seed is

used as a high-protein animal feed. The fully refined oil is bland, pale yellow and free from waxes, phosphorus and sulphur. Until the early 1970's the rapeseed oil high in erucic acid had been used freely in a number of countries for edible purposes but biological tests on animals highlighted a potential danger to the human heart. Consequently, national legislation and FAO/WHO recommended using low erucic acid rapeseed oil varieties for edible purposes. On the other hand, rapeseed oil high in erucic acid (C22:1) content is more favourable for biodiesel production than rapeseed oil high in linoleic acid (C18:3) because the greater the degree of unsaturation, the greater the susceptibility to oxidation.

Introduction of low erucic acid rape varieties means that the composition of rapeseed oil can vary enormously, as shown in Table 1.4.[70, 71] Low-erucic rapeseed oil (LEAR) contains less saturated acid (~6% w/w) than any other commodity oil. It is typically rich in oleic acid (>60% w/w) and contains linoleic (~22% w/w) and linolenic acids (~10% w/w).

Table 1.4. Fatty acid composition of a range of rapeseed oils.[70, 71]

	LEAR[c]	HEAR[d]	LLAR[e]	HOAR[f]
Saturated acids[a]	6.3	7.1	6.6	7.7
16:0	3.6	4.0	3.9	3.4
18:0	1.5	1.0	1.3	2.5
Monounsaturated[b] ***acids***	62.4	69.7	63.1	79.9
18:1	61.6	14.8	61.4	77.8
20:1	1.4	10.0	1.5	1.6
22:1	0.2	45.1	0.1	0.1
Polyunsaturated acids	31.3	23.2	30.2	12.4
18:2	21.7	14.1	28.1	9.8
18:3	9.6	9.1	2.1	2.6

[a] Also 14:0, 20:0, 22:0, and 24:0 all at low levels.
[b] Also 16:1.
[c] Low-erucic rapeseed oil.
[d] High-erucic rapeseed oil.
[e] Low-linolenic rapeseed oil.
[f] High-oleic rapeseed oil.

1.1.7. Biodiesel Standards

Biodiesel is produced from different vegetable oils, varying in origin and quality. Hence, variation in the physical properties of biodiesel based on its oil source is obvious and needs to be determined. Irrespective of the oil source, the presence of glycerol, monoacylglyceride (MAG), diacylglyceride (DAG), triacylglycerides (TAG) in biodiesel, after the transesterification reaction, can lead to severe operational and environmental problems. Therefore, the quality of biodiesel should meet certain standards in order to ensure better engine performance.[72] The American Society for Testing and Materials Standards (ASTM D6751:2008) and the European Standards (EN 14214:2008) are, currently, the most commonly used biodiesel standards around the globe.[73, 74] The parameters, which are included in the aforementioned standards, can be divided into two groups. The first group specifies properties such as viscosity and density, and the second group deals with the purity and chemical composition of fatty acid alkyl esters. Specifications for biodiesel quality according to ASTM D 6751 and EN 14214 standards published in 2008 are illustrated in Table 1.5.

Table 1.5. Biodiesel specifications according to ASTM D6751 and EN 14214 standards.[75]

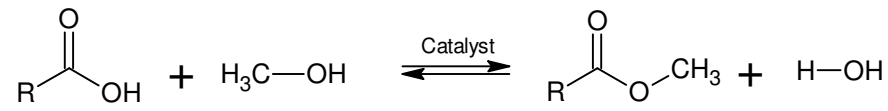
Property	ASTM D 6751		EN 14214	
	Test method	**Limits**	**Test method**	**Limits**
Ester content	-	-	EN 14103	96.5% (mol mol $^{-1}$) min
Linolenic acid content	-	-	EN 14103	12.0% (mol mol $^{-1}$) max
Content of FAME with ≥4 double bonds	-	-	-	1.0% (mol mol $^{-1}$) max
MAG content	-	-	EN 14105	0.80% (mol mol $^{-1}$) max
DAG content	-	-	EN 14105	0.20% (mol mol $^{-1}$) max
TAG content	-	-	EN 14105	0.20% (mol mol $^{-1}$) max
Free glycerol	ASTM D 6584	0.020% (w/w) max	EN 14105	0.020% (mol mol $^{-1}$) max
Total glycerol	ASTM D 6584	0.240% (w/w) max	EN 14105	0.25% (mol mol $^{-1}$) max
Water content	ASTM D 2709	0.050% (v/v) max	EN ISO 12937	500 mg Kg^{-1} max
Methanol content	-	-	EN 14110	0.20% (mol mol $^{-1}$) max
Na, K content	UOP 391	5.0 mg Kg $^{-1}$ max	EN 14108	5.0 mg Kg $^{-1}$ max
Ca, Mg content	-	-	Pr EN 14538	5.0 mg Kg $^{-1}$ max
P content	ASTM D 4951	0.001% (w/w) max	EN 14107	10.0 mg Kg $^{-1}$ max
Oxidative stability	-	-	EN 14112	6 h min
Density	-	-	EN ISO 3675	860-900 Kg m $^{-3}$
Kinematic viscosity	ASTM D 445	1.9-6.0 mm^2 s^{-1}	EN ISO 3104	3.5-5.0 mm^2 s^{-1}
Flash point	ASTM D 93	130° C min	EN ISO 3679	120° C
Cloud point	ASTM D 2500	Not specified	-	-
Sulphur content	ASTM D 5453	0.05% (w/w) max	EN ISO 20684	10.0 mg Kg $^{-1}$ max
Carbon residue	ASTM D 4530	0.050% (w/w) max	EN ISO 10370	0.30% (mol mol $^{-1}$) max
Cetane number	ASTM D 613	47 min	EN ISO 5165	51 min
Sulphated ash	ASTM D 874	0.240% (w/w) max	ISO 3987	0.20% (mol mol $^{-1}$) max
Total contamination	-	-	EN 12662	24 mg kg^{-1} max
Copper strip corrosion	ASTM D 130	No. 3 max	EN ISO 2160	1 (degree of corrosion)
Acid value	ASTM D 664	0.50 mg KOH g^{-1} max	EN 14104	0.50 mg KOH g^{-1} max
Iodine value	-	-	EN 14111	120g I^2 100g^{-1} max
Distillation temperature	ASTM D 1160	360° C max	-	96.5% (mol mol $^{-1}$) min

1.2. PROCESS CHEMISTRY

Plant and animal oils have to be modified in order to be used in a modern diesel engine because the triglycerides in the oils have a molecular weight between 660 and 980 which is higher than diesel fuel (mol. wt. 140-208). To reduce the viscosity of the oil, the ester linkage between the fatty acid and glycerol bonds need to be hydrolysed and replaced. This can be achieved either by transesterification of the triglycerides or by direct esterification of the fatty acids. The transesterification of triglycerides is preferred as they are more readily available than free fatty acids (1-2% w/w) in the oil.[76]

1.2.1. Esterification of Free Fatty Acids

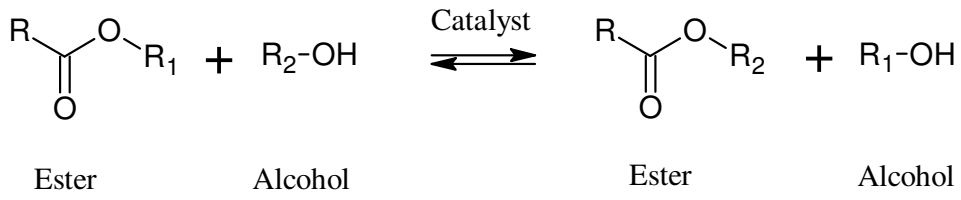
The esterification reaction (Scheme 1.1) involves the reaction of a free fatty acid (FFA) with an alcohol (usually a low molecular weight alcohol, such as MeOH, EtOH, n-PrOH, or n-BuOH)[77] to produce an alkyl ester (biodiesel) and water. Either base or acid catalysts can be used for the reaction. More commonly, acid catalysts such as sulphuric acid are employed to carry out the esterification reaction under mild conditions.



Scheme 1.1. Esterification reaction of a FFA with methanol.

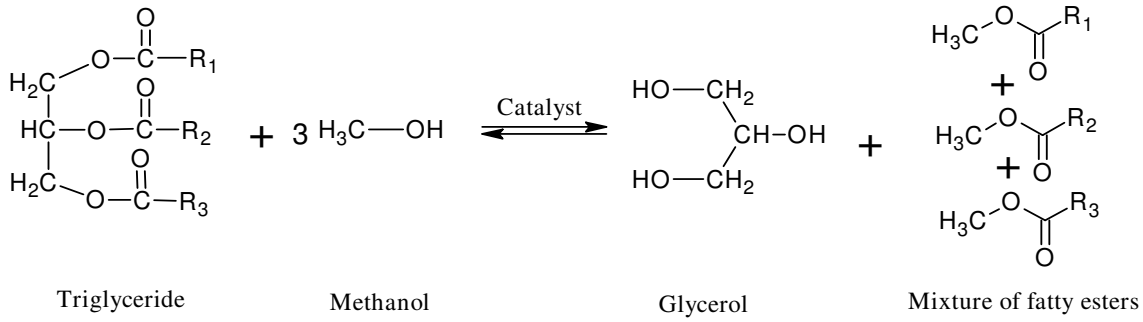
1.2.2. Transesterification of Triglycerides

Transesterification, also called alcoholysis, is the displacement of an alcohol from an ester by another alcohol in a process similar to hydrolysis except that alcohol is used instead of water.[78, 79] The transesterification reaction is represented as:

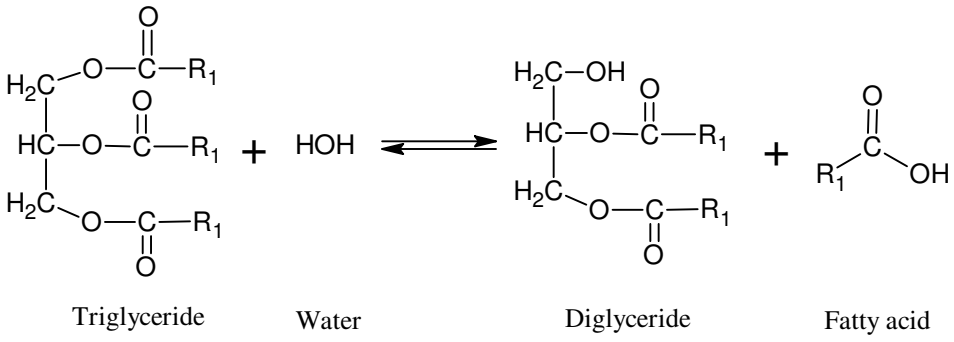


Scheme 1.2. Process of transesterification reaction.

In the presence of methanol or water, the ester linkage between the glycerol and fatty acid of the triglyceride molecule breaks. In this process, if methanol is used then it is called methanolysis and if water is used then it is termed as hydrolysis. Methanolysis and hydrolysis of triglycerides are represented in Schemes 1.3 and 1.4, respectively.



Scheme 1.3. Methanolysis of triglycerides.

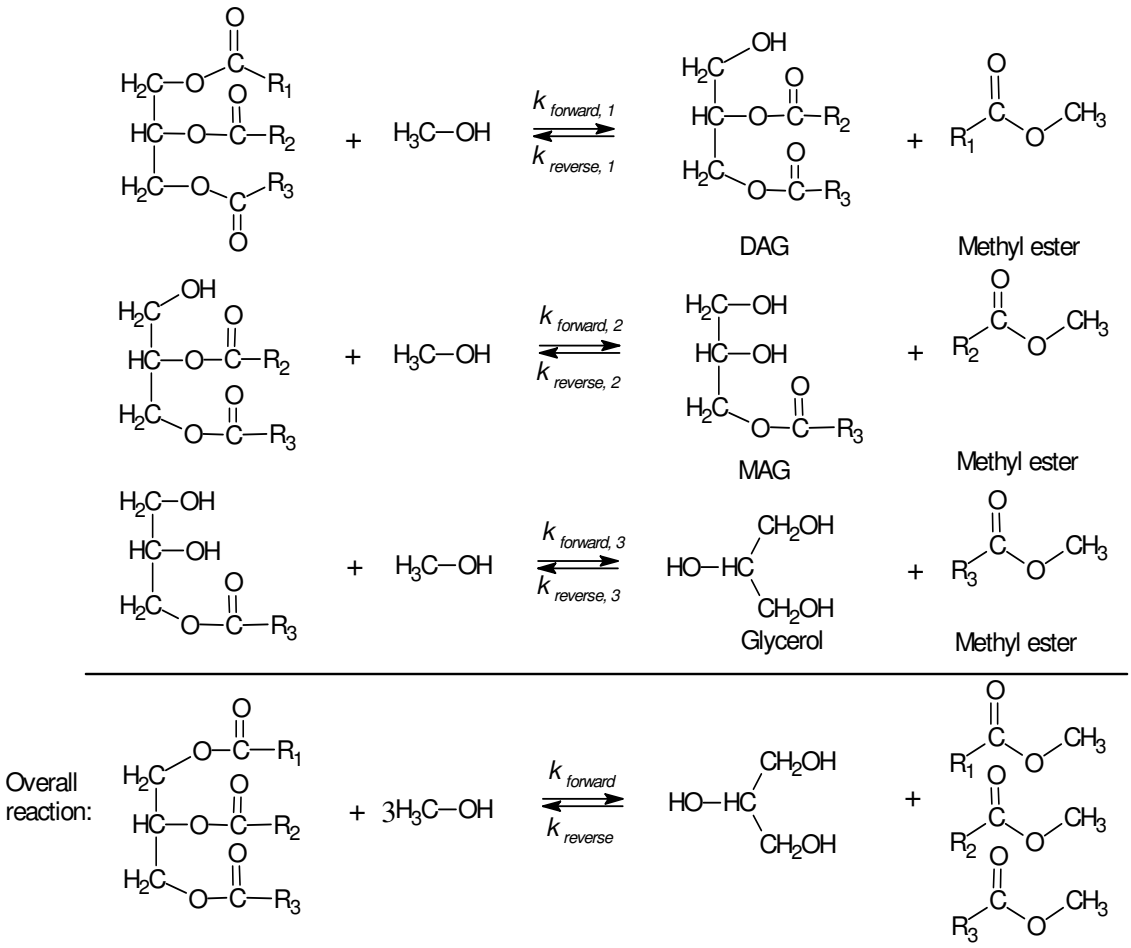


Scheme 1.4. Hydrolysis of triglycerides.

Transesterification is a reversible reaction which proceeds essentially by mixing the reactants.[74, 80] However, the presence of a catalyst (a strong acid or base) accelerates the conversion.

1.3. CHEMISTRY OF THE TRANSESTERIFICATION PROCESS

The overall transesterification reaction is composed of three consecutive reversible steps, in the presence of either an acid or base catalyst (Scheme 1.5).



Scheme 1.5. Overall transesterification reactions of triglycerides with methanol.[81]

The first step is the conversion of triglycerides to diglycerides followed by the conversion of diglycerides to monoglycerides and then to glycerol yielding one methyl ester molecule from each glyceride at each step.[67] Each step is an equilibrium described by its equilibrium constant *K*, and it's forward ($k_{forward}$) and backward ($k_{backward}$) rate constants.

Most studies show that the kinetics of alkali-catalysed transesterification is categorised by three regimes.[82, 83] In the initial reaction phase, mass-transfer is limited

due to the low solubility of reagents *i.e.* the non-polar oil phase is immiscible with the polar alcohol-catalyst phase and slows down the reaction. As the reaction proceeds, sufficient amounts of methyl esters are accumulated in the reaction mixtures and act as emulsifying agents; ultimately forming a single-phase system that favours the kinetically controlled process. Finally, the last stage of the reaction is characterised by a slower reaction rate because glycerol is formed and a phase separation phenomenon takes place between polar glycerol and non-polar esters. For comparison, the values of rate and equilibrium constants for the three steps are given in Table 1.6.[82]

Table 1.6. Comparison of rate and equilibrium constants of three steps in the transesterification of triglycerides.[82]

Vegetable oil	k_1[a]	k_2[a]	k_3[a]	k_{-1}[a]	k_{-2}[a]	k_{-3}[a]	K_1[b]	K_2[b]	K_3[b]	T °C	KOH %
Rapeseed	5.01	4.93	29.7	3.55	2.99	0.79	1.06	1.71	132.8	23	1.5
Pongamia	0.029	0.0058	0.011	0.014	0.021	0.00051	1.99	0.27	21.74	60	1-2
Palm[c]	0.036	0.070	0.141	-	-	-	-	-	-	60	1
Palm	0.011	0.018	0.131	0.000	0.082	0.002	-	-	-	60	1[d]
Soybean	0.050	0.215	0.242	0.110	1.228	0.007	-	-	-	50	-

[a] Units: (L mol $^{-1}$ min $^{-1}$).

[b] Calculation of equilibrium constant based on the final concentration.

[c] Units: (wt% min) $^{-1}$.

[d] NaOH.

After the completion of the transesterification reaction, the products are a mixture of esters, glycerol, unreacted alcohol, catalyst and tri-, di- and monoglycerides. Saydut *et al*. reported that obtaining pure esters is not an easy process since reaction intermediates such as diglycerides and monoglycerides always remain in the ester phase. The monoglycerides cause turbidity in the esters mixture. After the completion of the transesterification reaction, when the mixture is allowed to cool down to room temperature, it separates into a crude ester phase and a glycerol phase.[84] The glycerol and ester phase are usually separated by gravitational settling or centrifugation. To meet the EN standard, the ester and glycerol phase needs to be separated and esters purified.

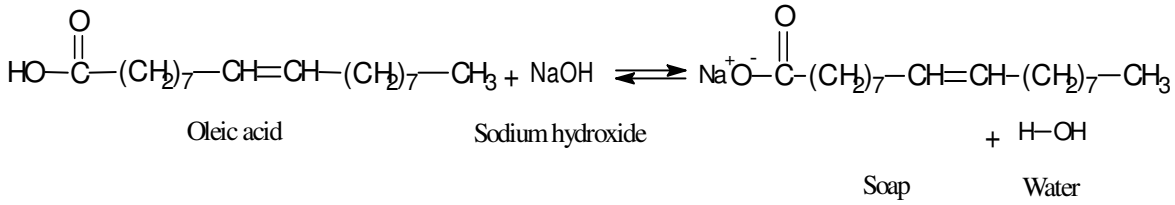
1.4. PHYSIO-CHEMICAL EFFECT ON THE TRANSESTERIFICATION REACTION

It has been reported that the transesterification process depends on several parameters that can affect the conversion rate of FAMEs (biodiesel): [85-91]

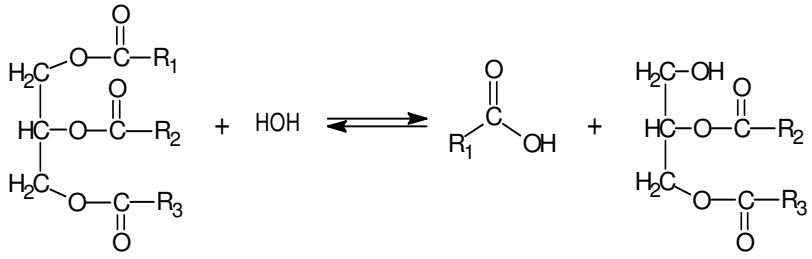
- free fatty acids content.
- moisture content.
- reaction time & temperature.
- mixing parameters.
- ratio of alcohol to oil.
- nature and concentration of catalyst.

1.4.1. Free Fatty Acids Content

The free fatty acid content in the oil plays an important role in the transesterification reaction, especially when a base catalyst is used for the reaction. FFAs can react with the alkali catalyst giving rise to saponification (soap formation), as shown in Scheme 1.6a. This factor could have a serious impact on the rate of transesterification. Furthermore, the presence of soap can cause an increase in viscosity and often gives rise to gel formation, which complicates the glycerol-monoalkyl ester separation process.[92, 93]



a) Soap Formation



b) Hydrolysis of a Triglyceride to form free fatty acids

Scheme1.6. (a) Reaction of the base catalyst (NaOH) with FFAs to produce soap and water, (b) hydrolysis due to reaction with water forming FFAs.

The acid value of the oil should be less than 1% (w/w); [94] otherwise a large amount of alkali catalyst will be consumed to neutralise the free fatty acids. An example of the effect of FFAs on the yield of methyl ester during alkali-catalysed transesterification is shown in Figure 1.3. There is a significant decrease in ester conversion when the free fatty acids are present beyond 2% (w/w).[95] Demirbas also reported a similar decrease in yield of the alkyl ester due to the presence of FFAs as they reduce the effectiveness of the catalyst.[96]

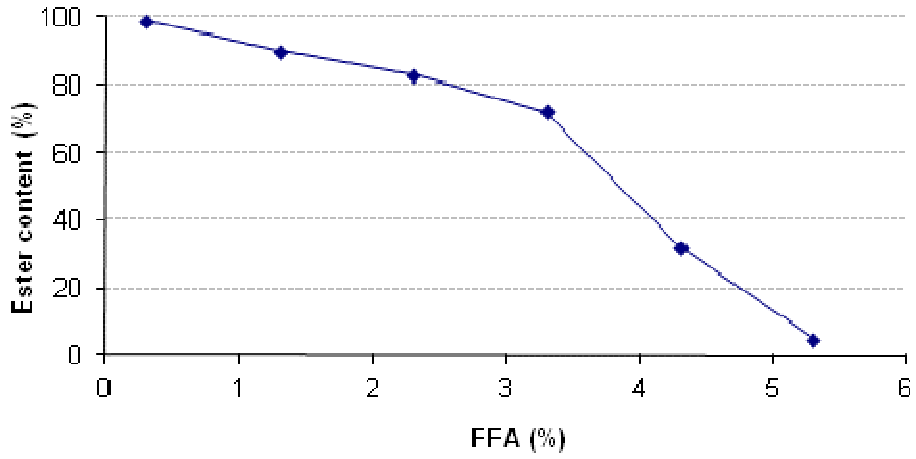


Figure 1.3. Effects of FFA on the yield of methyl ester during alkali-catalysed transesterification (6:1 CH_3OH : oil molar ratio, 1% (w/w) KOH).[95]

1.4.2. Moisture Content

Water can cause a more adverse effect than the presence of free fatty acids as water will compete with methanol to react with triglycerides to form FFA[97] (Scheme 1.6 b). Figure 1.4 shows the influence of water content on the yield of methyl esters.

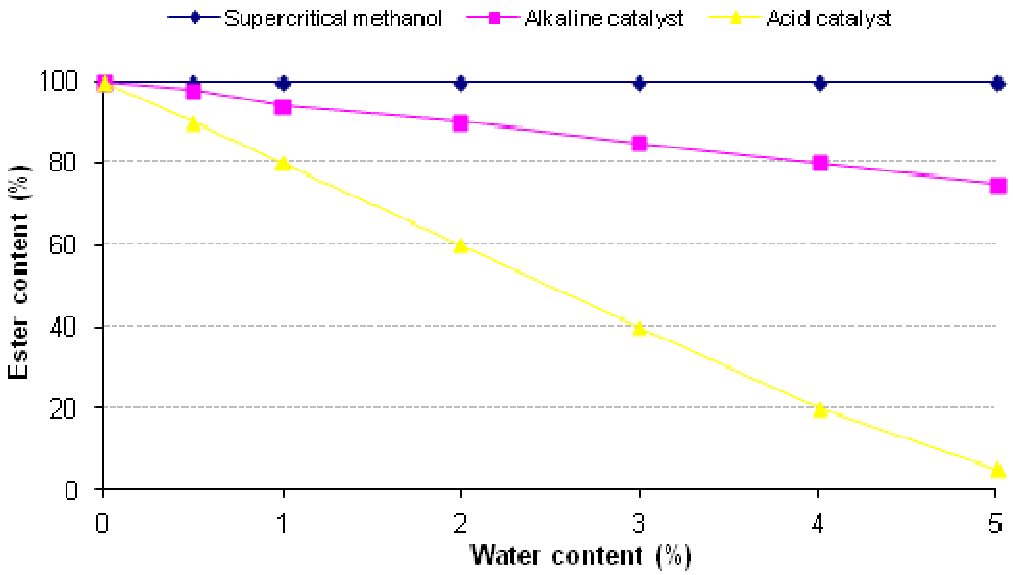


Figure 1.4. Yields of methyl esters as a function of water content in the transesterification of triglycerides.[87]

Different studies[98, 99] have shown that the presence of traces of water (0.1% w/w) in the transesterification reaction decreases the ester conversion from vegetable oil. Therefore, Srivastava and Verma[100] removed the moisture content from vegetable oil by heating in an oven for 1 hr at 110 ºC prior to the transesterification reaction. Meher *et al.*[101] also reported a precautionary step to prevent moisture being absorbed and maintenance of catalytic activity by using a potassium hydroxide in a fresh solution of methanol. Figure 1.4 shows that an acid catalyst is more sensitive to the presence of water when compared with an alkaline catalyst. No effect on the ester content was observed when supercritical methanol (non-catalytic transesterification) was used because this process does not require any alkali-catalyst. The presence of water had a negligible effect on the conversion while using lipase as a catalyst.[102] Thus, it is concluded that, for both acid and alkali catalysed transesterification, the concentration of FFA should not exceed 0.5% (w/w),[103] and moisture content in the feedstock oil should be kept to a minimum. Otherwise, production yields are proportionally affected as they deactivate catalysts and pose problems in the separation of pure products.

1.4.3. Reaction Time and Temperature

The rate of the transesterification reaction is strongly controlled by the reaction time and temperature. The reaction can be conducted at room temperature if sufficient time is provided.[22] In most cases, the reaction temperature is kept close to the boiling point of methanol (64.6 °C), if methanol is used as an alcohol at atmospheric pressure. The transesterification of waste cooking oil (WCO)[104] has been carried out successfully by using methanol at 60 °C under elevated pressure of 400 kPa. The maximum yield of esters was achieved at temperatures ranging between 60 and 80 °C at a molar ratio of alcohol to oil of 6:1.[105] Leung and Guo[91] investigated the effects of reaction time on the ester content and the product yield by using WCO (Figure 1.5). They reported that sufficient time (15-20 min) should be provided for the reaction to occur. However, excess reaction time does not increase the conversion but favours the backward reaction (hydrolysis of esters) which results in a reduction of product yield.

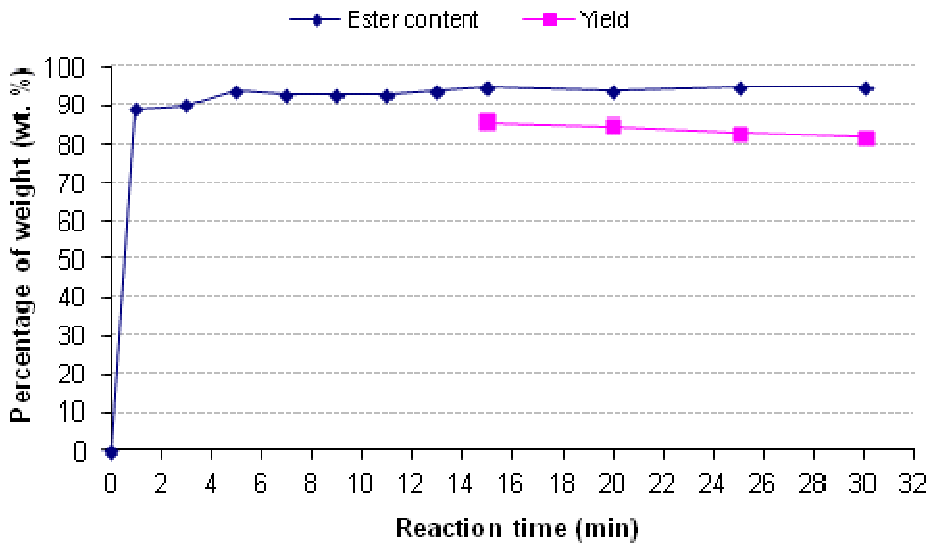


Figure 1.5. Effect of reaction time on ester content and isolated product yield. Ester content (% w/w) was determined by calculating the concentration of methyl esters in biodiesel sample whereas yield (% w/w) was estimated by the biodiesel weight yield relative to initial amount of WCO used.[91]

Felizardo *et al*. reported that after 1 hr of reaction, at a methanol/oil molar ratio of 4.2 and using a catalyst concentration of 0.6% (w/w) the highest yield (92% w/w) of methyl esters (MEs) was obtained which allowed an efficient separation of the ester phase.[106]

The transesterification can be conducted at various temperatures ranging from room temperature to the boiling point of the alcohol used or even higher. The temperature positively influences the yield of biodiesel almost up to the boiling point of the alcohol if other parameters (ratio of alcohol to oil, nature and concentration of catalyst, mixing intensity) are kept unchanged.

1.4.4. Mixing Parameters

Mixing is very important in the transesterification reaction, as oils or fats are immiscible with NaOH–CH_3OH solution. Once the two phases are mixed and the reaction is started, stirring is no longer needed. Meher *et al*. conducted the

transesterification reaction[101] using karanja oil (KOH 1% (w/w), 65 °C, 6:1 CH_3OH: oil molar ratio) at 180, 360 and 600 revolutions per minute (rpm) and reported an incomplete reaction at 180 rpm. The yield of methyl ester was the same at 360 or 600 rpm after 3hr of reaction. Sharma *et al*. also reported[107] that the mode of stirring also plays a vital role in the transesterification reaction. The yield of biodiesel increased from 85% (w/w) to 89.5% (w/w) when a magnetic stirrer (1000 rpm) was replaced with mechanical stirrer (1100 rpm). A proposed explanation may be a more thorough mixing of the reactants by using the mechanical stirrer.

1.4.5. Ratio of Alcohol to Oil

Another important parameter that has a significant influence on the yield of ester is the molar ratio of alcohol to oil. The stoichiometric ratio for transesterification requires three moles of alcohol and one mole of triglyceride to yield three moles of fatty acid alkyl esters and one mole of glycerol. However, transesterification is an equilibrium reaction in which an excess amount of alcohol is required to drive the reaction in the forward direction. Widyan *et al*. reported that when 100% excess alcohol was used *i.e.* 6 mol of alcohol to 1 mol of triglyceride, the reaction proceeded faster. The presence of a sufficient amount of methanol during the transesterification reaction is essential to break the glycerol-fatty acid linkages.[108] However, excess methanol should be avoided, because by increasing the molar ratio of methanol/oil beyond 6:1 neither increases the product yield nor the ester content, but rather makes the ester recovery process complicated and raises cost. Methanol has a polar hydroxyl group which can act as an emulsifier (causing emulsification).[91] Thus, separation of the ester layer from the water layer becomes difficult. Maio *et al*. reported that the addition of a large quantity of methanol, *i.e.* 70:1 and 84:1 molar ratio slowed down the separation of the ester and glycerol phases during the production of biodiesel.[109]

Different alcohols (methanol, ethanol, propanol and butanol) can be used for this process but methanol is widely used due to its low cost and its physical and chemical advantages as it is a polar and short chain alcohol.[77] Ramadhas *et al*. and Sahoo *et al*.,

determined that a 6:1 molar ratio and a 9:1 molar ratio (alcohol: oil) during acid esterification and alkaline esterification was the optimum amount for biodiesel production from high FFA rubber seed oil and polanga seed oil, respectively.[65, 110] Sharma and Singh[107] also used a similar two step transesterification process as discussed by Ramadhas *et al*. and Sahoo *et al*. They used 8:1 molar ratio for acid esterification and 9:1 molar ratio for alkaline esterification to obtain an optimum yield of biodiesel production from karanja oil.[65, 110] Veljkovic *et al*.[111] used a 18:1 molar ratio during acid esterification and a 6:1 molar ratio during alkaline esterification. Meng *et al*.[112] conducted transesterification of WCO with a 6:1 CH_3OH: oil molar ratio in the presence of NaOH and reported the foregoing as an optimum ratio corresponding to 89.8% (w/w) conversion. Similarly, transesterification of pre-treated waste rapeseed oil was carried out where maximum conversion has been estimated at a 6.5:1 ratio of methanol to oil.[109] For acid catalysed conversion of WCO with a high FFA content, a higher alcohol to oil ratio is required compared with base catalysed operation for a better yield of biodiesel. However, the effect of an increase of alcohol to oil ratio on the production of biodiesel becomes less significant for alkaline processes as compared to the corresponding acid catalysed process.[113]

1.4.6. Nature and Concentration of Catalyst

The catalyst plays a major role in the transesterification of vegetable oils. Generally two types of catalysts are used for chemical transesterification *viz.* acid catalysts and base catalysts.[114] The type and amount of the catalyst to be used depends on the nature of the oil. For example, for oil samples with FFA below 2.0% (w/w), alkaline transesterification is preferred over the acid catalysed transesterification to avoid the competing reaction *i.e.* saponification. The former catalytic process is reported to proceed 4000 times faster than the latter because an alkoxide acts as a stronger nucleophile than the alcohol itself.[115-116]

1.5. CATALYSIS

1.5.1. Homogeneous Base Catalysis

1.5.1.1. Reaction Mechanism

The accepted mechanistic route for transesterification under alkaline conditions is presented in Scheme 1.7.[45, 116]

The sequence of steps can be summarised as follows. First, the catalytically active species, RO^- is generated:

(a) when the base is an alkaline alkoxide, simple dissociation gives rise to the catalytically active species RO^-,

$$ROM \rightleftharpoons RO^- + M^+$$

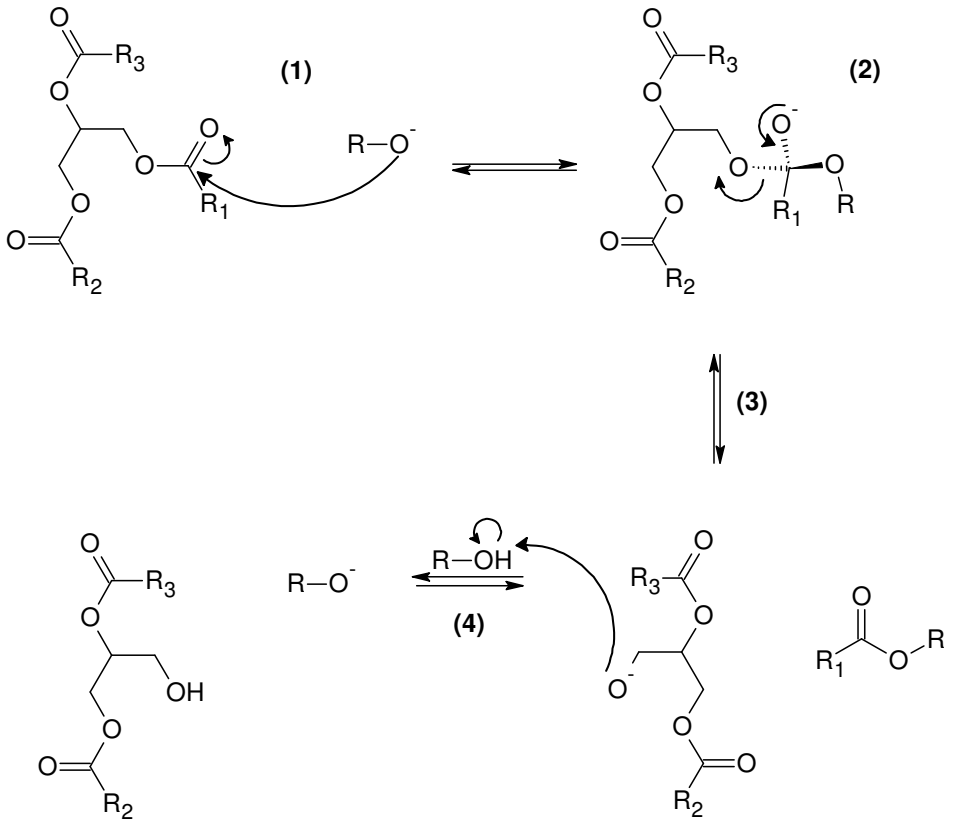
(b) the base catalyst deprotonates the alcohol producing the RO^- species,

$$MOH + ROH \rightleftharpoons RO^- + H_2O + M^+$$

(c) alkaline carbonates react with methanol.

$$M_2CO_3 + ROH \rightleftharpoons RO^- + HMCO_3 + M^+$$

Second, a tetrahedral intermediate is formed by nucleophilic attack on a carbonyl carbon in the TG. Third, the tetrahedral intermediate breaks down into a fatty acid ester and a diglyceride anion. Fourth, proton transfer to the diglyceride ion regenerates the RO^- catalytically active species. This sequence is then repeated twice to yield first a monoglyceride intermediate, and then finally the glycerol product and biodiesel.



R = Alkyl group on alcohol
R_1 R_2 R_3 = Fatty acid alkyl groups
M = Na, K

Scheme 1.7. Homogeneous base-catalysed reaction mechanism for transesterification of triglyceride.[116]

1.5.1.2. Base Catalysed Biodiesel Processing

Homogeneous base catalysts used for the methanolysis of lipids include alkaline metal compounds such as CH_3ONa, CH_3OK, NaOH, KOH, Na_2CO_3 or K_2CO_3.[117, 118] One particular advantage of using alkaline catalysts is that they give rise to a relatively fast reaction just by increasing the catalyst concentration to 1 or 2 mol% and can be carried out at low temperatures and pressures (60-65 °C and 1.4-4.2 bar).[119, 120] Currently, most commercially available biodiesel is produced by base-catalysed processes that employ NaOH as the active catalyst due to its lower cost. Another reason for using NaOH is well described by Leung and Guo. They used three different homogeneous catalyst *i.e.* sodium hydroxide, potassium hydroxide and sodium methoxide and proposed that the amount of NaOH required was less than the amounts

of either CH_3ONa or KOH for the same conversion of fatty acid methyl ester because NaOH has a lower molar mass (40 g/mol), compared to CH_3ONa (54 g/mol) and KOH (56 g/mol).[91] Table 1.7 shows the reaction conditions for base-catalysed transesterification processes in biodiesel synthesis and also highlights the fact that if all of these reaction parameters are fulfilled, more than 95% (w/w) methyl esters can be expected.

Table 1.7. Typical reaction conditions for biodiesel synthesis using homogeneous base catalysis.[64]

Base Catalysed Biodiesel Synthesis	
Feedstocks	Triglyceride mixtures with low free fatty acid contents (≤ 0.5% w/w) *e.g.*, Refined vegetable oils + Anhydrous short chain alcohol (generally, methanol)
Alcohol–to–oil molar ratio	6:1
Temperature	60-65 ºC
Pressure	1.4- 4.1 bar
Catalyst	NaOH (most common)
Catalyst concentration (by weight of lipid feedstock)	0.5- 2% (w/w)
Conversion	≥ 95% (w/w) can be expected after 1 hr reaction

When using hydroxides as a catalyst, small amount of soaps are expected to be produced, as mentioned in Section 1.4.1. In contrast, alkaline carbonates reduce the impact of soap production by forming bicarbonates instead of water (step 1(c), Scheme 1.7).[116] However, the carbonate anion is a weaker base, which translates into lower concentrations of the active RO^- species, slower reaction rates, and the need for higher amounts of the carbonate catalyst (2–3 % w/w) in order to achieve yields comparable to those obtained with alkoxides or hydroxide catalysts.

1.5.2. Homogeneous Acid Catalysis

1.5.2.1. Reaction Mechanism

The accepted chemical mechanism for the homogeneous acid-catalysed transesterification is given in Scheme 1.8.[116, 120] The sequence of steps can be summarised as follows:

1) the TG carbonyl group is protonated by the acid catalyst.

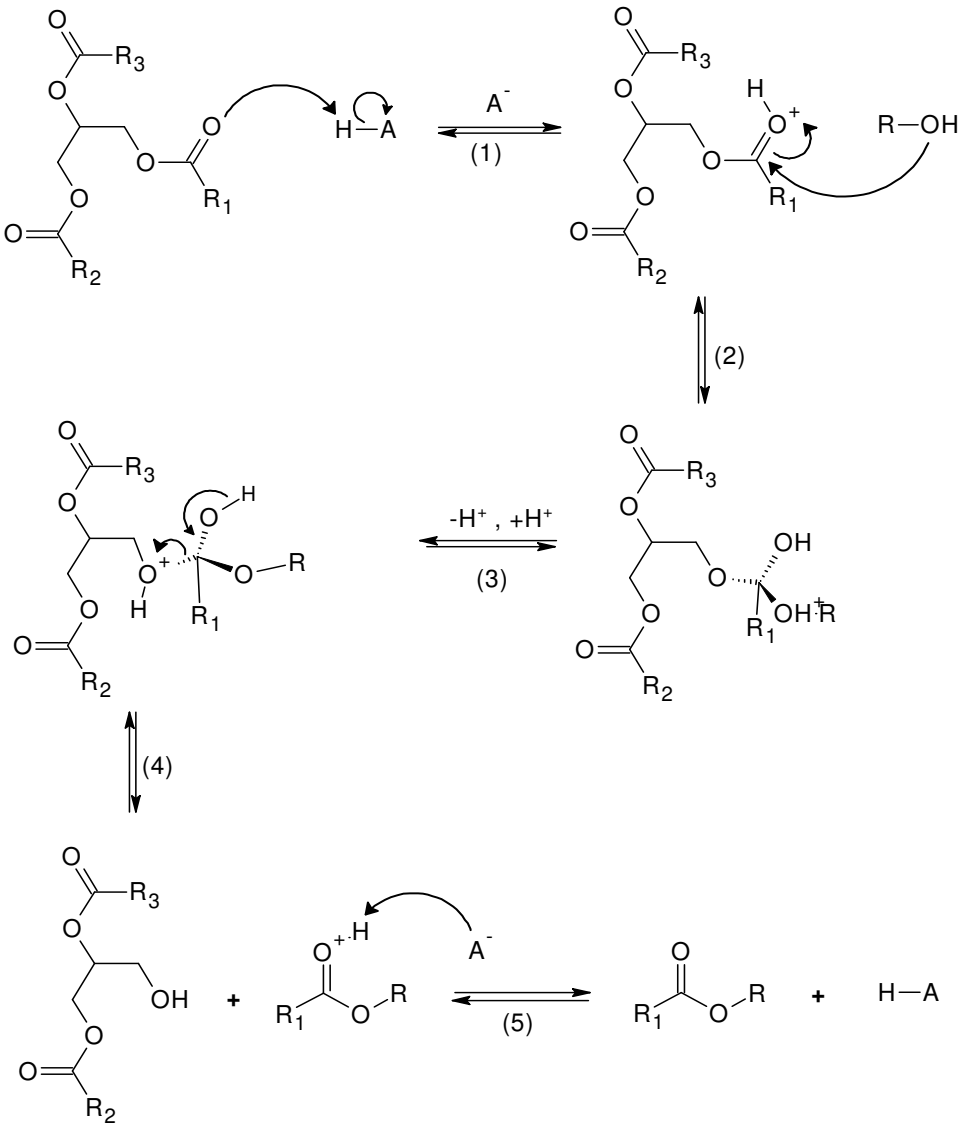
2) the activated carbonyl group undergoes nucleophilic attack from an alcohol molecule, forming a tetrahedral intermediate.

3) solvent assisted proton migration gives rise to a good leaving group

4) promoting the cleavage of the hemiacetal species (tetrahedral intermediate) and yielding a protonated alkyl monoester and a diglyceride molecule.

5) proton transfer regenerates the acid catalyst.

This sequence is repeated twice, to ultimately yield three alkyl monoesters and glycerol as products.



Scheme 1.8. Homogeneous acid catalysed reaction mechanism for transesterification of triglyceride.[116, 120, 121]

The important factor that promotes the catalytic effect in the reactions given in Scheme 1.8 is the protonation of the carbonyl group in the TG. Such catalyst-substrate interaction increases the electrophilicity of the adjacent carbonyl carbon atom, making it more susceptible to nucleophilic attack. This is in comparison to the base-catalysed mechanism where the base catalyst employs a more direct route to activate the reaction, creating an alkoxide ion that directly acts as a strong nucleophile (Scheme 1.7).

1.5.2.2. Acid Catalysed Biodiesel Processing

Compared with the base-catalysed synthesis of biodiesel, fewer studies have dealt with the subject of acid-catalysed transesterification of lipid feedstocks. Homogeneous acid catalysts, such as sulphuric acid, phosphoric acid, hydrochloric acid, organo sulfonic acids and others, can be used to catalyse the transesterification of TGs and the esterification of FFAs to produce biodiesel type monoesters.[20, 64, 122, 123] Typically, acid-catalysed process is a two-stage process as in the first stage it can be used to esterify the FFAs and in the second stage used for the transesterification of TGs. However, acid catalysts give very high yields of alkyl esters, but the reactions are slow, requiring higher temperatures and more than 3 hr to reach completion.[124]

The reason that the acid-catalysed process is not as commercially viable compared to the base-catalysed process is due to the need for longer reaction times. Additionally, when a higher concentration of catalyst is used to drive the transesterification reaction towards completion it can lead to increased corrosion. Acid catalysis is recommended for the transesterification of feedstock containing higher FFA content to avoid soap formation and reduce costs *e.g.* low-cost, low-quality feedstocks (generally high in FFAs like WCO).[64, 125] Therefore, homogeneous acid catalysis is still applicable for specific applications and provides options to the operators. The factors that need to be considered in choosing homogeneous acid catalysis for transesterification are given in Table 1.8.

Table 1.8. Favourable and unfavourable factors for homogeneous acid catalysed transesterification.[22, 104, 125]

Favourable factors	Unfavourable factors
1. Transesterification can be carried out using oils with high free fatty acid content.	1. Acid catalysed transesterification is about 3 orders of magnitude slower than the alkali catalysed reaction for comparable amounts of catalyst.[74]
2. Acid catalysis is preferred when oil component is low grade material like sulphur olive oil or yellow grease.	2. High temperature and concentration of the acid catalyst could burn some of the oil which results in a low yield of biodiesel.
3. The esterification and transesterification proceed simultaneously. This avoids the use of pre-extracted seed oil.	3. Corrosiveness of strong liquid acids and the environmental threat that they pose have also been limits to their use

In general, acid catalysed reactions are performed at high alcohol-to-oil molar ratios, low-to-moderate temperatures and pressures, and high acid catalyst concentrations. Table 1.9 summarises reactions conditions proposed by Zhang *et al*. to prepare biodiesel from WCO using sulphuric acid as the catalyst.[64]

Table 1.9. Reaction conditions for biodiesel synthesis using homogeneous acid catalysis.[64]

Acid Catalysed Biodiesel Synthesis	
Feedstocks	Triglyceride mixtures with high free fatty acid contents (≥ 4% w/w) *e.g.*, waste cooking oil + Anhydrous short chain alcohol (generally, methanol)
Alcohol–to–oil molar ratio	50:1
Temperature	80 ºC
Pressure	4.0 bar
Catalyst	H_2SO_4 (most common)
Catalyst load	1.3 : 1 molar ratio of H_2SO_4 to waste oil
Conversion	97% (w/w) can be expected after 4 hr reaction

1.5.3. Disadvantages of Homogeneous Catalysts

The use of homogeneous catalysts involved in biodiesel synthesis currently presents separation and catalyst recovery issues. Although transesterification using a conventional alkali-catalysed process gives high conversion levels of triglycerides to their corresponding methyl esters in short times, the reaction has several drawbacks. It is energy intensive, recovery of glycerol is difficult, the catalyst has to be removed from the product, alkaline waste-water requires treatment and free fatty acids and water interfere with the reaction.[126, 127]

Despite the advantages of using acid catalysts, the two-stage method (esterification and transesterification) also faces the problem of catalyst removal. The problem of catalyst removal in the first stage can be avoided by neutralising the acid catalyst and using an alkaline catalyst in the second stage. However, the use of a greater amount of catalyst will increase the cost of biodiesel. Generally, the residue of either alkaline or acidic catalyst in the ester can cause engine problems. Alkaline catalyst can produce high levels of incombustible ash and acid catalyst attacks the engine's metallic parts. Therefore, the catalysts must always be eliminated from the biodiesel when the reaction is complete.

1.5.4. Heterogeneous Catalysis

Heterogeneous catalysts greatly simplify the post-treatment of the products (separation and purification) in the alcoholysis of triglycerides. They can be easily separated from the system at the end of the reaction and can also be reused. Moreover, the use of heterogeneous catalysts does not produce soaps *via* free fatty acid neutralisation or triglyceride saponification.[128] Notwithstanding the aforementioned comments, the performance is still unfavourable compared to alkaline homogeneous catalysts because heterogeneous catalysts are less active and require relatively higher temperature and pressure. Furthermore, they can suffer from deactivation phenomena

such as poisoning, coking and leaching.[120] The other major problem is associated with diffusion limitations between different phases that exist during the reaction, which in turn significantly reduces the surface area of the catalyst to promote the transesterification of TGs.

1.5.4.1. Heterogeneous Base Catalysis

Many types of heterogeneous catalysts have been tested in the esterification and transesterification reactions of fatty acids, triglyceride feedstock and simple esters. Ideally, an active solid catalyst should be able to perform transesterification and esterification simultaneously (similar to homogeneous acid-catalysed processes), but allow elimination of the post-treatment steps. It is likely that heterogeneous catalysts that perform well in esterification should also be good candidates for transesterification since the mechanisms for both reactions are quite similar.[129]

Classic heterogeneous base catalysts, where the solid contains either Lewis or Brønsted base sites, have been the most extensively tested solid catalysts for the transesterification reactions of TGs. Many type of heterogeneous solid base catalysts such as alkaline and rare earth oxides like MgO, CaO, SrO, various alkali metal compounds supported on alumina or zeolites, hydroxides, alkoxides and hydrotalcites have been studied in relation to the transesterification of vegetable oils.[102, 130-133] Some solid bases, that showed good catalytic activity, catalysed the transesterification reactions *via* a homogeneous molecular pathway rather than a truly heterogeneous one, due to their negligible solubility in alcohols.[134]

1.5.4.1.1. Alkali Earth Metal Oxides

Alkaline earth oxides and hydroxides are potential base catalysts for use in TG transesterification. The origin of basic sites in alkaline earth oxides is generated by the presence of M^{2+}–O^{2-} ion pairs in different coordination environments. The basic

strength of the group IIA oxides and hydroxides increases in the order Mg<Ca<Sr<Ba.[55] This is because the ionic radii of alkaline earth metals increase and their electronegativity decreases in this order.

According to Lewis theory, oxides are stronger bases than their hydroxides. Alkaline earth metal methoxides are even more basic. Thus, calcium compounds, and similarly magnesium and barium compounds, can be ordered according to their alkaline power as follows: $Ca(OH)_2$<CaO<$Ca(OCH_3)_2$.[54] Magnesium oxide has the weakest basic strength and solubility among group II oxides and has been rarely used for biodiesel production.[135] MgO did not show significant catalytic activity in the transesterification of rapeseed oil with methanol under conditions normally used to prepare biodiesel.[136] Nano-particulate magnesium oxides have been used for the transesterification of soybean oil and yields of 99% (w/w) were obtained within 10 min at a supercritical temperature of 523 ºC and a pressure of 24 MPa. This result showed that this catalyst displays greater activity at high pressures and temperatures.[135]

Ca-derived bases are the most promising since they possess relatively high basic strengths and lower environmental impact due to their low solubility in methanol. Moreover, they can be synthesised from cheap sources like limestone and calcium hydroxides.[137] Barium hydroxide has been reported to catalyse the methanolysis of rapeseed oil at 65 ºC with over 80% (w/w) of oil conversion in less than a hour.[54, 138] Unfortunately, barium hydroxides are much more soluble in methanol than all other alkaline earth metal compounds.[54] The HO-Ba-OH bonds are strongly polarised and show ionic character. They can undergo dissociation easily, particularly in methanol, (Scheme 1.9) which is characterised by a relatively high solvation power.[139] Barium alcoholates can form *via* the reactions shown in Scheme 1.9:

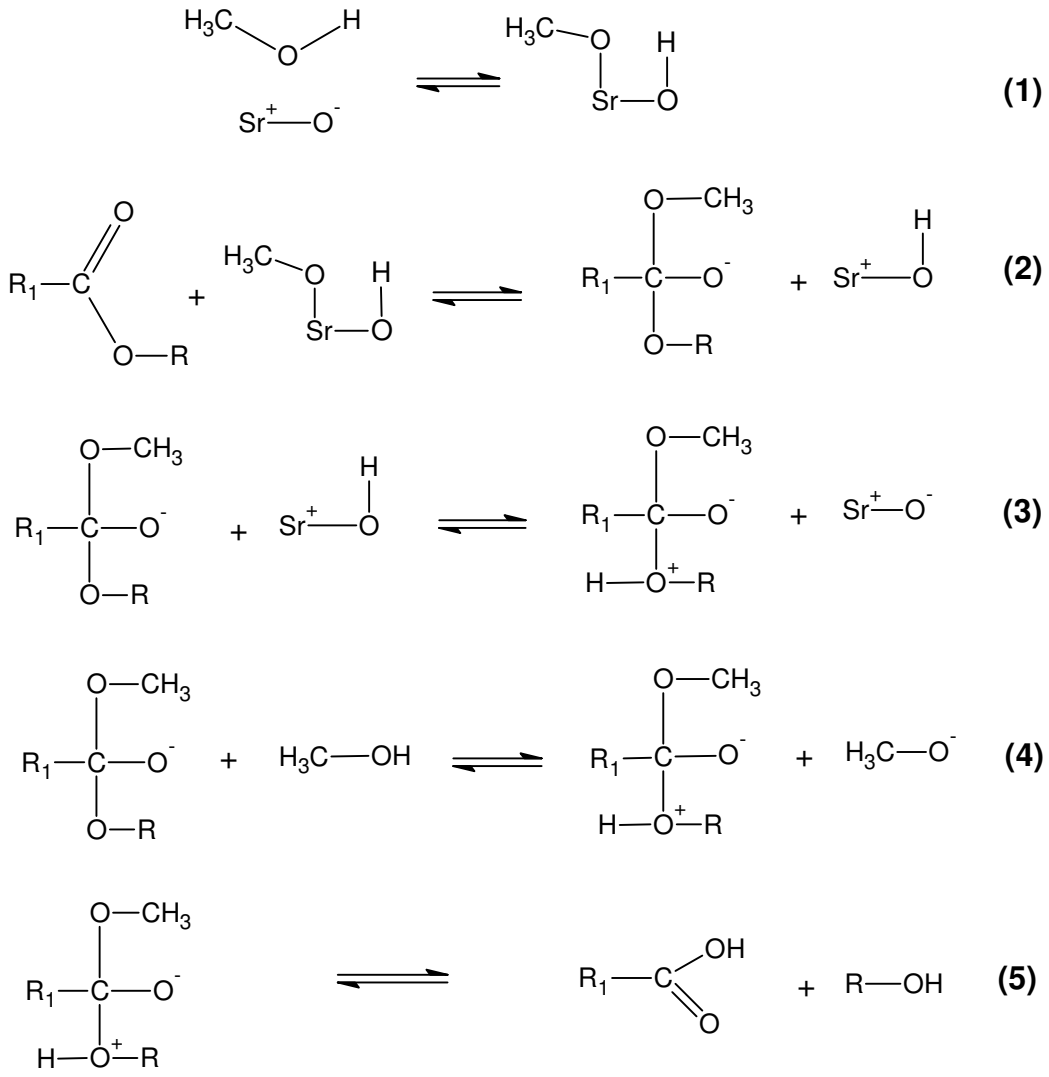
$$Ba(OH)_2 + CH_3OH \rightleftharpoons CH_3OBaOH + H_2O$$

$$CH_3OBaOH + CH_3OH \rightleftharpoons Ba(OCH_3)_2 + H_2O$$

Scheme 1.9. Formation of barium alcoholates.

The solubility of barium hydroxides results in high toxicity, therefore barium hydroxide cannot be used as catalyst for the transesterification process.[140]

Strontium oxide has also attracted attention as a heterogeneous catalyst owing to its high basicity and insolubility in methanol, vegetable oil and methyl esters. Liu *et al*. studied SrO for the transesterification of soybean oil. Catalytic reactions take place on the surface of the solid base catalyst. The mechanism proposed by the authors is described in Scheme 1.10.[141-143]



Scheme 1.10. Mechanism of SrO catalyst for the transesterification reaction (R_1= alkyl group of fatty acid, R= alkyl esters of triglyceride).[141-143]

In the first step, surface O^{2-} extracts H^+ from CH_3OH to form surface CH_3O^- (Eq. 1), which is strongly basic and has high catalytic activity in the transesterification reaction. In the second step, the carbonyl carbon atom of the triglyceride molecule attracts a methoxide anion from the surface of the SrO to form a tetrahedral intermediate (Eq. 2), where R_1 represent the long chain alkyl group. In the third step, the tetrahedral intermediate accepts H^+ from the surface of the SrO (Eq. 3). The tetrahedral intermediate can also react with methanol to generate a methoxide anion (Eq. 4). In the last step, rearrangement of the tetrahedral intermediate results in the formation of biodiesel (Eq. 5).

1.5.4.1.2. Zeolites

Zeolites, due to their uniform pore structure, appear to have definite advantages for both acid and base catalysis. In order to favour catalysis, the surface should be made hydrophobic to promote preferential adsorption of oily hydrophobic species on the catalyst surface and to avoid deactivation of catalytic sites by strong adsorption of polar by products such as glycerol or water.[144, 145]

Zeolite X and microporous titanosilicate ETS-10, in their as-prepared forms and ion-exchanged with K^+ and Cs^+, have been examined for their catalytic activities in the transesterification of soybean oil with methanol.[140] Non-thermally treated zeolites showed no activity, probably because the basic sites in zeolites are poisoned from exposure to CO_2 and moisture in the air during handling.[146, 147] For the K^+-exchanged X zeolite, catalytic activity was higher than the Cs^+-exchanged and Na^+ forms, in that order. However, the same trend was not observed for ETS-10. The parent ETS-10 material, which contains Na^+ and K^+ ions in a ratio of approximately 3:1, was the most active catalyst followed by the K^+- and Cs^+-exchanged materials. In all cases, the ETS-10 titanosilicate showed much higher activity than the X zeolite. Superior performance of ETS-10 was expected since it is known that ETS-10 is about four times more basic than NaX.[148]

1.5.4.1.3. Carbonate Salts

Simple carbonate salts have also been used with success as base catalysts for transesterification reactions. For instance, the alcoholysis reaction of soybean oil and beef tallow with ethylene-, diethylene-, triethylene-glycol and glycerol was carried out using M_2CO_3 (M = K, Na) and MCO_3 (M = Mg, Ca, Zn) base catalysts at temperatures above 200 ºC and alcohol/TG ratios >8. High triglyceride conversions (total conversion >95% w/w) were achieved under these conditions in less than three hours.[149] Sodium and potassium carbonates catalysed significant hydrolysis side reactions, which lowered their efficiency considerably. However, Mg, Ca and Zn carbonates produced a clean reaction. It is also reported that carbonate catalysts can be used efficiently in the production of biodiesel from TG mixtures with high FFA contents. But, the fact that esterification of FFA produces water raises the question of carbonate solubility as carbonate species have shown leaching phenomena under mild conditions.[140]

1.5.4.1.4. Alkaline Metal Salt on Porous Support

One of the ways to minimise the mass transfer limitation for heterogeneous catalysts in liquid phase reactions is to use catalyst supports. Supports can provide higher surface area *via* the existence of pores where metal particles can be anchored.[137] Supports such as alumina,[150, 151] silica,[134] zinc oxide[152-154] and zirconium oxide[155] have been used in biodiesel production. Table 1.10 summarises different types of heterogeneous catalysts supported on porous substrates and the conversion rate of substrates under different operating parameters.

Table 1.10. Different heterogeneous catalysts used for transesterification of vegetable oils.

Vegetable oil	Catalysts	Ratio CH_3OH/Oil	Reaction time, hr	Temperature ºC	Conversion % (w/w)	Reference
Soybean oil	La/Zeolite beta	14.5	4	160	48.9	[156]
Palm oil	Hydrotalcite	30	6	100	86.6	[157, 158]
Rapeseed oil	$CaTiO_3$, $CaMnO_3$, Ca_2Fe_2O, $CaZrO_3$, $CaO\text{-}CeO_2$	6	10	60	90	[159]
Soybean oil	$MgO.MgAl_2O_4$	3	10	65	57	[160]
Sunflower oil	CaO/SBA-14	12	5	160	95	[161]
Soybean oil	MgO, ZnO, Al_2O_3	55	7	70,100,130	82	[162]
Jatropha curcas oil	CaO	9	2.5	70	93	[163]
Rapeseed oil	Mg-Al HT	6	4	65	90.5	[164]
Soybean oil	CaO, SrO	12	0.5-3	65	95	[141, 165]
Soybean oil	ETS-10	6	24	120	94.6	[140]

1.5.4.2. Heterogeneous Acid Catalysis

Solid acid catalysts have the potential to replace strong liquid acids to eliminate the corrosion problems and consequent environmental hazards posed by liquid acids. Among the solid acid catalysts available are functionalised polymers, such as the acid forms of resins[119, 125, 166-170], as well as inorganic materials, such as zeolites, modified oxides and clays. Some of these solids have already been found to be effective in transesterification reactions of simple esters and β-ketoesters.[129] The other types of solid acid catalysts that have been exploited for use in esterification and transesterification reaction studies includes, Amberlyst115,[166, 167] Lewatit GF 101, sulfonated saccharides,[171-174] and organosulphonic functionalised mesoporous silicas.[175-178]

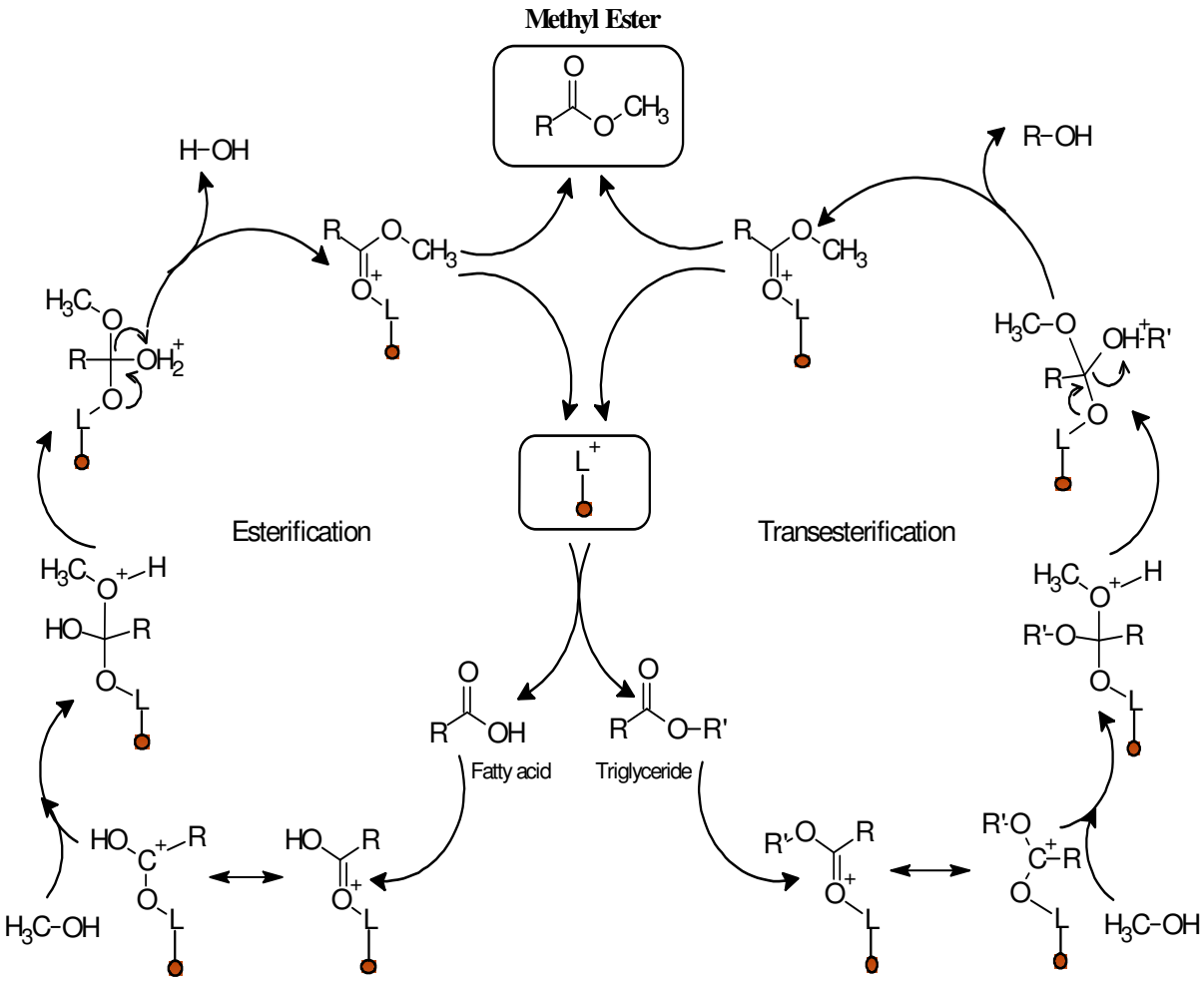
However, efforts at exploiting solid acid catalysts for transesterification have been limited due to pessimistic expectations on the possibility of low reaction rates and

adverse side reactions. In particular, the double dehydration of the by-product glycerol to produce acrolein and water (reaction catalysed by acids) has been a major concern.[177] As a result, the factors governing the reactivity of solid catalysts are not fully understood. For example, simple correlations between acid strength and activity of the catalyst have not been clearly formulated. Second, due to diffusional restrictions, the catalyst must have a porous system with interconnecting pores, so that the entire surface of the solid is available for promoting the transesterification reaction. Even though it is possible to generate these features in the solids, it is not yet routinely possible to obtain uniform pore architecture with absolute control over the size, diameter or geometry of the pores as well as the stability of the solids in the system.

Solid acid catalysts have been applied effectively in the esterification of carboxylic acids, but for transesterification they require higher reaction temperatures due to their lower activity.[179] The lower activity is because solid acids have an even smaller population of acid sites per gram of catalyst as compared to a homogeneous liquid acid (HCl, H_2SO_4). Some resins, such as Amberlyst115, may be considered an exception, as these catalysts catalyse both esterification and transesterification reactions, under mild reaction conditions, due to the possession of high concentrations of acid sites.[166, 168] However, thermal stability becomes an issue when resin-type catalysts are used at higher temperatures[180] in order to achieve higher reaction rates in an application such as reactive distillation. The other issue is associated with catalyst regeneration.

1.5.4.2.1. Reaction Mechanism

The reaction mechanism for simultaneous esterification and transesterification using a Lewis acid is shown in Scheme 1.11.[181] The esterification takes place between free fatty acids (RCOOH) and methanol (CH_3OH) whereas transesterification takes place between triglyceride and methanol adsorbed on acidic sites (L+) of the catalyst surface. The interaction of the carbonyl oxygen of free fatty acid or triglyceride with the acidic site of the catalyst forms a carbocation.



L^+ =acid site on the catalyst surface
R= alkyl group of fatty acid
R' = alkyl esters of triglyceride

Scheme 1.11. Esterification and transesterification using Lewis acid.[181]

The nucleophilic attack of alcohol with the carbocation produces a tetrahedral intermediate. During esterification the tetrahedral intermediate eliminates water molecule to form one mole of ester ($RCOOCH_3$). In the reaction sequence, the triglyceride is converted stepwise to di-, mono- and finally glycerol. In esterification and transesterification reaction, the final product *i.e.* methyl ester is the same. The catalyst is also regenerated after the simultaneous esterification and transesterification reactions. The use of excess alcohol favours the forward reaction and thus maximises the yield of ester.

1.5.4.2.2. Heteropolyacids (HPA)

Heteropolyacids (HPA) and their salts are a class of highly acidic polyoxometalates compounds made up of heteropoly anions having metal–oxygen octahedra as the basic structural unit.[182] The HPA, which possesses a Keggin structure, is thermally stable and easy to synthesise. The major disadvantages of Keggin-type HPAs are low specific surface areas and solubility in polar media. These issues can be overcome by dispersing it on high surface area supports. Heteropoly acids appear to be an appropriate choice for water tolerant acid catalysts. Most of these systems have acidity in the range of super acids with the possibility of tailoring the porous architecture as well as solubility in water (such as that of the Cs salt).

An example is $Cs_{2.5}PW$ catalyst, which was chosen[183] on the basis of high activity, water tolerance, reusability and environmentally benign nature of this material for biodiesel production. The solid acid catalyst was so efficient that it produced 99% (w/w) yield of biodiesel with the advantage of only low catalyst concentration ($1.85x10^{-3}$:1 weight ratio of catalyst: oil), low methanol-to-oil ratio (5.3:1), low temperature (65 ºC), and relatively short reaction time (45 min). The process was economical since the activity of the $Cs_{2.5}PW$ was not greatly affected by the free fatty acid and moisture content of the vegetable oil. The catalyst could be separated easily from the product mixture and reused a number of times.[144]

1.5.4.2.3. Transition Metal Oxides

Zirconium oxide, titanium oxide and zinc oxide are among the transition metal oxides that have attracted attention for biodiesel production due to their acidic properties.[137] The use of zirconia as a solid catalyst for the transesterification of different oils is more efficient than other transition metal oxides due to its strong acidity. Furthermore, the acidity is promoted when the surface of these metal oxides contains anions such as sulphate and tungstate.[184]

The methanolysis of soybean oil was tested using tungstated zirconia-alumina (WZA), sulphated tin oxide (STO) and sulphated zirconia-alumina (SZA) as acid catalysts.[141] Among these, the WZA catalyst was the most effective as it achieved a conversion greater than 90% (w/w) after 20 hr at 250 ^{o}C.[185]

1.5.4.2.4. Clays

Clays such as montmorillonite have been tested with and without acid activation. The latter can be carried out by submerging the clays in a solution of sulphuric acid and methanol followed by methanol/water washing and drying at 70 ^{o}C. Montmorillonite KSF showed the highest activity for the alcoholysis of refined rapeseed oil with methanol. Using 5% (w/w), at 220 ^{o}C and 52 bar, an ester yield of almost 99.9% (w/w) was obtained after 6 hr. However, at this temperature, dimerisation of alkyl esters was observed. At lower temperatures and pressures (140 ^{o}C and 8.5 bar), ester yields were affected (70% w/w yield after 8 hr).[129]

1.5.5. Disadvantages of Heterogeneous Catalysis

Most research concerning the application of heterogeneous catalysts for biodiesel synthesis has focused on solid base catalysts rather than on solid acid catalysts, because acid catalysts exhibit slower reaction rates compared to base catalysts. Some of these catalyst provided promising results, but at the expense of high temperature and pressure. High temperatures and, more importantly, high pressures translate into high equipment costs, hazardous working conditions and high-energy demands, outweighing the value of the end product.

There is also a dearth of systematic research for exploring the principles of solid catalyst activity for the transesterification of TGs and esterification of FFAs with alcohols. For instance, the most active heterogeneous catalyst reported is $Ba(OH)_2$ but its catalytic mechanism is still unclear. This catalyst is likely to operate *via* a

homogenous rather than a heterogeneous reaction mechanism due to its solubility in alcohols like methanol. Similar behaviour may occur with other solid bases that require higher temperatures to show measurable catalytic activity.

Studies carried out by Lotero *et al.* [120] suggest that the main problem for solid acid catalysts concern the diffusivity of large TG (and glyceride species in general) molecules through the pores of solid materials. Water and some polar compounds (methanol and glycerol) can have a deleterious effect by absorbing and clustering around acid sites (through the formation of strong hydrogen bonds), isolating and lowering the acid strength of these sites. In addition, water can promote the degradation and leaching of acid sites in sulphate based catalysts.

1.5.6. Enzymatic Catalysis

There has been a growing interest in the use of enzymes such as lipases as catalysis for biodiesel production. Sources of lipase include *Candida antartica*,[49, 51, 186] *Candida rugosa*,[187] *Pseudomonas cepacia*,[188-190] *Pseudomonas sp.*[191] or *Rhizomucor miehei.*[191, 192] The yield of biodiesel from enzyme catalysis can vary depending on the type of enzyme used, as summarised in Table 1.11. Some of the advantages of enzymatic transesterification over the chemically-catalysed reactions includes the fact that by-products are not produced, products can be easily extracted, mild reaction conditions can be adopted (temperature, 35–45 ºC), and the catalyst can be recycled.[193]

It has been reported that enzymatic reactions are insensitive to FFA and water content; therefore, they can be used in the transesterification of waste cooking oil.[194-196] To establish enzymatic catalysis at an industrial level, there is still a need to optimise the reaction conditions (temperature, alcohol: oil molar ratio, type of microorganism which generates the enzyme, enzyme amount, time, etc.).[197] Other conditions such as pH, use of solvent, use of immobilised or free enzyme and water content are also important in order to obtain higher conversion rates.[198-201]

Table 1.11. Summary of some studies using enzymes as a catalyst in the production of biodiesel.

Source of enzyme Microorganism	Oil	Temp °C	Time hr	Catalyst % (w/w)	Conversion % (w/w)	Ref
Candida antarctica	soybean	30	3.5	4% (w/w) lipase	97	197
Cryptococcus spp. S-2	ricebran	30	120	2000U of crude lipase	80.2	62
Candida antarctica	soybean	30	48	4% (w/w) lipase	93.8	49
Candida antarctica	cottonseed	50	7	30% (w/w) lipase	91.5	202
Rhizopus oryzae	palm	35	96	200 IU/mL lipase	55	203
Rhizomucor miehei	soybean	36.5	6.3	8% (w/w) lipase	92.2	204
Chromobacterium viscosum	jatropha	40	8	10% (w/w) lipase	92	205

1.5.6.1. Limitations of Enzymatic Catalysis

Enzyme reactions are highly specific and chemically clean; the main problem of the lipase-catalysed process is the high cost of the lipases.[79] Most lipases are also inhibited by alcohol. In order to overcome this issue, a typical strategy is to feed the alcohol into the reactor in three steps of 1:1 methanol: oil molar ratio each. The reactions are very slow in nature, with a three step sequence requiring from 4 to 40 hr or more to complete. If the reaction temperature is increased, enzymes can be denatured instead of increasing the rate of reaction.[206] Table 1.12 presents a comparison between enzymatic transesterification and alkaline transesterification.

Table 1.12. Comparison of enzymatic process versus conventional alkaline technology for biodiesel production.[207]

Key issues	Enzymatic process	Alkaline process
Presence of free fatty acid in the starting oil	FFAs are transformed to biodiesel	FFAs are transformed to soaps
Water content of the starting oil	It is not deleterious for lipase	Impact on the catalyst by forming soaps. It may hydrolyse the oil and ultimately more soaps are formed
Biodiesel yield[a]	High, usually around 90% (w/w)	High, usually >96% (w/w)
Glycerol recovery	Easy, high grade glycerol	Complex, low grade glycerol
Catalyst recovery and reusage	Easy or not necessary when operating in a packed bed reactor (PBR). Reusability not sufficiently studied	Difficult or not profitable, usually it is neutralised by adding an acid after transesterification, its is partially lost as soaps or in the successive washing steps
Energy costs	Low, temperature range 20-50 ^{o}C	Medium, temperature range 60-80 ^{o}C
Catalyst cost	High	Low
Environmental impact	Low, waste water treatment not needed	Medium, alkaline and saline effluents are generated waste water treatment needed
Process productivity[b]	Low	High

[a] Percentage of starting oil transformed to biodiesel
[b] Mass of biodiesel produced per volume of reactor and per unit of time

1.5.7. Non-Catalytic Transesterification

The transesterification of triglycerides by supercritical methanol (SCM), ethanol, propanol and butanol has been investigated as another promising process.[208] The use of supercritical technology in biodiesel production is an emerging technology. The conversion of oil is a very slow reaction, due to the poor miscibility of methanol and oil. Therefore, non-catalyst options are designed to overcome the reaction initiation lag time caused by the extremely low solubility of the alcohol in the triglyceride phase.

This approach utilises methanol at very high temperature and pressure. Supercritical methanol not only acts as a solvent but also as an acid catalyst.[209] The reaction is reported to be completed in about 4 min by using a high (42:1) alcohol to oil ratio, under supercritical conditions (350-400 ºC and >80 atm or 1200 psi). Reaction in supercritical methanol offers some advantages. First, glycerides and free fatty acids react at equivalent rates. Second, the homogeneous phase eliminates diffusion problems. Third, the process tolerates high percentages of water in the feedstock catalytic process that otherwise would require the periodical removal of water in the feedstock or at an intermediate stage to prevent catalyst deactivation. Fourth, the catalyst removal step is eliminated. And fifth, if high methanol: oil ratios are used, total conversion of the oil can be achieved in a few minutes.[29, 97, 210]

The use of supercritical alcohol for the transesterification of neat vegetable oil is widely reported in the literature.[55,96,97,206] However, its application in the transesterification of waste cooking oil is not well documented.

Another approach, which is now commercialised, is the use of a co-solvent that is soluble in both methanol and oil. The result is a fast reaction, of the order of 5-10 min, and there are no catalyst residues in either of the ester or the glycerol phase. One such co-solvent is tetrahydrofuran; used because it has a boiling point very close to that of methanol and the system requires a rather low operating temperature of 30 ºC.[206]

1.5.7.1. Limitations of Supercritical Methanol

Despite having all these advantages, the supercritical methanol method has some serious disadvantages.[79] These include the following:

(1) The process operates at very high pressures (25–40 MPa).

(2) The high temperatures (350–400 ºC) result in proportionally high heating and cooling costs.

(3) High methanol: oil molar ratios (usually set at 42:1) involve high costs for the evaporation of the unreacted methanol.[211]

1.6. METHODS FOR THE CHARACTERISATION OF BIODIESEL

The characterisation of FAMEs (biodiesel) after the transesterification reaction is an important issue for biodiesel quality control since potential contaminants of biodiesel can arise during the reaction process *i.e.* unreacted TAG, partial glycerides, FFA, residual methanol and catalyst. Monitoring allows biodiesel producers to recognise and correct problems at an early stage (before storage). Various analytical methods have been developed for analysing mixtures containing fatty acids esters and mono-, di-, and tri-glycerides obtained by the transesterification of vegetable oils.

1.6.1. Thin Layer Chromatography Method

The first method used for monitoring the transesterification reaction of vegetable oils was thin layer chromatography (TLC) by Freedman *et al.*[124] Using this method, fatty esters, tri- di-, and monoglycerides can be analysed. The analysis time is quite short; 30 samples could be analysed in 2–3 hr. However, this method shows lower accuracy, sensitivity to humidity, material discrepancies as well as high cost of the instrument. Therefore, other TLC technologies based on silica gel methodology were developed whereby the area of triacylglycerol spot from the mixture is compared to a standard.[212] However, the analysis is only qualitative and does not allow the exact determination of the degree of conversion. Actually, TLC is still used for qualitative analyses especially for the evaluation of oil conversion, since it is fast and effective.[213]

1.6.2. Gas Chromatography Method

Freedman *et al.*[74] developed the first GC methodology to monitor fatty acids, tri, di-, and monoglycerides in the transesterification reaction of soybean oil. Before

performing the analyses, mono- and diglycerides have to be silylated with *N*, *O*-bis (trimethylsilyl) trifluoroacetamide (BSTFA). The complete separation of acylglycerols and fatty esters was obtained in a run time of 12 min.[214]

A GC–FID method for the quantification of fatty acid methyl esters and glycerides in biodiesel, without the need for sample derivatisation, has also been described and now form the basis of the EN standard 14103 method.[215, 216] Evaluation is performed by measuring the peak areas of esters during the reaction. The advantages of this method are the use of cheap polar columns, high accuracy and precision as it does not require special preliminary sample preparation.[217] However, this method requires the use of several internal standards (lauric, myristic, palmitic, stearic, oleic, linoleic, and linolenic esters). This method also allows the determination of short-chain fatty acid esters (C_8–C_{12}), which occur in some biodiesel samples from coconut and palm oil. The use of fresh standard solutions is necessary as the stability of the methyl heptadecanoate (internal standard) influences the values of ester content.

Glycerol is a major by-product in the manufacturing of biodiesel. Its removal is necessary as it can cause engine damage and hazardous emissions. Plank *et al*. developed an important GC-FID procedure, which later became the EN 14105 and ASTM 6584 method. This method allows the simultaneous determination of glycerol, mono-, di-, and triglycerides in vegetable methyl esters.[218] The determination was achieved by the silylation of the hydroxyl groups, employing *N*-methyl-*N*-trimethylsilyltriflouroacetamide (MSTFA), followed by capillary GC analysis using a DB-5 column (10 m × 0.32 mm). Moreover, the use of 1,2,4-butanetriol and tricaprin, as internal standards, allowed for reliable quantitative analysis within a run time of 40 min. Therefore, this method is suited for the quality control of biodiesel.[219]

1.6.3. High Performance Liquid Chromatography

The transesterification reaction can also be characterised by using reversed phase-HPLC. The first reversed phase-HPLC with density detection (DD) method was developed by Trathnigg and Mittelbach[220] to determine the overall content of tri-, di-, monoglycerides and methyl esters in the biodiesel samples. Subsequently, other detection methods (UV at 205 nm, ELSD, APCI–MS and atmospheric pressure chemical ionization–mass spectrometry) were developed to monitor the transesterification of rapeseed oil to methyl esters and to quantify the residual content of triacylglycerol.[221] These detection methods are suitable for the analysis of complex mixtures due to their compatibility with gradient elution, which is necessary for good resolution of methyl esters and mono-, di, and triacylglycerols. Besides the non-quantification of saturates, the main disadvantage of UV detection is the weak absorbance of acylglycerols at wavelengths higher than 220 nm. However, the sensitivity of APCI–MS and ELSD decreases strongly with an increase in the number of double bonds in acylglycerols. Even so, APCI–MS is considered the most suitable detection method for biodiesel analysis.

1.6.4. Nuclear Magnetic Resonance Method

The first work on the utilisation of nuclear magnetic resonance was described in 1995, particularly ^{1}H NMR,[222] for monitoring the yield of the transesterification reaction. The peaks of the methylene group adjacent to the ester moiety in triacylglycerols (α-CH_2, 2.3 ppm, *t*) and the methoxy group in the esters (OCH_3, 3.7 ppm, *s*) were used to follow the progress of the reaction. The conversion was calculated from the areas of the aforementioned peaks, using the equation:

$$C = 100 \times (2A_{OCH_3}/3A_{\alpha\text{-}CH_2})$$

The use of ^{13}C NMR for monitoring rapeseed oil transesterification was mentioned by Monteiro *et al*. in 2008.[75] The signal at 14.5 ppm for the terminal methyl

groups, which are not affected by the reaction, was chosen as an internal standard, and the glyceridic carbons at 62–71 ppm together with the methoxy carbon of fatty esters at 51 ppm were selected to determine the conversion rate.[223] The authors state that this method is faster and simpler than the chromatographic based methods. Moreover, a small amount of sample is required and it could be analysed without a pre-purification process. However, instrumentation and maintenance costs are relatively high and must be evaluated.[224]

1.6.5. NIR Spectroscopy Method

The first reported work in this area is from Knothe,[225, 226] who developed a fiber-optic near infrared (NIR) method to monitor the transesterification reaction of soybean oil, based on the differences in the NIR spectra at 6005 and 4425–4430 cm^{-1}, where fatty esters display bands and triglycerides exhibit shoulders. The NIR method can also be used for the quantification of methanol in biodiesel, which can be an alternative to flash point evolution of biodiesel.

1.6.6. Measurement of Viscosity

The use of an acoustic wave solid-state viscometer (ViSmart^{TM}) to monitor the transesterification reaction has also been described.[227] The progress of the reaction is evidently indicated by the decreasing viscosity of the mixture. The endpoint of the transesterification reaction could be detected by using viscometry data and therefore could be used in the future to monitor the batch production process of biodiesel. The main advantage of the use of this kind of viscometer is that it does not require an extra step for measuring the density.

1.7. OBJECTIVES OF THE PROJECT

Substantial effort has been invested to find and/or develop homogeneous and heterogeneous catalysts for the transesterification of plant oil to form biodiesel. Few studies have reported the detailed kinetics of the reaction, which provides important insight into catalyst activation-deactivation and catalyst selectivity, both for homogeneous as well as heterogeneous catalysts. A comprehensive understanding is also unavailable in the databases regarding the function and possible improvements of solid catalysts in the biodiesel formation. Part of the problem lies in the lack of a suitable methodology to obtain sufficient measurements within the time-scale of the reaction(s).

The aim of this research work is to explore a suitable and efficient method for the production of FAMEs by understanding the activation route and kinetic profile of both homogeneous and heterogeneous catalytic systems.

Specific objectives related to the aim of this research are as follows:

1) To study the influence of reaction conditions on the production of biodiesel, using mainly, unrefined rapeseed oil. In relation to current methodologies used for producing FAMEs from rapeseed oil and other refined oils, specific parameters, listed below, will be examined in order to achieve ester content above 96.5% (w/w), EN standard 14103-but at a low cost:

- the amount of homogeneous catalyst (NaOH) used for maximum conversion.
- the amount of alcohol (CH_3OH) required for the transesterification reaction after the homogeneous catalyst (NaOH) has been optimised.

2) To explore the use of heterogeneous catalyst for the production of biodiesel, such as strontium oxide which has recently been shown to be an effective catalyst in biodiesel formation and which in turn provides scope for expanding the portfolio of

suitable catalysts for oil transesterification. Design of a biodiesel production process is relatively easy, but design of a heterogeneous catalyst for biodiesel production is not. The catalyst must be active, stable and relatively easy to procure on a large scale. It is difficult to design a catalyst for the transesterification reaction between methanol and vegetable oils because:

- triglycerides are bulky molecules, so internal diffusion limitations are significant.
- the reactants are initially in two phases and have very different polarities.
- the polar character of the undesired co-product, glycerol, could limit the ester conversion.
- feedstock may contain contaminants and water which may deactivate catalysts.

One of the major problems associated with heterogeneous catalysis is the formation of three phases with alcohol and oil. It can be postulated that the slow transesterification rate is mainly due to slow mass transfer between polar methanol/glycerol and non-polar oil phases. If the system methanol-oil-catalyst forms a homogeneous liquid phase, mass transfer limitation between the partially miscible reactants methanol and oils will be overcome. Therefore, further investigations of the transesterification reaction in a single phase will be carried out with the hypothesis that the reaction will proceed at a faster rate. The data obtained will allow the design of a continuous-flow process for biodiesel production.

3) The online monitoring of reaction kinetics will be investigated, to pave the foundations for reaction optimisation *i.e.* phase changes in going from oil to FAMEs. A study of the kinetics of the transesterification reaction will provide parameters that can be used to predict the extent of the reaction as a function of time under particular conditions.

4) In addition to the aforementioned objectives, studies to determine the affect of glucosinolate content in rapeseed oil will be conducted. It has been observed that glucosinolates significantly hamper the catalytic activity of metal oxides. Therefore, research to determine the effect of glucosinolates on catalyst deactivation would also be interesting to conduct.

5) Finally, to set up analytical methods according to European standards EN 14103 and EN 14105, in order, to characterise the product obtained were necessary.

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CHAPTER 2

EXPERIMENTAL AND INSTRUMENTATION

2.1. MATERIALS

The oils and chemicals used in the experimental studies are listed in Table 2.1 and 2.2, respectively.

Table 2.1. Oils used.

Oils	**Supplier**
Unrefined rapeseed oil	Weald Granary Ltd. Maidstone, UK
Refined rapeseed oil	Tesco Stores, UK
Refined sunflower oil	Tesco Stores, UK
Refined grapeseed oil	Tesco Stores, UK
Refined corn oil	Tesco Stores, UK
Refined soya oil	Tesco Stores, UK
Refined groundnut oil	Tesco Stores, UK

Table 2.2. Chemicals used.

Chemical Name	Chemical Formula	Molecular Weight	Chemical Supplier	Purity %
Methanol	CH_3OH	32.04	Fisher Scientific	≥ 99.0
n-Heptane	C_7H_{16}	100.21	Fisher Scientific	+ 99.5
Isopropyl alcohol	C_3H_80	60.1	Fisher Scientific	≥ 99.0
Sodium hydroxide	NaOH	39.99	Sigma Aldrich	≥ 98.0
Sodium methoxide	CH_3ONa	54.02	Fluka	≥97.0
Potassium hydroxide	KOH	56.11	Sigma Aldrich	≥90.0
Glycerol trioleate	$(C_{17}H_{33}COOCH_2)CHOCOC_{17}H_{33}$	885.43	Sigma	≥99.0
Methyl heptadecanoate	$CH_3(CH_2)_{15}COOCH_3$	284.48	Fluka	≥99.5
Strontium oxide	SrO	103.62	Aldrich	99.9
Lanthanum oxide	La_2O_3	325.81	Aldrich	99.9
Yttrium oxide	Y_2O_3	225.81	Aldrich	99.9
Gadolinium oxide	Gd_20_3	362.50	Aldrich	≥99.9
Titanium oxide	TiO_2	79.87	Fluka	≥99.9
Tin oxide	SnO_2	150.71	Aldrich	≥99.9
Zirconium oxide	ZrO_2	123.22	Aldrich	99.9
Cerium oxide	CeO_2	172.11	Aldrich	99.9
Pyridine	C_5H_5N	79.10	Aldrich	99.9

± -1,2,4-Butanetriol	$HOCH_2CH_2CH(OH)CH_2OH$	106.12	Aldrich	95.0
Tricaprin	$C_{33}H_{62}O$	554.847	Aldrich	99.9
N-methyl- *N*- (trimetyl silyl) triflouroacetamide	$CF_3CON(CH_3)Si(CH_3)_3$	199.25	Fluka	≥98.5
1- oleoyl-rac-glycerol	$CH3(CH2)7CH=CH(CH_2)COOCH_2CHOHCH_2OH$	356.54	Sigma	~99.0
1,3- Diolein	$C_{39}H_{72}O_5$	620.99	Sigma	≥99.0
Methyl palmitate	$CH_3(CH_2)_{14}CO_2CH_3$	270.45	Fluka	≥99.0
Methyl stearate	$CH_3(CH_2)_{16}CO_2CH_3$	298.50	Fluka	≥99.5
Methyl oleate	$CH_3(CH_2)CH=CH(CH_2)_7CO_2CH_3$	296.49	Fluka	≥99.0
Methyl linoleate	$CH_3(CH_2)_3(CH_2CH=CH)_2(CH_2)_7CO_2CH_3$	294.47	Fluka	≥98.5
Methyl linolenate	$CH_3(CH_2CH=CH)_3(CH_2)_7COOCH_3$	292.46	Fluka	≥99.0
Methyl behenate	$CH_3(CH_2)_{20}COOCH_3$	354.61	Fluka	≥99.0
Methyl erucate	$CH_3(CH_2)_7CH=CH(CH_2)_{11}COOCH_3$	352.59	Fluka	≥99.0
Methyl lignocerate	$CH_3(CH_2)_{22}COOCH_3$	382.66	Fluka	≥99.0
Glucosinolate	-	-	Sigma	-
EN 14105: 2003 Standard 4	-	-	Supelco	-
Biodiesel EN 14105 - Kit	-	-	Agilent Technologies	-
Monoglyceride stock solution	-	-	Supelco	-
Hydranal®- coulomat AG	-	-	Fluka	-
Hydranal® water standard	-	-	Fluka	-

2.2. EXPERIMENTAL PROCEDURES

2.2.1. Production and Purification of Biodiesel (FAMEs) by Using Homogeneous Catalysis

The transesterification reaction was carried out by preheating 100 g (0.113 mol, 1 eq) of triglycerides, at 60 ºC (333K) in a beaker on a hot plate with continuous magnetic stirring (600 rpm). Methanol and the catalyst were premixed separately and added to the oil. Each reaction was conducted for 60 min in order to achieve the conversion of the triglycerides into FAMEs in the selected time frame. To avoid the escape of methanol into a gas phase during the reaction, the reaction temperature was maintained below the boiling point of methanol.

After 60 min, the reaction mixture was allowed to cool down and equilibrate, which resulted in the separation of the two phases. The upper phase consisted of methyl esters, and the lower phase contained the glycerol, the excess methanol, the remaining catalyst together with the soaps formed during the reaction and some entrained methyl esters and partial glycerides. After separation of the two layers, the lower aqueous layer was removed. The remaining ester layer was washed with distilled water (3:1 biodiesel: water v/v) in order to remove any water soluble impurities. The washing of the ester layer was conducted three times to ensure the removal of catalyst. This was done by checking the pH of the ester layer. The residual water was eliminated by removing it using a separating funnel. The ester layer was dried at 60 ºC for 1-hour in a beaker over a hot plate with continuous stirring. Finally, the FAMEs content were determined by preparing samples for GC-FID.

The sets of experiments carried out by using this experimental procedure are detailed in Table 2.3.

Table 2.3. Experimental conditions used for the transesterification reaction by using homogeneous catalysts.

Experiments	Triglyceride type 100 g (0.013 mol)	Catalyst type and amount	Methanol amount
Transesterification of glycerol trioleate	glycerol trioleate	0.015 mol NaOH	6:1 CH_3OH/oil
Transesterification of unrefined rapeseed oil	unrefined rapeseed oil	0.0075-0.022 mol NaOH	3:1 CH_3OH/oil – 15:1 CH_3OH/oil
Comparison of catalysts	unrefined rapeseed oil	0.015 mol NaOH and CH_3ONa	6:1 CH_3OH/oil
Transesterification of refined vegetable oils	rapeseed oil, sunflower oil, corn oil, soya oil, grapeseed oil, groundnut oil	0.015-0.022 mol NaOH	6:1 CH_3OH/oil

2.2.2. Production and Purification of Biodiesel (FAMEs) by Using Heterogeneous Catalysis

Unrefined rapeseed oil (100 g, 0.113 mol, 1 eq) was heated to 60 °C (333K) with continuous stirring at 600 rpm. Then methanol (6 eq) and catalyst were transferred to the preheated oil. The amount of catalyst and reaction time chosen for these experiments was 3% (w/w) and 120 min, respectively. The type of catalysts used and their selection criterion is given in Table 2.4. After reaction completion, the mixture was centrifuged at 8000 rpm for 5 min, resulting in the separation of three phases. The methyl ester layer was decanted from the glycerol layer and catalyst. The methyl ester layer was used for chromatographic analysis.

Table 2.4. Type of catalysts studied for the transesterification reaction.

Catalysts	Reason
Gadolinium oxide (Gd_2O_3)	Mild basic oxide
Cerium oxide (CeO_2)	Mild basic oxide
Yttrium oxide (Y_2O_3)	Basic oxide
Titanium oxide (TiO_2)	Amphoteric oxide
Tin oxide (SnO_2)	Amphoteric oxide
Zirconium oxide (ZrO_2)	Amphoteric oxide
Lanthanum oxide (La_2O_3)	Strongly basic oxide
Strontium oxide (SrO)	Strongly basic oxide

2.2.3. Transesterification Reaction Using SrO Catalyst

The amount of catalyst and the reaction time used for the experiments are given in Table 2.5. All the other experimental procedures were the same as described in Section 2.2.2.

Table 2.5. Experimental conditions for the transesterification reaction using a SrO catalyst.

Oil :CH_3OH 1:6 molar ratio, Temperature = 60 ºC, Stirring speed = 600 rpm					
Experiment	**Amount of SrO used (% w/w)**	**Reaction time (min)**	**Experiment**	**Amount of SrO used (% w/w)**	**Reaction time (min)**
1	3	60	**10**	3	240
2	5	60	**11**	5	240
3	7	60	**12**	7	240
4	3	120	**13**	3	300
5	5	120	**14**	5	300
6	7	120	**15**	7	300
7	3	180	**16**	3	420
8	5	180	**17**	5	420
9	7	180	**18**	7	420

2.2.4. Addition of Glucosinolate in the Transesterification of Unrefined Rapeseed Oil

This set of experiments was carried out by using preheated (100 g, 0.113 mol, 1 eq) unrefined rapeseed oil at 60 °C. In the first experiment, 3% (w/w) SrO and 6 eq CH_3OH were added to the preheated oil. The second and third experiments were set up using similar amounts of SrO and methanol. Additionally, 10 mg (0.01% w/w) or 100 mg (0.10% w/w) glucosinolate were added, respectively with SrO and methanol to the preheated oil. All three experiments were terminated after 120 min and the mixture centrifuged at 8000 rpm for 5 min. The methyl ester layer was decanted from the glycerol layer and catalyst for chromatographic analysis.

2.2.5. Refractive Index Measurement of the Transesterification Reaction with SrO Catalyst

The control experiment was carried out by mixing a 6:1 molar ratio of methanol and unrefined rapeseed oil. The mixture was stirred continuously at 60 °C. The refractive index values were monitored every 15 sec for five minutes.

The set of experiments was designed to determine the refractive indices of the transesterification reaction in the presence of heterogeneous catalyst (SrO) at different time intervals. Unrefined rapeseed oil (25 g, 1 eq, 0.0282 mol) was heated at 60 °C. Then methanol (6 eq, 5.41g, 6.84 mol) and the catalyst (3% w/w) were added to the reactor under vigorous stirring. The reaction was stopped by centrifugation at 8000 rpm at different time intervals between 0 and 120 min. The methyl ester layer was decanted and the refractive index measured at 60 °C. For the purpose of correlation and verification of the results obtained by refractometry, the quantification of methyl esters was also carried out by GC on different samples collected between 0 to 120 min. Each experiment was conducted in triplicate.

2.2.6. Generation of a Phase Diagram

A series of experiments were carried out to determine the solubility of rapeseed oil and methanol at different concentrations of FAMEs at 60 °C.

The FAMEs were added using a micro-burette to a stirred mixture of methanol and oil of known composition in a sample bottle. The point, when the mixture changed from transparent to turbid, was considered the saturation point of FAMEs in oil and methanol solution. The volume of FAME required to achieve miscibility is given in Table 2.6. The compositions of the resulting mixtures obtained for methanol/oil combinations were plotted as a phase diagram (Chapter 5). In order to validate the results generated from varying the oil to methanol vol. %, another set of experiments was carried out by varying the FAMEs/methanol vol. % and then gradually adding the oil.

Table 2.6. Volume (%) of FAMEs used for miscibility of CH_3OH/oil mixture.

Data Entry	Oil (vol %)	Methanol (vol %)	FAME (vol %)
1	01	89	10
2	04	77	19
3	03	68	29
4	08	55	37
5	16	42	42
6	23	31	46
7	26	22	52
8	42	10	48
9	78	02	20

2.2.7. Transesterification Reaction Using SrO in the Miscibility of Oil and Methanol

The transesterification reactions were carried out by using the same vol. % of FAME, oil and CH_3OH (shown as the blue point on the phase diagram, Figure 2.1) for experiments **1**-**4**. In these experiments, 60 mL FAME and 33 mL oil, were added and heated to 60 °C.

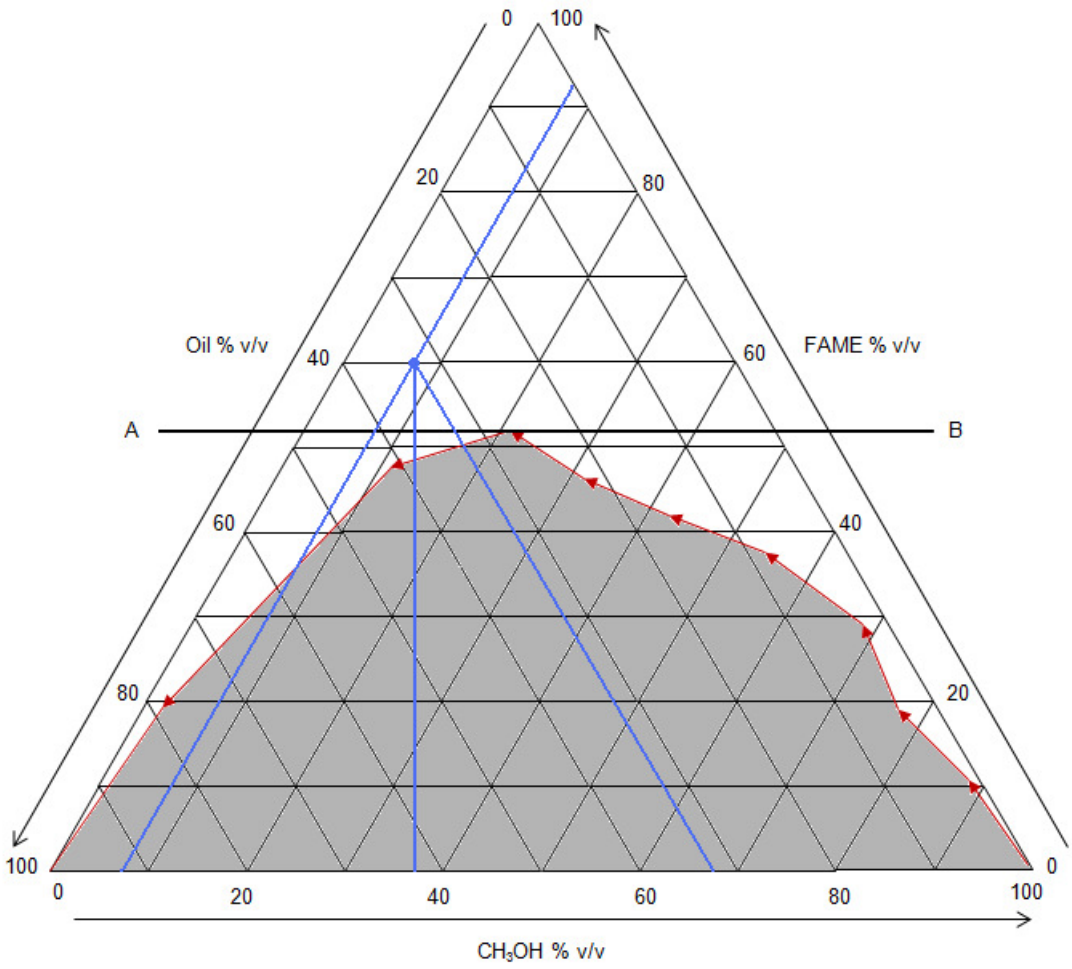


Figure 2.1. Ternary phase diagram to show the miscibility properties of rapeseed oil–methanol–FAME at 60 °C. Rapeseed oil–methanol–FAME was titrated to the point of miscibility determined by turbidimetric analysis using titration. Blue line intersect shows the miscible region (60 mL FAME: 33 mL oil: 7 mL) on the ternary phase diagram. Line A to B represents the point of miscibility. The shaded area is the immiscible region and the un-shaded area is the miscible region.

The FAME samples used in these experiments were prepared in advance by using a similar method to that stated in Section 2.2.1. However, the FAME samples used in these set of experiments differ from each other in term of ester content (%) detailed in Table 2.7. Once the reaction mixture attained the required reaction temperature, 7 mL CH_3OH and 3% (w/w) SrO was added at time zero. The reaction times are given in Table 2.7. In some experiments, the addition of methanol and

strontium oxide was carried out during the reaction. The amount of additional methanol and SrO added to the reactions are also given in Table 2.7, together with the timings of the additions.

A small amount of the reaction mixture was transferred at different time intervals on the prism cell to note the readings for refractive indices. In the case of chromatographic analyses, the reaction mixture was collected (approx. 1.5 mL) at different time intervals and centrifuged at 8000 rpm for 10 min to remove the methyl ester layer.

Table 2.7. Reaction conditions for experiments **1**-**4**.

Reactants: 60 mL FAME: 33 mL oil: 7 mL CH_3OH, Reaction temp: 60 °C, Catalyst: 3% (w/w) SrO			
Experiments	**FAME purity % (w/w)**	**Reaction time**	**Addition of CH_3OH and SrO during reaction**
1	88.6	45 and 60 min	-
2	93.6	90 min	3% (w/w) SrO at 50 min and 7 mL CH_3OH at 60 min
3	95.4	120 min	3% (w/w) SrO at 60 min and 7 mL CH_3OH at 80 min
4	95.4	120 min	7 mL CH_3OH at 60 min and 3% (w/w) SrO at 100 min

2.2.8. Comparative Studies of Miscible and Non-Miscible Phases with the Addition of Methanol during the Reaction

Experiments **5**-**8** were designed in order to compare transesterification reactions carried out in miscible and non-miscible phases. Experiments **5** and **7** carried out without using the phase diagram, whereas experiments **6** and **8** were carried out in a miscible region of a phase diagram. The reaction conditions of all experiments are specified in Table 2.8. Additional methanol was also added during the reaction in

experiments **5** and **6**. In the case of non-miscible experiments (**5** and **7**) 3.0 g (3% w/w) of SrO was used whereas for experiments in the miscible region (**6** and **8**) 0.904 g (3% w/w) was used. The method to conduct these experiments was the same as mentioned in Section 2.2.7 except for the reactant quantities.

Table 2.8. Reaction conditions for experiments **5**-**8**.

Experiments	Reactants			Extra addition of CH_3OH
	Oil	FAME	Methanol	
5	100 g	-	27.5 mL	-
6	33 mL	60 mL (95.4%)*	7 mL	27.5 mL at 60 min
7	100 g	-	27.5 mL	-
8	33 mL	60 mL (96.1%)*	7 mL	7 mL at 60 min

* Ester content (%) of FAME (reactant).

2.2.9. Transesterification in the Miscible Region of the Phase Diagram

Six data points were selected to carry out these experiments in the miscible region of a ternary phase diagram. Figure 2.2 shows the six data points on the phase diagram obtained after the intersection of three axes (Oil, CH_3OH and FAME). The volumes calculated from these points were used to set up the reactions.

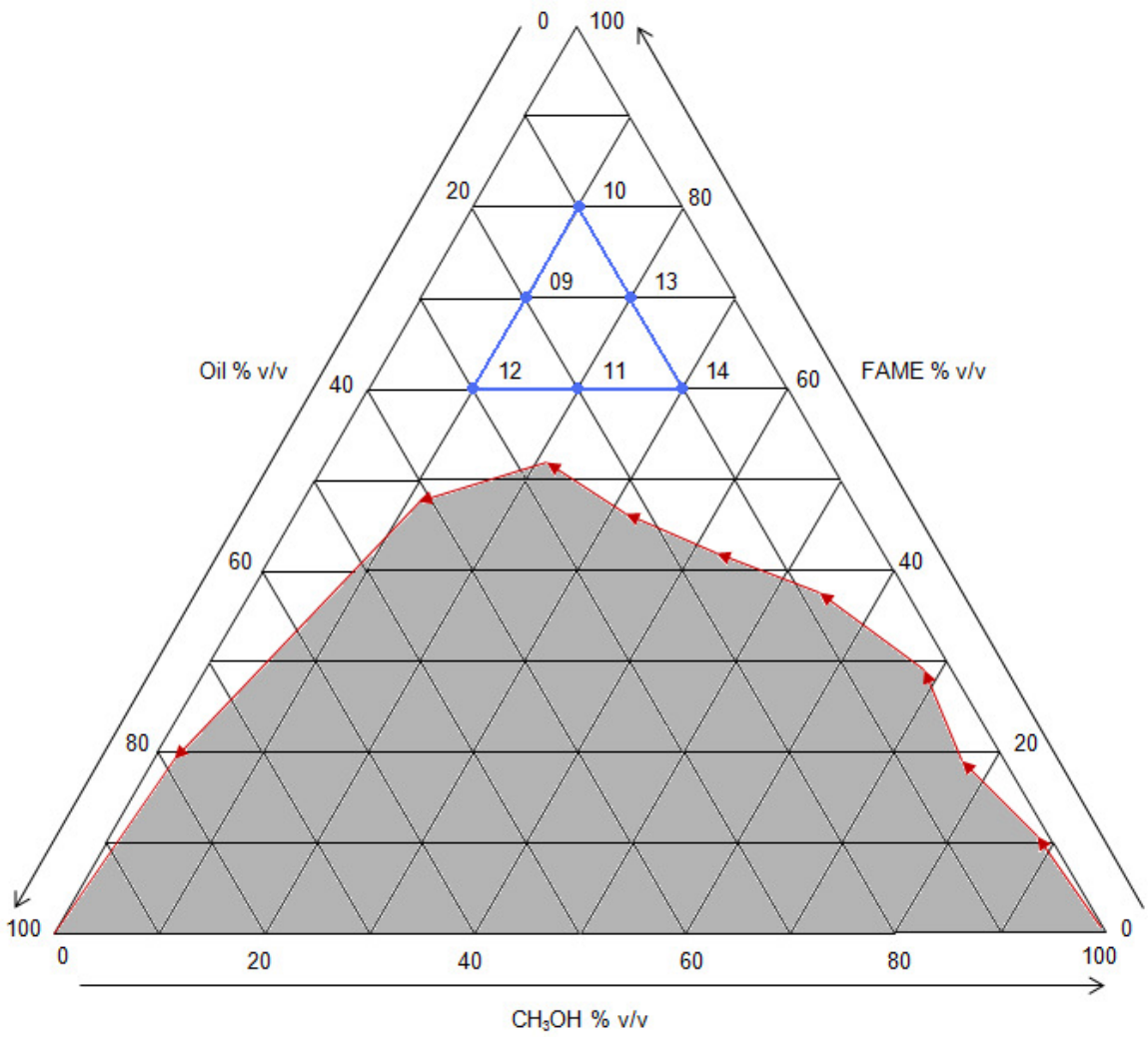


Figure 2.2. Data points of experiments **9**-**14** (shown in blue dots) in the miscible region of the phase diagram. The shaded area is the immiscible region and the un-shaded area is the miscible region.

Each experiment differs from each other in terms of vol. % ratio of the reactants used. The sample of FAME used as the reactant in this set of experiments had an ester content of 94.0% (w/w) and the other 6% (w/w) was total glycerol and glyceride content. The strontium oxide used in these experiments was with respect to the total amount of triglycerides used *i.e.* vol. % ratio of oil + 6 % unreacted glycerides present in the FAME sample. The amount of the reactants (FAME, oil, CH_3OH and strontium oxide) used are given in Table 2.9.

Table 2.9. Volume (%) ratio of FAME: oil: CH_3OH and SrO used for experiments **9**-**14**.

Experiments	FAME (vol %)	Oil (vol %)	CH_3OH (vol %)	SrO (g)
9	70	20	10	0.728
10	80	10	10	0.454
11	60	20	20	0.728
12	60	30	10	1.0026
13	70	10	20	0.454
14	60	10	30	0.454

2.2.10. Transesterification in the Miscible Region of Phase Diagram with Different Metal Oxides

The transesterification reactions were carried out by premixing 70 mL FAME and 20 mL oil in a beaker. The reaction mixture was heated to 60 °C. Subsequently, the prism cell for refractive index measurements was also heated up to 60 °C. After the temperature was reached, the catalyst and 10 mL CH_3OH were added at time t=0. The catalysts used were 0.728 g of CeO_2, Gd_2O_3, La_2O_3, ZrO_2, SnO_2, Y_2O_3, TiO_2, SrO in each experiment. During the experiments, the temperature was kept constant. 1.0 mL samples were collected from the reaction mixture to place on the prism cell for online monitoring of refractive indices. After a specific contact time (10, 20, 30, 40, 60 or 90 min), 1.5 mL of the reaction mixture was collected and centrifuged at 8000 rpm for chromatographic analysis.

2.2.11. Effect of Glucosinolate on the Ester Content (%) in a Miscible Region of Phase Diagram

Two different set of experiments were carried out by adding 20 mL oil and 70 mL FAME (94.0% w/w) in a reaction vessel. The reaction mixtures were heated to 60 °C. Once the reaction temperature was attained, 10 mL CH_3OH and 0.728 g SrO

were added in both experiments. Additionally, 100 mg of glucosinolate was added to the second experiment. After the addition of catalyst and methanol, 1.0 mL of the sample was transferred to the prism cell for the refractive index measurement. Meanwhile, at different time intervals (20, 30, 40, 50, 60, 80 and 120 min) a sample of approx. 1.5 mL was collected for chromatographic analysis. These samples were centrifuged at 8000 rpm for 10 min to decant the methyl ester layer.

2.3. ANALYTICAL METHODS

The biodiesel quality was evaluated according to the European biodiesel standard EN 14214 (2008).

2.3.1. Determination of Moisture Content

The coulometric Karl Fisher titration method (Table 2.10) was used for the determination of moisture content. The method used was derived from BS EN 12937: 2008 which meets the requirements of the European Standard. According to the European standard, the maximum water content in biodiesel should not be greater than 500 mg/kg (0.05 wt %) biodiesel. It is the most accurate method available to measure moisture content as low as 1 ppm of free, emulsified and dissolved water.

Table 2.10. Instrumental conditions used for moisture determination.

Instrument	Metrohm KF Coulometer 831
Sample preparation	None
Calibration	Hydranal® water standard 0.1% (w/w)
Analysis	The instrument was conditioned before use. A known weight (1-2 g) of the FAMEs or oil sample was injected into the titration cell. Each experiment was carried out in triplicate.

2.3.1.1. Calculation of Moisture Content

The mass fraction of water, *w*, expressed as a percentage, was determined using the following equation;

$$w = 100\ m_2 / m_1 \times 10^6$$

$$w = m_2 / m_1 \times 10^4$$

Where m_1 is the mass of the test sample, expressed in grams (g) and m_2 is the mass of water obtained by titration, expressed in micrograms (μg).

2.3.2. Determination of Acidity

The acid value is defined as the mass (mg) of potassium hydroxide required to neutralise the free fatty acids present in 1 g of sample. The acidity (Table 2.11) of the triglycerides and FAMEs were determined by volumetric titration. To meet the required European standard EN 14104, the acid value should not be greater than 0.50 mg KOH per g biodiesel.

Table 2.11. Instrumental conditions used for determination of acid value.

Instrument	Metrohm titrino 794 auto titrator
Sample preparation	KOH solution, 0.1 mol/L in isopropyl alcohol 0.1 M solution of KOH was prepared by dissolving 5.61 g with isopropyl alcohol in a 1000 mL volumetric flask.
Calibration	None
Analysis	An appropriate quantity of a sample was accurately weighed and dissolved in a *ca.* 50 mL of isopropyl alcohol. The solution was titrated with 0.1M KOH and the acid value of the sample were obtained as mg KOH/g. Each experiment was carried out in triplicate.

2.3.2.1. Calculation of Acidity

The acid value is reported as:

$$56.1 \times V \times C / \text{m}$$

Where V is the volume, in milliliters of standard volumetric potassium hydroxide solution used, C is the exact concentration, in moles per litre, of the standard volumetric potassium hydroxide solution used, m is the mass, in grams, of the test portion and 56.1 is the molecular mass of KOH.

2.3.3. Determination of Ester and Linolenic Acid Methyl Ester Content

The purity of biodiesel samples is measured in terms of their methyl ester content (% w/w), Tables 2.12/2.13**,** and is a very important parameter to ensure biodiesel quality. The European biodiesel standard EN 14103:2008 specifies a minimum purity of 96.5 (% w/w). For the linolenic acid methyl esters the European biodiesel standard specifies that values should be below the maximum limit of 12.0 (% w/w).

Table 2.12. System used for the determination of ester and linolenic acid methyl ester content.

Gas Chromatograph	Fison GC 8000
Injector	Split mode, 1.0-µl syringe with 0.47 mm ID needle
Detector	FID
Pneumatics	Carrier gas – Helium, FID gases – Air and Hydrogen
Guard Column	5m (Length) × 0.32mm I.D
Analytical Column	30m L× 0.32mm I.D × 0.25µm F.T DBWAX column

Table 2.13. Conditions used for the determination of ester and linolenic acid methyl ester content.

GC oven	200 °C
Carrier Gas	Helium at 2 mL/min with constant flow
Injector	Split mode 32:1, Temperature – 250 °C, Injection volume – 1.0-µL.
Detector	FID Range- ×1, Attn- ×4, Temperature – 250 °C, Air – 60 KPa, H_2 - 50 KPa.
Wash solvent	n- Heptane, Rinse – 3, Pump – 3.
Run time	22 min

2.3.3.1. Preparation of Internal Standard and Samples

The internal calibration standard for GC was prepared by diluting 500 mg of methyl heptadecanoate in 50 mL heptane. Methyl heptadecanoate solution 5 mL was added to 250 mg biodiesel sample and the volume was made up to 10 mL with heptane.

2.3.3.2. Preparation and Analysis of Standard Solution

Fatty acid methyl esters were identified by comparing their relative and absolute retention times with those of authentic standards. The standards used were methyl palmitate (C16:0), methyl stearate (C18:0), methyl oleate (C18:1), methyl linoleate (C18:2), methyl linoleneate (C18:3), methyl behenate (C22:0), methyl erucate (C22:1) and methyl lignocerate (C24:0). The standard samples were prepared in the same way as the biodiesel samples for chromatographic analyses (Section 2.3.3.1). Peak areas were integrated by using the Clarity software (version 2.5.6) manufactured by Data Apex Ltd.

2.3.3.3. Calculation of Fatty Acid Methyl Ester Content

The ester content (C), expressed as a mass fraction in percent, is calculated using

the following formula:

$$C = (\Sigma A)\text{-}A_{EI} \ / A_{EI} \times \ C_{EI} \times V_{EI} / m \times 100\%$$

Where ΣA is the total peak area from the methyl ester in C_{14} to that in $C_{24:1}$, A_{EI} is the peak area corresponding to methyl heptadecanoate, C_{EI} is the concentration, in milligrams per millilitres, of the methyl heptadecanoate solution being used, V_{EI} is the volume, in millilitres, of the methyl heptadecanoate solution being used and m is the mass, in milligrams, of the sample.

2.3.3.4. Calculation of Linolenic Acid Methyl Ester Content

The linolenic acid methyl ester content L, is expressed as a mass fraction in percent, is calculated using the following formula:

$$A_L / (\Sigma A)\text{-}A_{EI} \times 100\%$$

Where ΣA is the total peak area from the methyl ester in C_{14} to that in $C_{24:1}$, A_{EI} is the peak area corresponding to methyl heptadecanoate and A_L is the peak area corresponding to linolenic acid methyl ester.

2.3.4. Determination of Free and Total Glycerol and Mono-, Di-, Triglyceride Content

The concentrations of free/ total glycerol and mono-, di-, and triglycerides were determined by gas chromatography (Tables 2.14/2.15) based on European standard EN 14105. The European biodiesel standard EN 14105:2008 specifies a minimum free glycerol content of 0.020 (% w/w). For the mono-, di and triglycerides the European biodiesel standard specifies that values should be below the maximum limit of 0.80 (% w/w), 0.20 (% w/w) and 0.20 (% w/w), respectively.

Table 2.14. System used for the determination of free and total glycerol and mono-, di-, triglyceride content.

Gas Chromatograph	Agilent Technologies 7890A GC system equipped with 7683B series injector
Injector	Programmable on column (POC), 10.0-µl syringe
Detector	FID
Pneumatics	PPC for POC Carrier gas – Helium, PPC FID gases – Air and Hydrogen
Guard Column	2m (Length) integrated column
Analytical Column	14m L× 0.53mm I.D × 0.16µm F.T MET-biodiesel column

Table 2.15. Conditions used for the determination of free and total glycerol and mono-, di-, triglyceride content.

GC oven	50 °C (1) 15 °C/min 180 °C (1) 7 °C/min 230 °C (5) 10 °C/min 370 °C (5)
Carrier Gas	Helium at 3cm^3/min with constant flow
Injector	Cold on column: Oven tracking mode, Injection volume – 1.0-µL, Speed – Fast.
Detector	FID Temperature – 380 °C Nitrogen served as detector makeup gas, Air – 400 KPa, H_2 - 30 KPa.
Wash solvent	n- Heptane, Rinse – 3, Pump – 6.
Run time	42 min

2.3.4.1. Solution Preparation

Internal standard No. 1 stock solution, 1mg/mL

1,2,4-butanetriol 50 mg was pipetted into a 50 mL volumetric flask and made up to the mark with pyridine.

Internal standard No. 2 stock solution, 8mg/mL

1,2,3- tricaprinoyl glycerol 80 mg was weighed in a 10 mL volumetric flask and made up to the mark with pyridine.

2.3.4.2. Sample Preparation

Biodiesel sample 100 mg were mixed with 100 μL of 1,2,4-butanetriol (1 mg/mL, standard 1) and 100 μL of 1,2,3-tricaprinoylglycerol (8 mg/mL, standard 2). Other 100 μL of *N*-methyl-*N*-trimethylsilyl trifluoroacetamide (MSTFA, derivatisation grade) was added to convert both free and total glycerol into volatile compounds. After 15 min, 8 mL of heptane was added as a solvent. Samples (1 μL) were injected into the gas chromatograph analyser for glycerol, TG, DG, and MG determination.

2.3.4.3. Calibration

For the quantitative determination of free glycerol, mono-, di-, and triglycerides in FAMEs a calibration using reference standards of glycerol, mono-, di-, and triolein was carried out. Freshly prepared standard solutions (4 concentration levels), containing known amounts of the standards glycerol, mono-, di-, and triolein and both internal standards (1, 2, 4-Butanetriol and tricaprin) were analysed three times. The calibration graphs for glycerol, mono-, di-, and triolein are shown in Figure 2.3. The correlation coefficient (R^2) values for glycerol and mono-, di-, and triolein were according to the specification of EN 14105 standard.

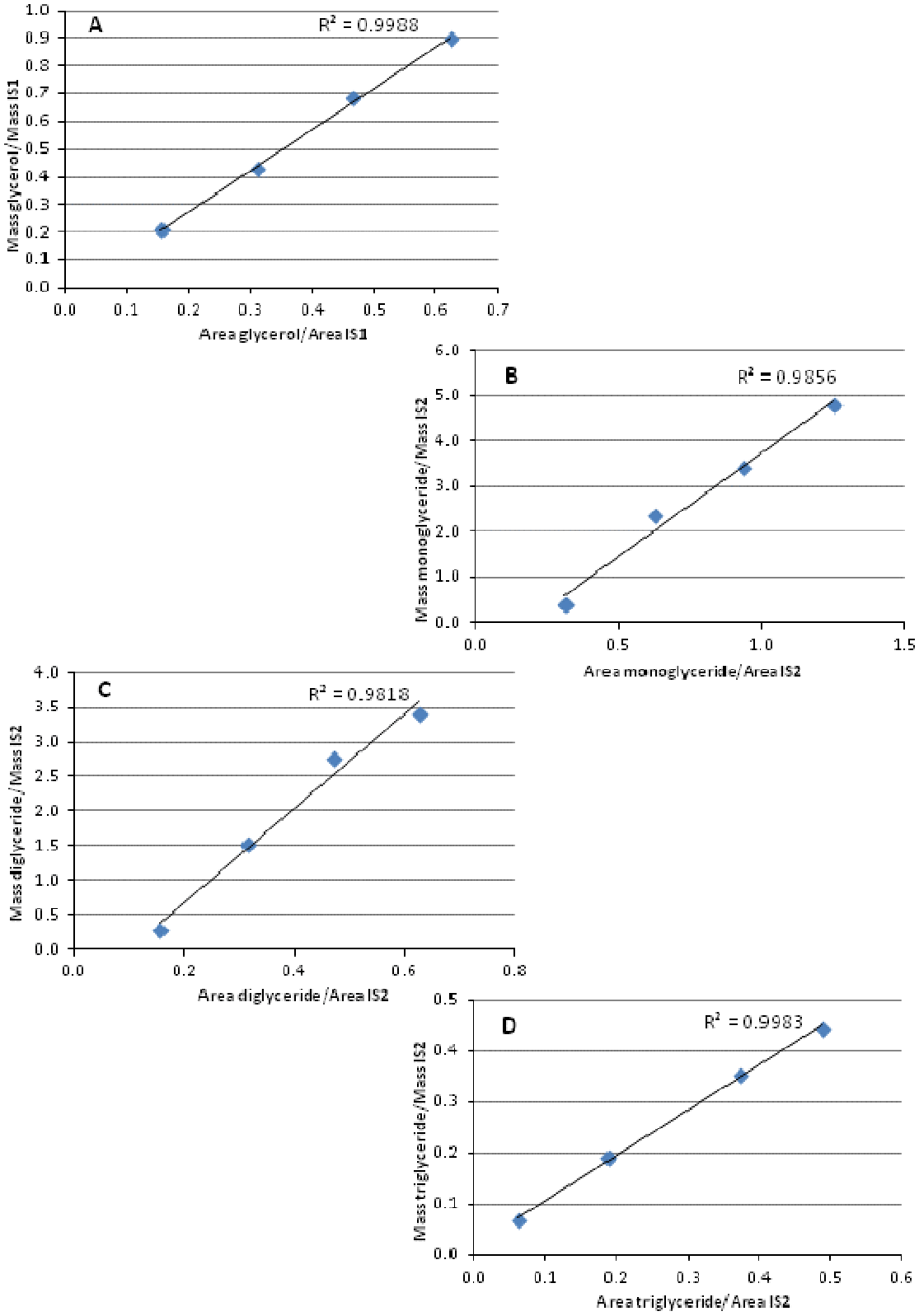


Figure 2.3. Calibration graphs for glycerol and glyceride content. **A**: glycerol content, **B**: monoglyceride content, **C**: diglyceride content, **D**: triglyceride content. Measured points by analysis of a known quantity of standard, (linear line of best fit—).

2.3.4.4. Glycerol Calibration Function

The calibration function is given by the following expression (obtained from the experimental data using the linear regression method):

$$M_g/M_{ei1} = a_g\,(A_g/A_{ei1}) + b_g$$

Where M_g is the mass of glycerol (mg); M_{ei1} is the mass of internal standard No.1 (mg); A_g is the peak area of glycerol; A_{ei1} is the peak area of internal standard No. 1; a_g and b_g are constants obtained from regression analysis for glycerol.

2.3.4.5. Glycerides Calibration Function

The calibration functions are given by the following expressions, obtained from the experimental data using linear regression analysis:

$$M_m/M_{ei2} = a_m\,(A_m/A_{ei2}) + b_m$$
$$M_d/M_{ei2} = a_d\,(A_d/A_{ei2}) + b_d$$
$$M_t/M_{ei2} = a_t\,(A_t/A_{ei2}) + b_t$$

Where M_m, M_d, M_t are, respectively, the mass of monoolein, diolein and triolein (milli grams); M_{ei2} is the mass of internal standard No.2 (milligrams); A_m, A_d, A_t are the peak areas, respectively, of monoolein, diolein and triolein; A_{ei2} is the peak area of the internal standard No. 2; a_m and b_m are constants obtained from regression analysis of monoglycerol; a_d and b_d are constants obtained from regression analysis for diglycerol; a_t and b_t are constants obtained from regression analysis for triglycerol;

2.3.4.6. Calculation of the Percentage of Free Glycerol

The percentage (*w/w*) of free glycerol in the samples can be calculated by using the following expression:

$$G = [a_g (A_g/A_{ei1}) + b_g] \times (M_{ei1}/m) \times 100$$

Where G is the percentage (*w/w*) of free glycerol in the sample; A_g is the peak are of the glycerol; A_{ei1} is the peak are of internal standard No. 1; M_{ei1} is the mass of internal standard No. 1 (milligrams); m is the mass of sample (milligrams); a_g and b_g are constants obtained from regression analysis for glycerol.

2.3.4.7. Calculation of the Percentage of Glycerides

The percentage (*w/w*) of the mono-, di- and triglycerides can be calculated by using the following expressions:

$$M = [a_m (\sum A_{mi}/A_{ei2}) + b_m] \times (M_{ei2}/m) \times 100$$
$$D = [a_d (\sum A_{di}/A_{ei2}) + b_d] \times (M_{ei2}/m) \times 100$$
$$T = [a_t (\sum A_{ti}/A_{ei2}) + b_t] \times (M_{ei2}/m) \times 100$$

Where *M, D, T* are the mono-,di-and triglycerides percentage (*w/w*) in the samples; $\sum A_{mi}$, $\sum A_{di}$, $\sum A_{ti}$ are the sums of the peak areas of the mono-,di-and triglycerides; A_{ei2} is the peak are of internal standard No. 2; M_{ei2} is the mass of internal standard No. 2 (milligrams); m is the mass of the sample (milligrams); a_m and b_m are constants obtained from regression analysis for monoglycerol; a_d and b_d are constants obtained from regression analysis for diglycerol; a_t and b_t are constants obtained from regression analysis for triglycerol.

2.3.4.8. Calculation of the Percentage of Total Glycerol

The percentage (*w/w*) of total glycerol in the sample can be calculated by using the following expressions:

$$G_T = G + 0.255\ M + 0.146\ D + 0.103\ T$$

Where G_T is the percentage (*w/w*) of the total glycerol (free and bound) in the sample; *G* is the percentage (*w/w*) of free glycerol in the sample; *M* is the percentage (*w/w*) of monoglycerides in the sample; *D* is the percentage (*w/w*) of diglycerides in the sample and T is the percentage (*w/w*) of triglycerides in the sample.

2.3.5. Determination of Refractive Index

Refractive index measurements were obtained using a Bellingham and Stanley RFM 390 refractometer equipped with a circulating water bath controlled to ± 0.05 °C. The refractometer was calibrated at 20 °C to the refractive index of water (η^{20} = 1.33330) and with the provided standard samples. The sample (approx. 1.0 mL) was added to the measuring cell. This step was carried out without introducing air bubbles on the surface of the prism. The measurements were carried out in triplicate. Figure 3.2 shows a typical calibration curve constructed using different concentrations of unrefined rapeseed oil and methanol.

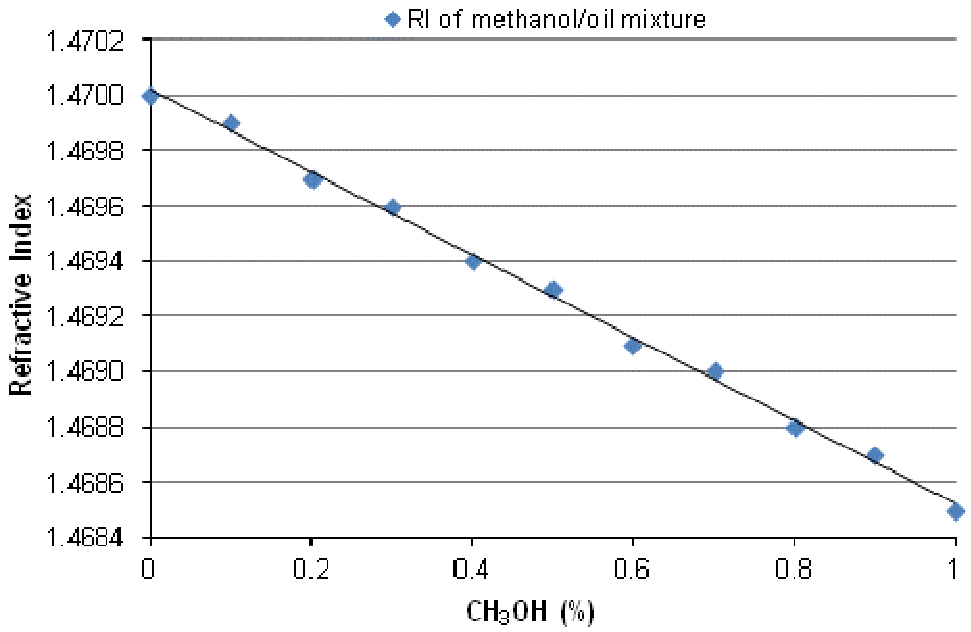


Figure 2.4. Calibration curve relating values of refractive index with the concentration of methanol in rapeseed oil at 20 °C.

2.3.6. Refractive Index Measurement for the Phase Solubility Studies

These studies were carried out by setting the prism temperature to 60 °C using the temperature controlled water bath attached to it. The refractive indices were logged electronically by using the software, RFM 300 Utility Program (version 13.0.0).

CHAPTER 3

BIODIESEL PRODUCTION USING HOMOGENEOUS CATALYTIC SYSTEMS

3.1. INTRODUCTION

When optimising the commercial performance of any process, the goals are typically to achieve a rapid rate of reaction, high yield of product, and little/no inhibition or loss of catalyst, all at a low cost. This requires a clear understanding of the principles and parameters governing the kinetics and thermodynamics of the reaction. In the case of the transesterification of vegetable oil, the situation is complex, since reaction rate and equilibrium yields are affected by numerous chemical and physical factors. The classical reaction protocol using a homogeneous catalyst, such as sodium hydroxide, requires mixing and stirring the reagents in a batch reactor. In the first few minutes of the reaction, the system has been shown to be two-phase, but because the accumulating methyl esters act as a mutual solvent, the reaction transforms to a single phase.[1] Glycerol, also a product of the reaction is, however, immiscible with the methyl esters and consequently a phase-separation phenomenon takes place again as the glycerol accumulates.[2] The immiscible glycerol phase solubilises the homogeneous base catalyst, withdrawing it from the reaction medium. At the end of the reaction, the non-polar phase containing the ester and the polar phase containing glycerol, methanol and catalyst are separated, and the ester further purified by washing to remove the remaining glycerol and other impurities.

The transesterification of vegetable oil also depends on the concentration of contaminating free fatty acids, moisture content, reaction time and temperature, ratio of alcohol to oil, and concentration of catalyst.[3-8] Most systems employ an alcohol/triglyceride molar ratio of 6:1 in order to shift the reaction equilibrium in the direction of product.[9] Bouaid *et al.*,[10] and Rashid *et al.*,[11] used a 6:1 CH_3OH/oil molar ratio for the transesterification of rapeseed oil, but whereas the former reported a yield

of 97.0% (w/w) ester content, the latter obtained only 83.0% (w/w) ester content. The difference between their systems was the amount of catalyst, 1.5% (w/w) alkali catalyst in the former case, and 1% (w/w) in the latter. On the other hand, Jeong *et al.*[12] reported 98.0% (w/w) ester content using 1.0% (w/w) alkali catalyst with an 8:1 CH_3OH/oil molar ratio.

The main objective of the work reported in this chapter was to obtain basic information concerning the transesterification reaction of crude and refined vegetable oils and resolve inconsistencies in the literature, such as molar ratios of CH_3OH/oil. Moreover, another objective was to delineate optimum reaction conditions for maximum conversion of triglycerides to fatty esters using alkali catalysed reactions. Reactions were analysed by gas chromatography using EN standard 14103 and 14105 methods.

3.2. EXPERIMENTAL AND INSTRUMENTATION

3.2.1. Materials and Experimental Procedure

See Sections, 2.1 and 2.2.1 for Materials and Experimental procedure in Chapter 2 respectively.

3.3. ANALYTICAL METHODS

3.3.1. Determination of Moisture Content, Acidity, Ester/ and Linolenic Acid Methyl Ester Content, and Free/ and Total Glycerol and Mono-, Di-, Triglyceride Contents

See Sections, 2.3.1-2.3.4 in Chapter 2, respectively.

3.4. RESULTS

3.4.1. Transesterification of Pure Glycerol Trioleate

In order to establish an experimental system for the transesterification reaction with the homogeneous base catalyst (NaOH), a high purity triglyceride was sought.

Commercially available glycerol trioleate met this criterion. Glycerol trioleate is comprised of three molecules of oleic acid, which reduces the complexity that may arise during the calculation of fatty acid methyl ester amounts from the analysis of peak areas displayed on the GC elution profile. In addition, the sample of glycerol trioleate selected was anhydrous and contained no free fatty acids. Optimised experimental conditions using a 6:1 methanol to oil molar ratio and 0.015 mol of NaOH for 60 min at 60 ºC were used, which Keera *et al.*,[13] and Agarwal *et al.*,[14] reported as being sufficient to drive the reaction in the forward direction within 60 min.

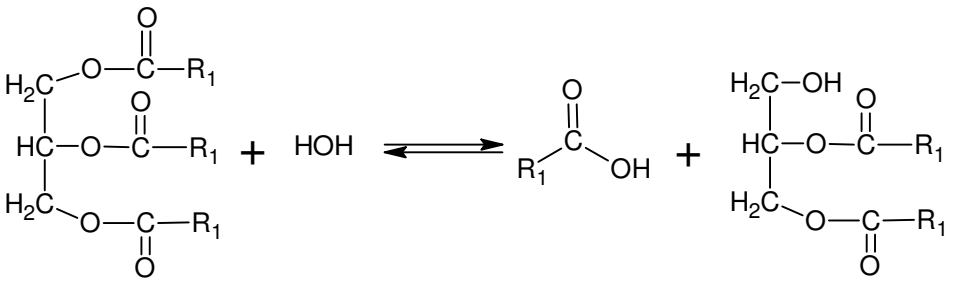
Table 3.1 shows that contrary to expectations, a yield of only 95.2% (w/w) methyl ester was obtained. This value was less than the expected value of 100% based on the purity of the sample (≥99.0 % w/w). It was also below the limit (96.5% w/w) set by the EN 14103 standard. Since the percentage product recovery (wt. of biodiesel relative to initial amount of glycerol trioleate used) was 98.0±0.01%, the lower yield of methyl esters obtained could not be attributed to experimental error. The data in Table 3.1 also shows that, after extraction, the amount of free glycerol (0.001% w/w) was insignificant, indicating that a high level of purification of methyl ester fraction was achieved.

Table 3.1. Properties of washed and dried biodiesel obtained from glycerol trioleate *via* the transesterification reaction.

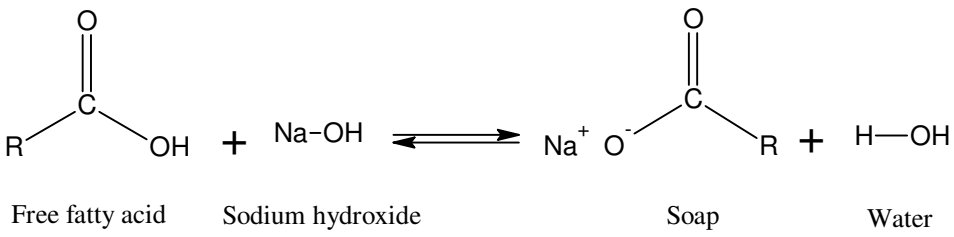
Property	Value [a]	Limits	Standard
Acidity mg KOH/g	0.25 ± 0.01	0.5 max	EN 14104
Moisture content ppm	116 ± 5.03	500 max	EN 12937
Chemical composition	(% w/w)		-
Methyl palmitate % (C16:0)	0.14	-	-
Methyl oleate % (C18:0)	99.86	-	-
Total FAME content (% w/w)	95.2 ± 0.11	96.5 min	EN 14103
Product recovery (% w/w)	98.0 ± 0.01	-	-
Monoglycerides (% w/w)	1.062 ± 0.003	0.8 max	EN 14105
Diglycerides (% w/w)	1.179 ± 0.009	0.2 max	EN 14105
Triglycerides (% w/w)	1.904 ± 0.099	0.2 max	EN 14105
Free glycerol (% w/w)	0.001 ± 0	0.020 max	EN 14105
Total glycerol (% w/w)	0.640 ± 0.011	0.25 max	EN 14105

[a] Mean of several analyses (n= 3) together with the standard deviation value.

To understand the reasons for the lower value, the methyl ester fraction was further analysed to determine the total acidity number (TAN) and moisture content. The acidity of the methyl ester fraction was 0.25±0.01 mg KOH/g indicating the presence of free fatty acids. Since the triglyceride was ≥99.0 % (w/w) pure with no free fatty acids, the result implies that FFAs were formed during the course of the reaction. Similarly, water was also formed during the reaction (Table 3.1, 116±5.03 ppm), though anhydrous conditions were used. Both the presence of free fatty acids and moisture content in the methyl ester fraction and lower than expected yield of methyl esters are consistent with the hydrolysis of triglycerides (Scheme 3.1) with water as opposed to methanol and saponification of free fatty acids with base catalyst (Scheme 3.2). Ongoing saponification at the onset of the transesterification reaction would in turn consume the base catalyst and increase the water content in the reaction.



Scheme 3.1. Hydrolysis of glycerol trioleate to form free fatty acid (R_1= fatty acid alkyl group).



Scheme 3.2. Saponification reaction of free fatty acid (R= carbon chain of the fatty acids).

The data in Table 3.1 show that *ca.* 4.14% (w/w) of unreacted glycerides remained in the extracted purified methyl ester fraction. The presence of intermediates *i.e.* mono-, di-, and triglycerides was also confirmed. These data show that the reaction had not gone to completion within the time frame (60 min) of the reaction. The data also support the notion that the water was formed during saponification (Scheme 3.2). Water tends to drive the transesterification reaction in the reverse direction *i.e.* formation of glycerides as opposed to methyl esters. The tendency for saponification is known to increase when using the base catalyst NaOH.[15]

In summary, these data show that:

1) the methodology adopted in this project for the transesterification of oil with methanol and subsequent purification of FAMEs was sound because product recovery was high, even though a lower than expected yield of ester was obtained.
2) using NaOH as a catalyst introduced the possibility of competing side reactions with water that is generated in the course of the reaction, resulting in a lower than expected yield of ester from pure oil on a mole for mole basis.

3.4.2. Transesterification of Unrefined Rapeseed Oil

3.4.2.1. Analysis of Unrefined Rapeseed Oil

The data in Table 3.2 shows the acidity and moisture content of unrefined rapeseed oil prior to transesterification reaction whereas the values obtained for the composition of fatty acids were after the transesterification reaction by using varying molar ratios of methanol to oil (3:1 to15:1) and varying concentration of NaOH (0.0075 to 0.022 mol).

The results showed that crude rapeseed oil was anhydrous (*ca.* 0.005% v/v). However, the acid value was higher than that of glycerol trioleate reported in section

3.4.1. Since the acid value of crude rapeseed oil was determined as 1.05 mg KOH/g, indicating the presence of FFAs, the neutralisation of FFA with NaOH could be substantial. High levels of FFAs can affect the conversion into FAMEs. Farag *et al.*, reported that FFAs amount greater than 1.0 mg KOH/g result in high amounts of undesirable soap produced simultaneously with the transesterification reaction.[16]

Table 3.2. Properties and composition of fatty acids of unrefined rapeseed oil.

Properties of crude rapeseed oil	
Acidity mg KOH/g	1.05 ± 0.01
Moisture content ppm	50 ± 1.45
Chemical composition (%w/w)[a]	
Palmitic ester % (C16:0)	5.14 ± 0.05
Stearic ester % (C18:0)	1.7± 0.00
Oleic ester % (C 18:1)	62.28 ± 0.01
Linoleic ester % (C 18:2)	18 ± 0.02
Linolenic ester % (C 18:3)	8.76 ± 0.01
Arachidic ester % (C 20:0)	0.55 ± 0.01
Gadoleic ester % (C 20:1)	1.47 ± 0.02
Behenic ester % (C 22:0)	0.34 ± 0.01
Erucic ester% (C 22:1)	1.12 ± 0.06
Lignoceric ester % (C 24:0)	0.07 ± 0.01
Nervonic ester % (C 24:1)	0.57 ± 0.01
Average (% total esters)	282.43 ± 0.05

[a] Standard error calculated for n=48 experiments together with the standard deviation value..

The data reported in Table 3.2 shows that the biodiesel derived from the samples of crude rapeseed oil contains oleic acid (*ca.* 62% w/w) followed by linoleic acid (*ca.* 18% w/w), linolenic acid (*ca.* 8% w/w), palmitic acid (*ca.* 5% w/w) and stearic acid (*ca.* 1.7% w/w). The remaining fatty acids comprised *ca.* 5% w/w. These results showed that the crude rapeseed oil contains a high degree of unsaturated fatty acids (oleic, linolenic and linolenic) comprising *ca.* 92% (w/w) of total fatty acids. Moreover, these results are similar to the composition of rapeseed oil fatty acids reported by Singh *et al*., and Ma *et al*., (94% unsaturated and 6% saturated fatty acids)[6,17] and are within the range reported in the literature.[18] The higher unsaturated fatty acids in the crude rapeseed oil makes it highly susceptible to oxidation as reported by Kiss *et al*.[19]

There was no significant correlation found between the change in total ester content (%) and the fatty acid composition with varying concentration of NaOH (0.0075 to 0.022 mol) and CH_3OH: oil molar ratio (3:1 to 15:1). This shows that all the fatty acids are released at the same rate for conversion into esters.

3.4.2.2. Effect of Varying Amounts of NaOH and CH_3OH on the Transesterification Reaction

In order to probe the effect of varying the molar ratio of methanol to oil (3:1 to 15:1) and varying concentration of NaOH (0.0075 to 0.022 mol) on the ester content (%) and acidity, a series of experiments were undertaken. The reaction time (60 min), temperature (60 ºC) and mixing intensity (600 rpm) were kept constant during this study.

3.4.2.2.1. Effect of varying amounts of NaOH on the Transesterification Reaction using 3:1 CH_3OH: Oil Molar Ratio

Using 3:1 methanol: oil molar ratio no phase separation was observed using 0.0075-0.011 mol of NaOH. At concentration of NaOH higher than 0.011-0.022 mol, an emulsion formed. Glycerol formation is thought to drive phase separation of the reaction as it proceeds in the forward direction. Therefore, the absence of phase separation at low NaOH concentration points to an insufficient accumulation of glycerol and, in turn, incomplete reaction. On the other hand, the formation of an emulsion as the concentration of NaOH is increased suggests that hydrolysis and saponification reactions dominated over transesterification.

In these reactions, the concentration of methanol was very much greater than that of water: the water concentration of the oil was less than 0.005% (v/v), and added reagents were dried before use. Nevertheless, with increasing NaOH concentration the tendency for saponification dominates. NaOH contains the necessary hydroxide group

for the saponification reaction, and water is less sterically hindered than methanol. Both these factors would increase the tendency for saponification over methanolysis and they rationalise the observation. These results highlight the need for strict control of the concentration of NaOH catalyst relative to methanol concentration in this type of catalytic system to ensure maximum conversion of vegetable oil to methyl ester.

3.4.2.2.2. Effect of varying amounts of NaOH on the Transesterification Reaction using 6:1 CH_3OH: Oil Molar Ratio

When the methanol: oil molar ratio was increased to 6:1, phase separation became evident, at all concentrations of NaOH investigated, and allowed a fraction of the methyl ester to be extracted from the reaction mixture and purified. The data in Figure 3.1 shows that the ester content (%) increased with an increase in the concentration of NaOH up to a maximum of 93.3% (w/w) with 0.015 mol of NaOH (0.6% w/w NaOH), then plateau'd off with a further increase in the concentration of NaOH.

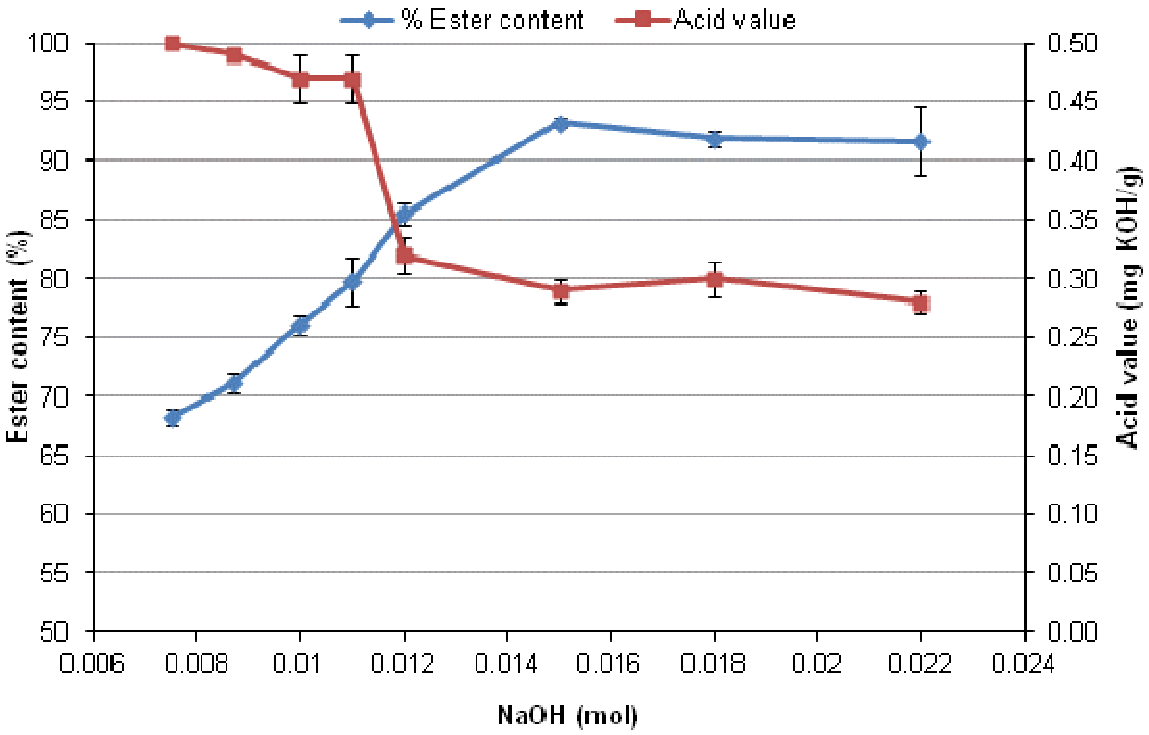


Figure 3.1. Influence of using 6:1 CH_3OH: oil molar ratio with varying concentration of NaOH on the ester content (%) and acidity (reaction conditions: 60 min, 60 ºC, 600 rpm). Values are the mean of three replicates, error bars indicate standard deviations.

The data in Figure 3.1 also shows that the acidity of the methyl ester fraction decreases as the concentration of NaOH increased. The acidity of the oil was 1.05 mg KOH/g before the transesterification reaction but the addition of 0.0075 mol of NaOH lowered the acid value to 0.50 mg KOH/g. However, the profile of decrease in acidity was striking: it comprised a gradual decrease with increasing NaOH from 0.0075 to 0.011 mol NaOH added, followed by a drop (almost 75% of the total acidity decrease recorded) between 0.011 and 0.012 mol of NaOH before a final plateau. Several factors could be responsible for this profile:

a) saponification of free fatty acids in the starting oil by NaOH at the outset of the reaction, which would lower the concentration of NaOH catalyst. For example, at a concentration of 0.015 mol of NaOH, the yield of methyl ester was 93.3% (w/w) and

the corresponding acid value was 0.29 mg KOH/g whereas at 0.0075 mol of NaOH, the methyl ester content was lower (68.2% w/w) and the corresponding acid value was 0.50 mg KOH/g.

b) the competing hydrolysis reaction due to the presence of water that can be generated during the saponification reaction of FFAs; this would become more severe as the concentration of methanol reduced in the course of the reaction and that of catalyst was experimentally increased. The presence of water could also favour the reverse reaction *i.e.* formation of diglycerides and FFA as shown in Scheme 3.1.[20]

c) phase separation with accumulating glycerol that will tend to separate catalyst from the non-polar phase of the reaction mixture, again reducing the concentration of NaOH catalyst.

The data in Table 3.3 shows that the final yield of methyl ester was only 93.3% (w/w) and unreacted glycerides *i.e.* tri- (5.13 % w/w), di- (0.33% w/w), and monoglyceride (0.55% w/w) were also present in the reaction mixture. As with pure glycerol trioleate (see Section 3.4.1) these results suggest that there might be insufficient concentration of catalyst to shift the equilibria for tri-, di-, and monoglyceride transesterification in the forward direction of formation of methyl esters and glycerol within the time frame of the reaction (60 min). The presence of FFAs increases the saponification or esterification reaction, enough water is formed to prematurely stop the production of methyl esters by inhibiting the forward reaction with methanol, leaving a large quantity of unreacted materials. Canakci and Van Gerpen determined that as little as 0.1% (w/w) of water in the reactants or during the reaction could reduce methyl ester production.[21]

Table 3.3. Properties of washed and dried biodiesel obtained from unrefined rapeseed oil *via* the transesterification reaction.

Property	Value [a]	Limits	Standard
Total FAME content (% w/w)	93.3 ± 0.80	96.5 min	EN 14103
Monoglycerides (% w/w)	0.55 ± 0.009	0.8 max	EN 14105
Diglycerides (% w/w)	0.33 ± 0.002	0.2 max	EN 14105
Triglycerides (% w/w)	5.13 ± 0.30	0.2 max	EN 14105
Free glycerol (% w/w)	0.05 ± 0.011	0.020 max	EN 14105
Total glycerol (% w/w)	0.766 ± 0.025	0.25 max	EN 14105

[a] Mean of several analyses (n=3) together with the standard deviation value.

Therefore, from these results it was hypothesised that the competing reactions *i.e.* saponification and hydrolysis of FFA offset the transesterification reaction in the presence of sodium hydroxide catalyst.

3.4.2.2.3. Effect of varying amounts of NaOH on the Transesterification Reaction using 9:1 -15:1 CH_3OH: Oil Molar Ratio

When the methanol: oil molar ratio was increased from 9:1-15:1 (Figure 3.2-3.4), the ester content (%) was *ca.* 93.2-93.4% (w/w) at 0.015 mol of NaOH. These results were similar to that achieved by using 6:1 molar ratio of CH_3OH to oil at this point.

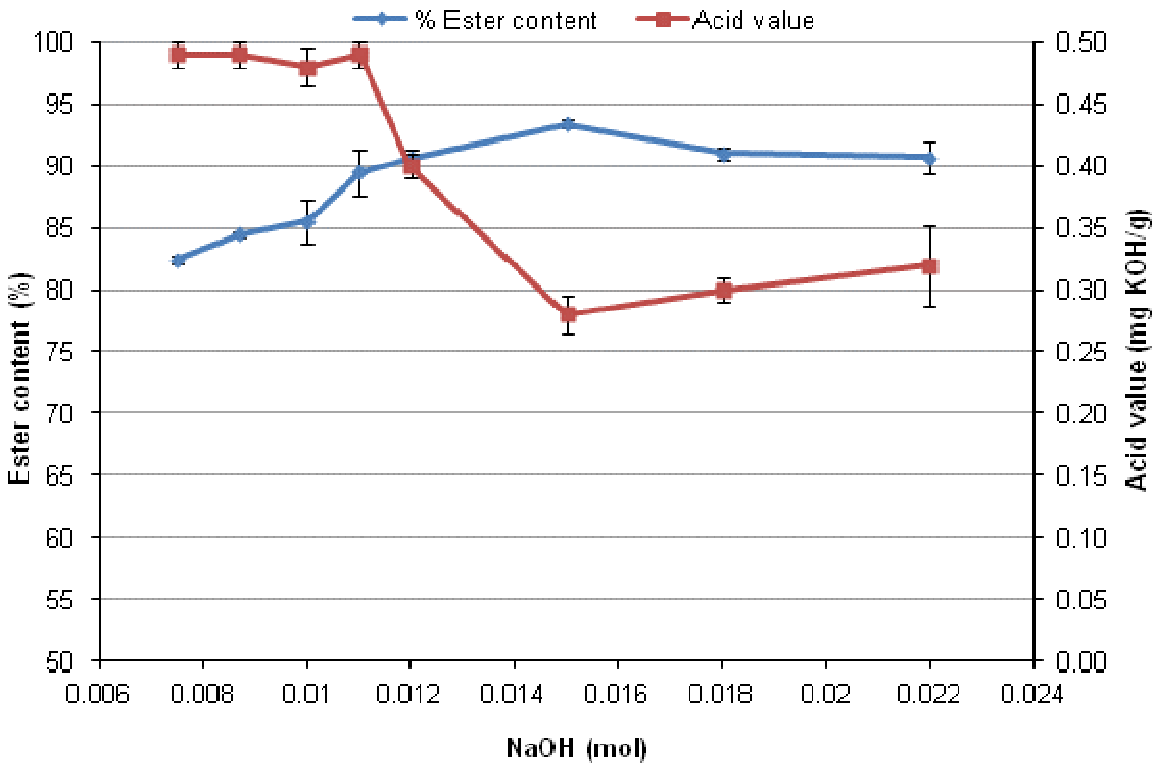


Figure 3.2. Influence of using 9:1 CH_3OH: oil molar ratio with varying concentration of NaOH on the ester content (%) and acidity (reaction conditions: 60 min, 60 ºC, 600 rpm). Values are the mean of three replicates, error bars indicate standard deviations.

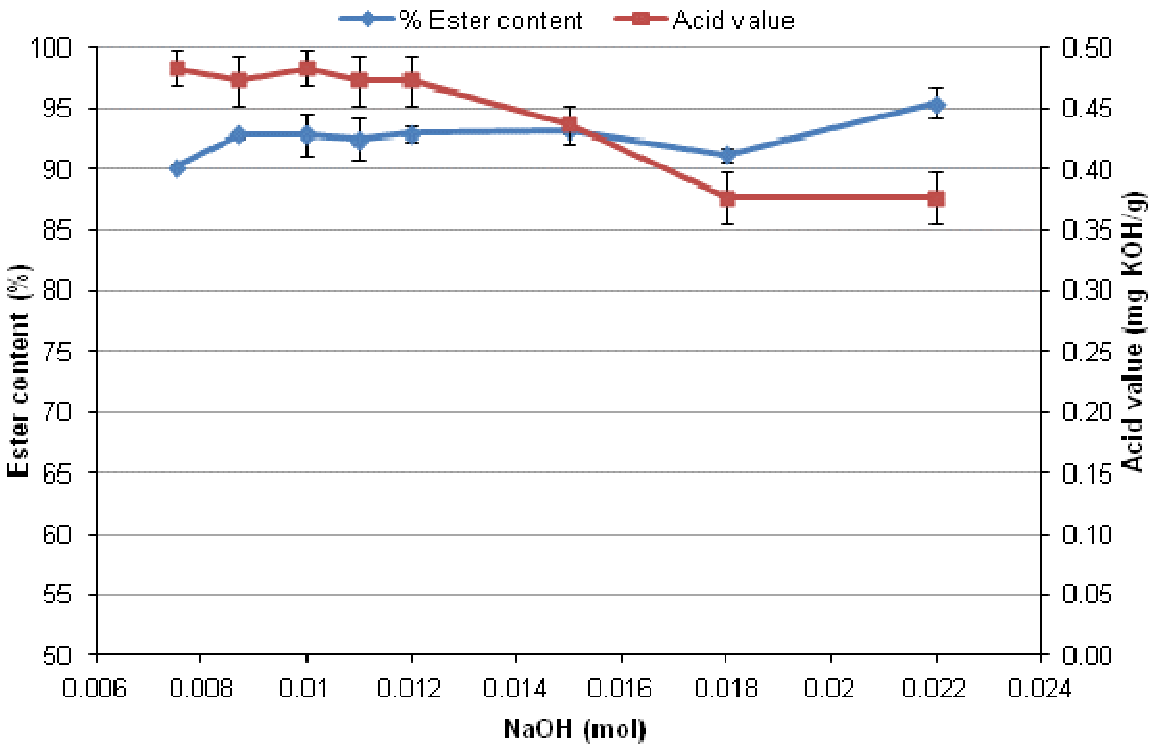


Figure 3.3. Influence of using 12:1 CH_3OH: oil molar ratio with varying concentration of NaOH on the ester content (%) and acidity (reaction conditions: 60 min, 60 ºC, 600 rpm). Values are the mean of three replicates, error bars indicate standard deviations.

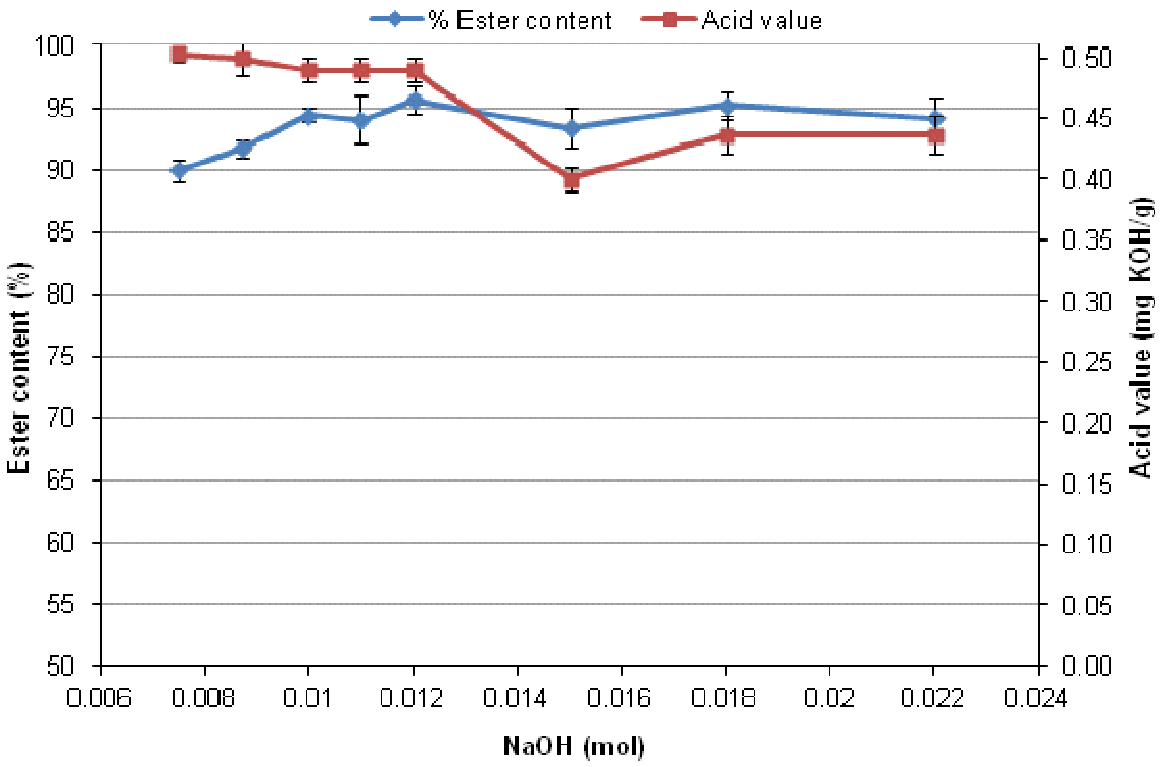
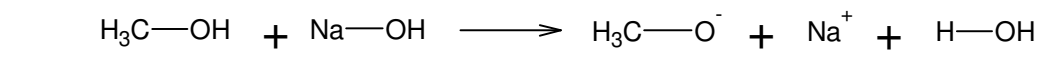
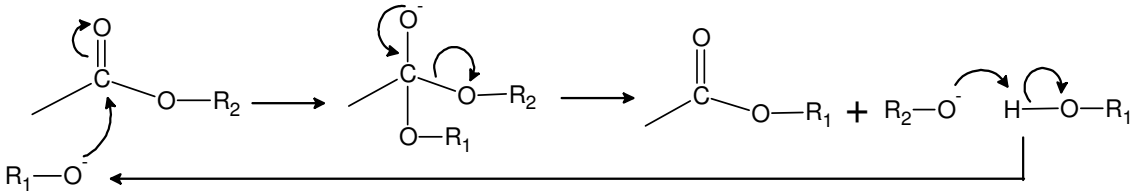


Figure 3.4. Influence of using 15:1 CH_3OH: oil molar ratio with varying concentration of NaOH on the ester content (%) and acidity (reaction conditions: 60 min, 60 ºC, 600 rpm). Values are the mean of three replicates, error bars indicate standard deviations.

However, in contrast to the situation with 6:1 molar ratio of CH_3OH: oil, at the lower concentration of NaOH (0.0075-0.012 mol), the ester content (%) were relatively higher (82-90% w/w of ester content for 9:1-15:1 CH_3OH: oil molar ratio compared with 68.2% w/w for 6:1 CH_3OH: oil molar ratio). These results indicate an excess amount of methanol can drive the reaction towards completion even if a lower amount of NaOH is used. A higher molar ratio of CH_3OH: oil utilises the base catalyst in the formation of methoxide ions (Scheme 3.3) instead of dissipating the catalyst in the saponification reaction. Methoxide ions would speed up the transesterification reaction because methoxide is a better nucleophile than the alcohol itself and can be regenerated in the reaction, as shown in Scheme 3.4. The methoxide ions acts as a nucleophile that attack on carbonyl carbon atom of triglyceride for the formation of methyl esters.



Scheme 3.3. Formation of methoxide ion.



Scheme 3.4. Carbonyl substitution reaction by alkoxide ion.

In the case of 9:1-15:1 CH_3OH: oil molar ratio, further increase in concentration of NaOH from 0.015 mol, showed no significant change in ester content (%) as shown in Figures 3.2-3.4. Nevertheless, the ester content was 90% (w/w) or more for 12:1 and 15:1 CH_3OH: oil molar ratio at all the levels of NaOH investigated.

However, the higher amount of CH_3OH used (12:1-15:1 CH_3OH: oil molar ratio) made the recovery of methyl ester layer difficult during purification *i.e.* washing of ester layer. Even though excess CH_3OH is necessary for the transesterification reaction to break the glycerol-fatty acid linkages but it aggravates the separation of glycerol by increasing its solubility in alcohol. This causes the reaction equilibrium to be shifted in the direction that favours the product decomposition reaction, with a consequential decrease in the concentration of methyl esters. This is the reason why at 15:1 CH_3OH: oil molar ratio, the ester content (%) was lower at 0.022 mol of NaOH.

Figures 3.2-3.4 also shows the TAN for 9:1-15:1 CH_3OH: oil molar ratio with varying amounts of NaOH. The acid values were higher (0.47-0.48 mg KOH/g) over the range of 0.0075-0.015 mol of NaOH as shown in Figures 3.2.-3.4. For 9:1 CH_3OH: oil molar ratio, a similar profile for acidity, *i.e*. a decline, was observed after using 0.011 mol of NaOH. The most likely explanation for a drop in acidity is that increasing the amount of NaOH increases the tendency for saponification of free fatty acid. In turn, the

saponification reaction at higher concentration of NaOH made the separation and purification of the methyl ester layer difficult.

However, by increasing the methanol ratio to 12:1 or 15:1, the decrease in TAN was significantly less as compared to when employing a 6:1 and 9:1 CH_3OH: oil molar ratio. The methanol concentration plays a very important role in relationship to the NaOH catalyst because the hydroxide ions that are responsible for the saponification reactions were not readily available due to its consumption in the formation of methoxide ions by increasing the methanol ratio.

Figures 3.2-3.4 show that with further increase in the amount of NaOH, above 0.015 mol, the trend for decreasing amounts of free fatty acids in the reaction mixture was reversed. This result was striking as it signifies an increasing tendency for hydrolysis instead of methanolysis, which in turn increases the concentration of free fatty acid in the reaction. Hydrolysis instead of methanolysis points to a shift in the ratio of water to methanol with increasing NaOH in the reaction mixture. This was verified by examining the moisture content of the reactions.

3.4.2.3. Determination of Moisture Content

The determination of water content after the transesterification reaction is very important as water can have a negative influence on the transesterification reaction.[22,23] This leads to the idea of other competing reactions, *i.e.* saponification, ongoing with the transesterification reaction. The triglyceride used in these experiments contained less than 0.005% (v/v) water content and all the other added reagents were anhydrous. Nevertheless, the moisture content ranged between 100 mg/Kg to 500 mg/Kg in the entire methyl ester layer (biodiesel) at different concentrations of CH_3OH and NaOH used, as shown in Figures 3.5 and 3.6.

All the methyl ester samples fulfilled the established EN standard limit for moisture content (500 mg/Kg). In the case of 6:1 CH_3OH: oil molar ratio (Figure 3.5), the water content generally decreased with increasing concentration of catalyst. At the lowest catalyst concentrations, the moisture content was highest, consistent with saponification and formation of free fatty acids (see Figures 3.1-3.4).

For 9:1 CH_3OH: oil molar ratio the moisture content showed no significant difference at all concentrations of NaOH investigated and was low (250-300 ppm, see Figure 3.5). For molar ratios of 12:1 and 15:1 CH_3OH: oil a different trend was observed. With increasing amounts of NaOH the moisture content gradually increased (Figure 3.6) to 440-460 ppm, in parallel with the trends observed for the acidity values (Figure 3.3 and 3.4). The data are consistent with an increasing tendency for esterification with increasing concentration of NaOH.

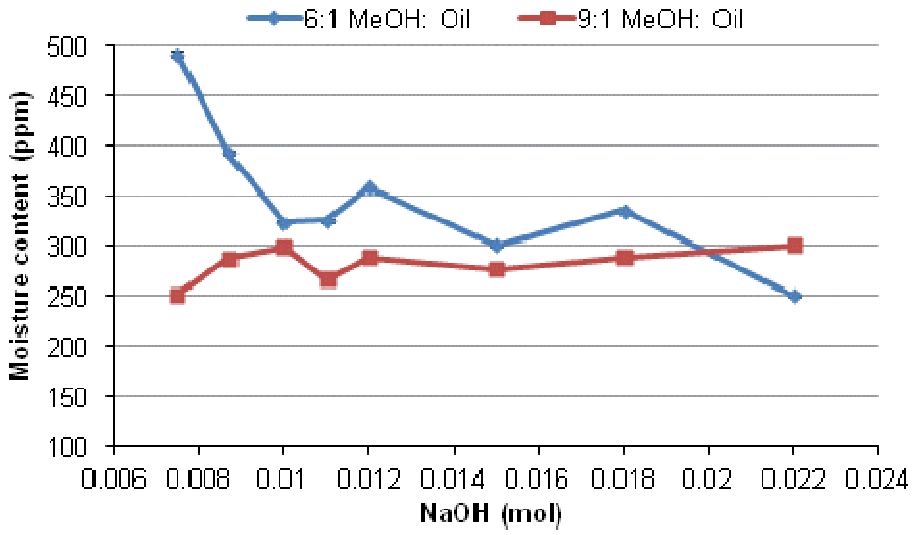


Figure 3.5. Moisture content for 6:1 and 9:1 CH_3OH: oil molar ratio with varying concentration of NaOH (values are the mean of three replicates, error bars were not plotted since the standard deviation was very low).

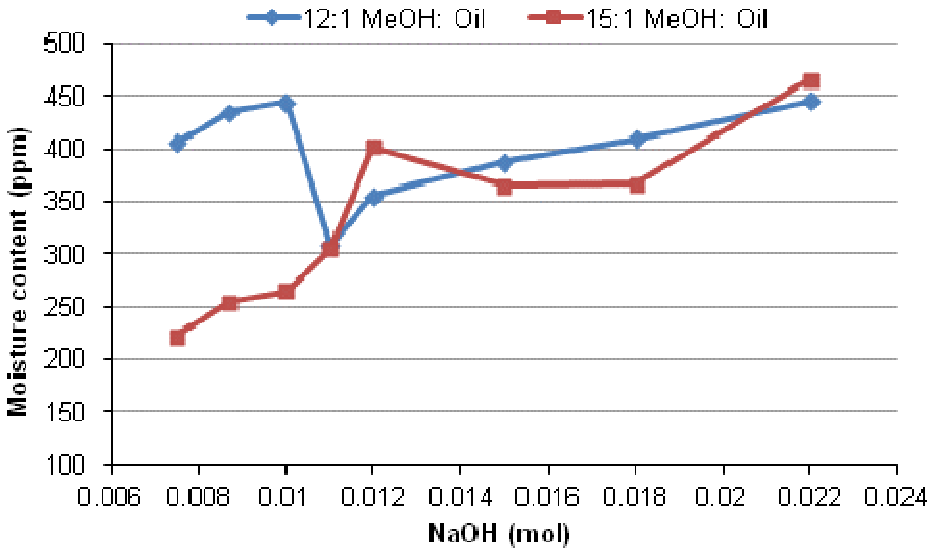
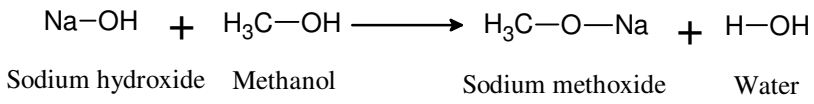


Figure 3.6. Moisture content for 12:1 and 15:1 CH_3OH: oil molar ratio with varying concentration of NaOH (values are the mean of three replicates, error bars were not plotted since the standard deviation was very low).

However, the result obtained implies that water molecules were formed during the transesterification reaction. There are two possibilities for this observation:

a) NaOH, when dissolved in methanol, contributes water in the reaction medium (Scheme 3.5).

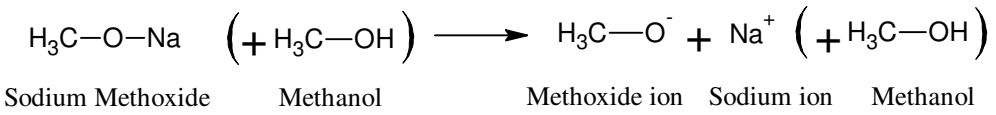


Scheme 3.5. Formation of water in the presence of NaOH and CH_3OH.

b) the presence of free fatty acids in the reaction mixture will consume the catalyst resulting in the production of soap and water, as shown earlier in Scheme 3.2.

3.4.2.4. Comparison of Sodium Methoxide *vs*. Sodium Hydroxide Catalyst

Using sodium methoxide (CH_3ONa) as a catalyst reduces the possibility of producing water (Scheme 3.6) compared to using NaOH (Scheme 3.5) and, therefore, could increase the methyl ester content (%).



Scheme 3.6. Dissolving solid sodium methoxide in the given alcohol.

Table 3.4 shows that the product recovery (%) was relatively higher in the case of CH_3ONa (97% w/w) than with NaOH (88% w/w). Additionally, on completion of reaction, the ester and glycerol layer were easily separated for CH_3ONa mediated reaction due to less soap formation as compared with NaOH.

Table 3.4. Comparison between the effect of CH_3ONa and NaOH catalysts on the transesterification reaction.

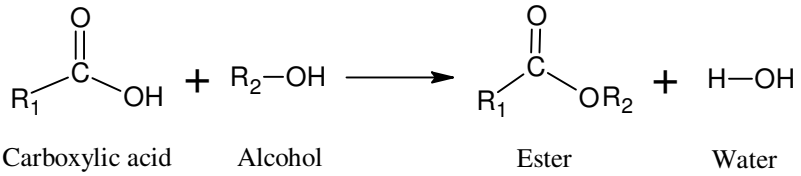
Catalyst	Product recovery (% w/w)[a]	Ester content (% w/w)[b]
NaOH	85.4±0.01	93.3±0.55
CH_3ONa	97.3±0.01	94.1±0.46

Conditions: crude rapeseed oil, 6:1 CH_3OH: oil molar ratio, 0.015 mol catalyst, 60 °C, 1 hr.

[a] Product recovery % (w/w) = wt. of methyl ester/ wt. of oil × 100
[b] Ester content % (w/w) = calculated as described in chapter two, section 2.3.3.3.
Error given as standard error (n= 3 experiments).

Table 3.4 shows that the ester content (%) obtained after 60 min was 93% (w/w) and 94% (w/w) for NaOH and CH_3ONa, respectively. This result showed insignificant difference in ester content (%) even though using CH_3ONa reduces the possibility of additional water being formed that is responsible for the saponification reaction. Notably, the esterification of FFAs (Scheme 3.7) could be the only substantive reason for the hydrolysis of triglyceride as the TAN in the crude rapeseed oil was 1.05 mg

KOH/g. Consequently, the water generated by the saponification reaction could in turn lower the ester content (%).



Scheme 3.7. Esterification of fatty acid (R_1= carbon chain of fatty acids, R_2= alkyl group of the alcohol).

3.4.3. Transesterification of Refined Vegetable Oils

3.4.3.1. Analysis of Refined Vegetable Oils

The moisture content and acidity of refined oils prior to the transesterification reaction are given in Table 3.5. The data shows that the moisture content and acidity was ≤ 0.005% (v/v) and ≤ 0.10 mg KOH/g, respectively. However, the values obtained for moisture content and TAN reduces the possibility of competing reactions (saponification and hydrolysis of TGs) which was observed earlier in the case of unrefined rapeseed oils due to higher acidity.

Table 3.5. Moisture content and acid value for refined vegetable oils prior to the transesterification reaction.

Refined vegetable oils	Moisture content ppm [a]	Acidity mg KOH/g [a]
Rapeseed oil	50 ± 1.22	0.08 ± 0.01
Sunflower oil	45 ± 2.30	0.05 ± 0.01
Grapeseed oil	35 ± 1.46	0.05 ± 0.01
Corn oil	44 ± 2.09	0.08 ± 0.02
Soya oil	50 ± 1.83	0.04 ± 0.02
Groundnut oil	40 ± 1.56	0.05 ± 0.01

[a] Standard error calculated for three replicates.

Table 3.6 shows the fatty acid composition (% w/w) derived after the transesterification reaction of various refined vegetable oils. The fatty acid composition of these oils differs from each other depending on the type of plant species and on the growth condition.[18] The fatty acid composition of the oils shown in Table 3.6 are within the range defined in the literature.[24] The unsaturated fatty acids were higher in refined rapeseed oil, *ca.* 92%, (w/w) compared to other refined oils. However, the fatty acid composition of refined rapeseed oil was similar to unrefined rapeseed oil reported in Table 3.2. The higher content of saturated fatty acids *ca.* 17% (w/w) were observed in groundnut oil followed by soya > corn > grapeseed > sunflower > rapeseed oil. Oils that are more unsaturated are oxidized more quickly than less unsaturated oil.[25] Moreover, saturated oil can increase the phase solubility issues more than the unsaturated oil.

The linolenic acid (%) was higher in refined rapeseed oil (*ca.* 9% w/w) but within the limit set by EN 14103 standard (12% w/w). In addition, the linolenic acid was less than 1% (w/w) in all other refined oils except corn oil (*ca.* 4% w/w). The higher content of linolenic acid in oil makes it highly susceptible to oxidation and promotes the formation of FFAs.

Table 3.6. Composition of fatty acids (% w/w) of different refined vegetables oils.[a]

Fatty acid (% composition by weight)	Refined Rapeseed Oil	Refined Sunflower Oil	Refined Grapeseed Oil	Refined Corn Oil	Refined Soya Oil	Refined Groundnut Oil
Palmitic ester % (C16:0)	4.73 ± 0.18	7.19 ± 0.00	7.79 ± 0.02	13.21 ± 0.34	12.30 ± 0.08	10.14 ± 0.14
Stearic ester % (C18:0)	1.67 ± 0.05	3.29 ± 0.00	3.13 ± 0.00	1.70 ± 0.01	2.83 ± 0.02	1.98 ± 0.01
Oleic ester % (C 18:1)	62.69 ± 0.03	24.26 ± 0.00	26.60 ± 0.04	27.56 ± 0.10	24.76 ± 0.07	59.60 ± 0.19
Linoleic ester % (C 18:2)	18.01 ± 0.66	63.52 ± 0.07	60.48 ± 0.11	55.83 ± 0.22	54.33 ± 0.22	20.50 ± 0.08
Linolenic ester % (C 18:3)	8.93 ± 0.58	0.39 ± 0.02	0.80 ± 0.11	0.84 ± 0.00	4.63 ± 0.00	0.62 ± 0.18
Arachidic ester % (C 20:0)	0.57 ± 0.01	0.17 ± 0.00	0.25 ± 0.00	0.31 ± 0.00	0.28 ± 0.00	0.94 ± 0.01
Gadoleic ester % (C 20:1)	1.48 ± 0.04	0.36 ± 0.00	0.15 ± 0.06	0.27 ± 0.01	0.28 ± 0.01	1.96 ± 0.02
Behenic ester % (C 22:0)	0.33 ± 0.02	0.54 ± 0.02	0.46 ± 0.00	0.11 ± 0.00	0.42 ± 0.00	2.60 ± 0.04
Erucic ester % (C 22:1)	1.15 ± 0.27	0.06 ± 0.00	0.19 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lignoceric ester % (C 24:0)	0.15 ± 0.00	0.22 ± 0.00	0.15 ± 0.00	0.16 ± 0.00	0.18 ± 0.01	1.65 ± 0.01
Nervonic ester % (C 24:1)	0.27 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Average FA RMM	282.38 ± 0.23	279.95 ± 0.01	279.79 ± 0.01	278.07 ± 0.09	278.42 ± 0.00	283.14 ± 0.01

[a] Standard error calculated for n=9 replicates.

3.4.3.2. Effect of Varying Amounts of NaOH on the Transesterification Reaction Using Refined Vegetable Oils

The transesterification reaction was carried out by using various types of commercially available refined vegetable oils namely rapeseed oil, sunflower oil, grapeseed oil, corn oil, soya oil, groundnut oil. The reaction parameters *i.e.* reaction time, temperature and mixing intensity were similar to those used earlier for unrefined rapeseed oil experiments (Section 3.4.2). The methanol to oil ratio was kept constant (6:1 CH_3OH: oil) with varying concentration of NaOH (0.015 to 0.022 mol).

The data in Figure 3.7 shows that the ester content (%) varies from 90.6 - 93.8% (w/w) for various refined oils and was the same order of magnitude as for unrefined rapeseed oil under these set of reaction conditions. The ester content (%) for all the refined vegetable oils decreased with an increase in the amount of catalyst except for the grapeseed oil. There was a sharp decrease, by 2% (w/w), in ester content as the concentration of catalyst increased from 0.015 to 0.018 mol (Figure 3.7) for refined rapeseed oil. Similar, results were obtained for sunflower, groundnut and soya oil. However, for the corn oil, the ester content remained the same at 0.015-0.018 mol and decreased by 1% (w/w) at 0.022 mol of NaOH. Equally, it has been shown that grapeseed oil shows an opposite trend *i.e.* at 0.015 mol of NaOH, the ester content (%) for grapeseed oil was 92.4% (w/w) but by increasing the catalyst amount to 0.018 mol it increased to 93.1% (w/w).

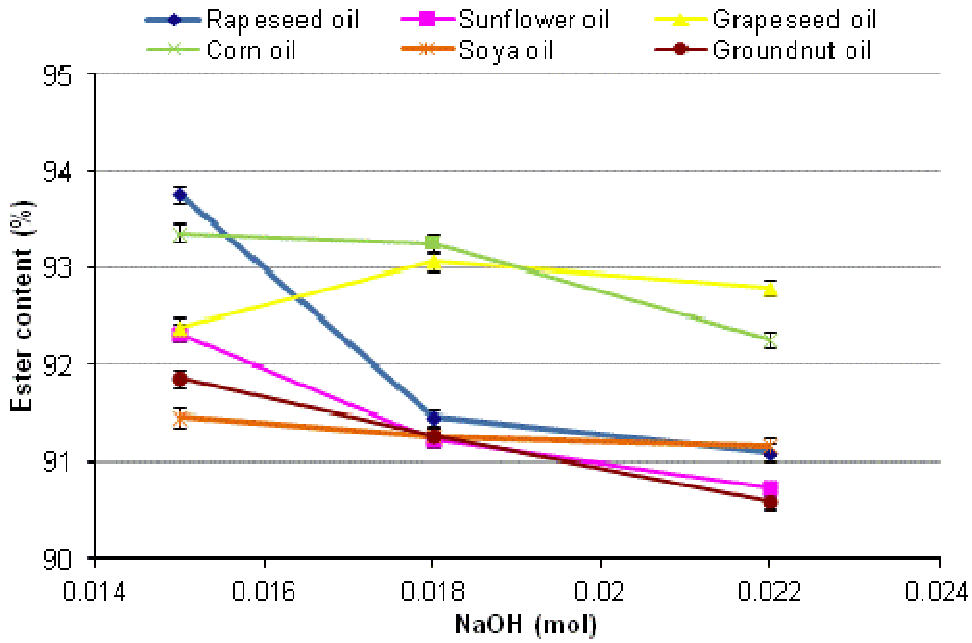


Figure 3.7. Ester content (%) of refined vegetable oils obtained by using 0.015-0.022 mol of NaOH catalyst (reaction conditions: 6:1 CH_3OH: oil molar ratio, 60 min, 60 ºC, 600 rpm). Values are the mean of three replicates, error bars indicate standard deviations.

This result also highlights that the composition of fatty acids in the oil plays a major role in the conversion of ester content. As the sunflower, groundnut, soya and corn oils had a higher content of saturated fatty acids compared to rapeseed oil (Table 3.6), the conversion into fatty acid methyl esters was lower than rapeseed oil. This is because the higher concentrations of saturated fatty acids in the oil make the oil more viscous, thus increasing the immiscibility of the oil with methanol.

Even though, the moisture content and acidity of refined vegetable oils was very low (Table 3.5) before the transesterification reaction, it was expected that the ester content (%) should be higher based on earlier results (Section 3.4.2). In the case of refined rapeseed oil, a 0.5% (w/w) increase in ester content was observed at 0.015 mol of NaOH (Figure 3.8). Above 0.015 mol of NaOH, there was a decrease of 0.5% (w/w) ester in refined rapeseed oil. However, this result did not show a significant difference in ester content (%) although they exhibited large differences in their acid values; 0.08

and 1.05 mg KOH/g for refined and unrefined rapeseed oil, respectively. The only possible reason is that the higher FFA content in oil (unrefined rapeseed oil) increases the probability of the esterification reaction. Therefore, simultaneous transesterification of TGs and esterification of FFAs (Scheme 3.7) increases the amount of ester and water in the reaction, which most probably happened in the case of unrefined rapeseed oil.

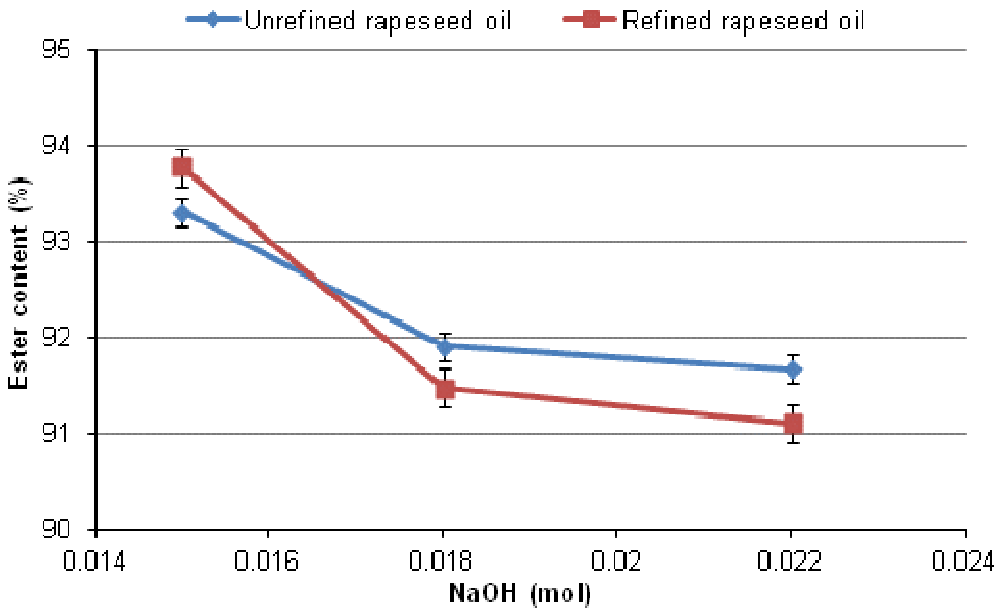


Figure 3.8. Ester content (%) of refined and unrefined rapeseed oil by using 0.015-0.022 mol of NaOH catalyst (reaction conditions: 6:1 CH_3OH: oil molar ratio, 60 min, 60 ºC, 600 rpm). Values are the mean of three replicates, error bars indicate standard deviations.

The moisture content and acidity increased for all refined vegetable oils after the transesterification reaction (Table 3.7), as was the case for glycerol trioleate (Section 3.4.1). The acidity and moisture content for corn oil was higher than the other refined vegetable oils. The only visible difference whilst undertaking the transesterification of refined and unrefined oils was the process of separation. The separation of the two phases was much easier before the crude biodiesel samples were washed, as there was less formation of soaps. This is due to the lower free fatty acid content in the refined vegetable oils than in the crude oil. However, if there is no saponification reaction then the water should not be present in the reaction mixture *via* the saponification reaction. Nevertheless, this was not the case as the moisture content was higher from the starting

oil for all the refined oils. As mentioned earlier, water can be present in the reaction mixture by mixing NaOH with methanol (Scheme 3.3). Therefore, the presence of water is responsible for the hydrolysis of TGs (Scheme 3.1) that increases the FFAs in the reaction. In turn, these FFAs undergo saponification (Scheme 3.2) to yield more water.

Table 3.7. Acidity and moisture content of various refined vegetable oils at different concentrations of NaOH (mol).

Type of oils	NaOH mol	Acidity mg KOH/g[a]	Moisture Content ppm[a]
Refined Rapeseed oil	0.015	0.10 ± 0.01	268.4 ± 1.22
	0.018	0.12 ± 0.01	265.1 ± 5.33
	0.022	0.12 ± 0.00	260.2 ± 3.45
Refined Sunflower oil	0.015	0.10 ± 0.01	374.5 ± 4.59
	0.018	0.10 ± 0.00	368.3 ± 3.80
	0.022	0.09 ± 0.00	375.1 ± 2.98
Refined Grapeseed oil	0.015	0.08 ± 0.01	250.9 ± 1.11
	0.018	0.08 ± 0.00	243.3 ± 6.80
	0.022	0.06 ± 0.00	231.5 ± 3.00
Refined Corn oil	0.015	0.10 ± 0.02	298.0 ± 2.37
	0.018	0.12 ± 0.01	301.4 ± 2.99
	0.022	0.10 ± 0.00	310.0 ± 6.70
Refined Soya oil	0.015	0.15 ± 0.00	401.2 ± 2.09
	0.018	0.16 ± 0.01	418.3 ± 3.44
	0.022	0.15 ± 0.01	398.0 ± 9.01
Refined Groundnut oil	0.015	0.07 ± 0.00	374.8 ± 1.04
	0.018	0.08 ± 0.01	333.6 ± 5.02
	0.022	0.07 ± 0.01	359.3 ± 4.65

[a] Standard error calculated for three replicates.

However, in summary, the results tabulated in Table 3.7 underline the fact that competing reactions *i.e.* esterification of FFAs and saponification or hydrolysis of TG take place during the transesterification, as was also observed when pure glycerol trioleate was used.

3.5. DISCUSSION

In this chapter research on the transesterification reaction has been reported using pure glycerol trioleate. The reason for carrying out this experiment was to ensure that the reported methodologies for the synthesis of biodiesel were reproducible. Despite of being free from impurities, even then the ester content (95.2% w/w) was not achieved according to EN 14103 standard specified for biodiesel. Moreover, the results showed that the content of individual glycerides (1.06% mono-, 1.17% di-, and 1.90% tri- glycerides) were also higher than the values mentioned by EN 14105 standard. Hence, higher values of glycerides in the FAMEs layer prove the reversible nature of the transesterification reaction by undergoing the hydrolysis of glycerol trioleate. However, for the hydrolysis reaction water is required. As the glycerol trioleate used was anhydrous, the only possibility for the presence of water is from the reaction of NaOH with CH_3OH. Therefore, the presence of water at the start of the reaction is likely to be responsible for the formation of intermediates (di- and mono- glycerides) and FFAs. In turn, the FFAs formed in the reaction give rise to the saponification reaction, which again increases the water content in the reaction mixture. The FFAs and water are generated during the transesterification reaction, as shown by the results of the experiments showing that competing reactions were ongoing. However, Leung and Guo (2006) reported that all the reactant, triglycerides reacts but not all the triglycerides undergo transesterification to form methyl esters.[5] Complete transesterification is assumed for the 97% (w/w) of triglyceride that forms methyl ester.

Therefore, in order to study the transesterification reaction in detail and to optimise the reaction conditions further studies were carried out by using unrefined

rapeseed oil instead of glycerol trioleate. Unrefined rapeseed oil was selected because it is cost effective and is better suited for examining the interferences caused by other chemical components present in the oil.

In order to optimise the reaction parameters involved in the transesterification reaction of indigenously available rapeseed oil, the amount of catalyst and alcohol were varied. These two factors are of immense importance and can affect the conversion of triglycerides into FAMEs.[26-28] Few parameters were not optimised during this study *i.e.* reaction time, temperature and agitation rate were taken from the literature. These parameters were selected from the literature.[4,29] Reaction time and temperature are significant operating parameters which are closely related to the energy cost of the production of biodiesel. All the experiments were conducted at 60 ºC for 60 min. Although, when small amounts of catalyst were used, 60 min was not enough time for the completion of the transesterification reaction. However, this time-frame was kept constant throughout the study, in order to monitor the effects of the amount of catalyst or an alcohol on the ester content (%).

The results obtained by varying the concentrations of catalyst and alcohol showed that the increase in catalyst concentration levels greater than 0.015 mol did not yield any further increase in ester content. This could be due to the competing reactions when an excessive amount of alkali catalyst is used. The saponification reaction increases the viscosity of the reaction mixture and hinders the glycerol separation from methyl ester phase and therefore makes the recovery of the methyl esters difficult. This in turn consumes the base catalyst and reduces product yields.[3,4,13] The other reason can be reversible nature of the transesterification reaction which can lead into the formation of mono-, di- and tri-glycerides as opined by Darnoko *et al.*[30] The glycerol and glyceride (%) formed in the transesterification reaction by using a molar ratio of 6:1 CH_3OH/oil and 0.015 mol of NaOH has proved the reversibility and incomplete transesterification reaction at 60 min.

In order to shift the equilibrium forward for the transesterification reaction, an excess stoichiometric ratio of methanol to oil is required but to prove this a 3:1 molar ratio of methanol to oil was used. As expected, there was no phase separation and hence no methyl esters could be determined for further studies. Monoglycerides, diglycerides, and triglycerides are not water-soluble. Consequently, when transesterification is incomplete these unreacted compounds are contained in the final biodiesel product since they are not washed away by water. Therefore, it is vital to employ a reaction mechanism that ensures that the transesterification reaction proceeds to completion. When the transesterification is complete, there should be no or only small traces of monoglycerides and only a small amount of diglycerides in the reaction product stream.[5,31,32]

At a 6:1 molar ratio of CH_3OH/oil and 0.015 mol of NaOH, a 93.3% (w/w) conversion of triglyceride to the ester was obtained. Using a higher molar ratio than 6:1, further methanol addition had no measurable effect on ester formation; rather it complicated ester recovery.[33,34] In so far as a longer time was required for the subsequent separation stage since separation of the ester layer from the glycerol was difficult. In the case of molar ratios ≥6:1, a dilution effect is the likely cause while for molar ratios ≤6:1, insufficient mixing of the reactants in the biphasic transesterification reaction system is the likely cause of lower ester content. A possible explanation for this behaviour may indicate the fact that excess methanol hinders the decantation by gravity so that the apparent yield of esters decreases because some of the glycerol may remain in the biodiesel phase.[35,36] However, the ester content (%) at 0.0075 NaOH (mol), 12: 1 and 15:1 CH_3OH: oil molar ratios were higher when compared to 6:1 or 9:1 CH_3OH: oil molar ratios. This is because the reaction takes place in the methanol phase. Since, NaOH is soluble in methanol so greater amount of methanol would provide higher methoxide ions for the transesterification reaction.

Hence, the optimium molar ratio of methanol/oil needed to produce higher ester content (93.3% w/w) from rapeseed oil was 6:1. The abovementioned results are in

agreement with the reports by Zhang *et al*.,[37] Freedman *et al*.,[38] and Boocock *et al*.,[22] Meher *et al*.,[7] and Usta[35] who obtained high yields of esters utilising molar ratios of 6:1 during the methanolysis of *P. pinnata* and tobacco seed oil, respectively. In the ethanolysis of used frying oil, Encinar *et al*.,[4] obtained yields of 94.2% (w/w), using an ethanol/oil molar ratio of 6:1 and 1.0% (w/w) potassium hydroxide as a catalyst.

Thus, by optimising the reaction conditions for the transesterification reaction, the ester content of 96.5% (w/w) was not achieved. Similarly, the glycerol and glycerides (%) measured in the sample at the optimum conditions for the transesterification was not in accordance with the EN standard specification. There can be several reasons for this, as the transesterification reaction is a complicated process. We used the unrefined rapeseed oil, which consist of FFAs. Therefore, the saponification (at higher concentrations of NaOH) or esterification reactions (at higher concentrations of CH_3OH) is known to occur. The results shown by the measurement of acidity and the moisture content proved the fact that the product entirely depends on the feedstock used for this process.

The important factor in the transesterification is the moisture content in the feedstock as it has a negative influence on the transesterification reaction and it is essential that anhydrous methanol and catalyst are used.[22,23] From the literature, it has been found that amongst alkaline metal alkoxides, NaOH when dissolved in methanol contributes water to the reaction medium, whereas by using CH_3ONa does not add water. To prove the effect of using NaOH or CH_3ONa on the ester content (%), a comparative study of using these catalysts were carried out. The result showed that FFAs that were already present in the oil initiated the saponification reaction, which in turn promoted the hydrolysis reaction. Hence, there was no significant difference in the ester content (%) either using both types of catalysts. However, the product recovery (%) was higher in the case of CH_3ONa than with NaOH. Theoretically, when NaOH is used it is expected that water can be contributed to the reaction medium (Scheme 3.5); by dissolving 0.015 mol (0.6 g) of NaOH in 0.54 mol (17.28 g) of CH_3OH produces

0.015 mol (0.27 g) of water and 0.015 mol (0.81 g) of CH_3ONa. Therefore, the presence of the hydroxide group is responsible for the hydrolysis of triglycerides and the saponification reaction, thus decreasing the yield of the product. Our results are in agreement with the investigations carried out by Vicente *et al*., for sunflower oil in which a 6:1 CH_3OH: oil molar ratio and 1% (w/w) catalyst were used. The yields were reported to be higher for methoxide (98% w/w) than hydroxide (85% w/w).[32]

Different refined vegetable oils were selected because they had lower acid values and moisture content. However, the results did not show a significant improvement in the ester content (%); except the process of separation easier. It was found that the moisture and TAN content was increased after the transesterification reaction. Therefore, demonstrating that competing reactions had taken place and cannot be stopped if alkali catalyst is used. In the case of high saturated oils (sunflower, groundnut, soya and corn oils), the ester content (%) were comparatively lower than the oil high in unsaturation (rapeseed oil). This showed that the viscosity of the oil also plays a major role in the conversion of triglycerides to FAME. Because the oil and CH_3OH are immiscible, the reaction rate is dependent on the phase behaviour of the reactants.

3.6. CONCLUSIONS

Considering the present work and from the perspective of optimising biodiesel production using the crude rapeseed oil, edible quality vegetable oils and the homogeneous catalysts, the following conclusions can be drawn:

- The result of the investigations reported herein show that if the starting material *i.e.* glycerol trioleate or refined vegetable oils used for the transesterification reaction are pure of FFAs and anhydrous, the ester content (%) should be higher than 96.5% (w/w) as specified according to EN 14103 standard. However, this was not the case. This is because the competing reactions were taking place

beside the transesterification reaction. The possibility of competing reactions arises when the water is introduced in the reaction at the start by mixing the CH_3OH with NaOH. This water starts the chain of competing reactions that are responsible for the lower ester content (%).

- The concentration of the alkali catalyst required for the transesterification reaction is dependent on the amount of FFA and water content in the oil used. The unrefined rapeseed oil used for this study displays a higher acidity than 1.05 mg KOH/g; this has inversely proportional effect on the production of FAMEs. If FFAs are present in the starting material, the neutralisation of FFAs is substantial. These FFAs increase the water content in the reaction, which in turn gives rise to the hydrolysis of TGs. The hydrolysis is responsible for the reversible reaction, forming intermediate (mono- and di- glycerides) in the reaction mixture.

- The amount of esters produced increases with an increase in concentrations of NaOH up to 0.015 (mol) but further increasing the catalyst concentration results in a slight decrease in FAMEs. The decrease in ester content is due to saponification whereby the FFAs in the reaction mixtures react with NaOH to form soaps, hence consuming the base catalyst.

- It has been observed from the results that acidity values decreases as the concentration of NaOH increases proving that FFAs are involved in the saponification reaction, which formed soaps and water. The data obtained from the determination of moisture content also complemented with the acidity data that at higher moles of NaOH and CH_3OH, the moisture of FAMEs was higher due to saponification reaction.

- There was no significant difference in methyl ester content when sodium methoxide instead of sodium hydroxide except that the production cost is higher when using the former catalyst.

- The stoichiometric ratio for transesterification requires three moles of alcohol and one mole of oil to drive the reaction to the equilibrium. However, experimentally three moles of alcohol were not sufficient to drive the reaction to completion. The ester yield increased as the molar ratio of methanol was increased. At higher methanol ratios (above 6:1 CH_3OH: oil molar ratio), the ester content (%) obtained at lower concentrations of NaOH was significantly higher. The reason is that the formation of methoxide ions occurs readily; they are needed to attack the carbonyl carbon of TGs and initiate the reaction mechanism. Moreover, the other reason for the higher ester content (%) at higher concentration of methanol is likely to be due to the esterification reaction. Because the concentration of FFAs is higher in unrefined rapeseed oil, there is a possibility that FFAs reacts with CH_3OH to yield esters and water. Nevertheless, from the results obtained, it has been shown that the water and ester content increases with an increase in concentration of methanol. However, the separation of the polar and non-polar phase was difficult at higher concentrations of methanol, as discussed earlier. Therefore, a molar ratio of 6:1 seems to be the most appropriate.

- The trend, assumed to be similar for all types of refined and unrefined oils, is that by increasing the concentrations of catalyst (NaOH) beyond 0.015 (mol) there is a decrease in the ester content.

- The process of separation of methyl ester and glycerol layer for refined oils after the completion of the transesterification reaction is much easier and quicker than using unrefined rapeseed oil due to the lower amounts of FFAs present in refined oils.

- By determining the fatty acid compositions of the oils, it has been shown that the concentrations of methanol or catalyst have no effect on the rate of release of the fatty acids. The results showed that the saturated refined oils have lower ester content (%) relative to unsaturated oil due to the viscosity of the oil. This highlights the hypothesis that the miscibility of the reactants is important in yielding the higher ester content.

- In using a homogeneous catalyst (NaOH), several drawbacks were observed. The FFAs and water interfere with the reaction such that the catalyst has to be removed from the reaction mixture by washing it several times with water. When used on an industrial scale, this alkaline water waste also needs treatment. In order to minimise the problems associated with the use of a homogeneous catalyst and in order to understand the kinetics of the reaction, attempts were made to use a heterogeneous catalyst; the results of such investigations are reported in Chapter 4.

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CHAPTER 4-A

BIODIESEL PRODUCTION USING HETEROGENEOUS CATALYTIC SYSTEMS

4A.1. INTRODUCTION

Heterogeneous catalysts commonly comprise metals or metal oxides that are thermally robust[1] which is frequently advantageous since the reaction rate increases with temperature. Heterogeneous catalysts are frequently less susceptible to decomposition by moisture or oxygen compared to homogeneous catalysts.[2,3]

Many types of heterogeneous solid base catalysts have been studied (see Section 1.5.4). The order of activity among alkaline earth oxide catalysts is BaO>SrO>CaO>MgO.[4-6] Peterson reported that magnesium oxide displays low activity for the transesterification of vegetable oils to biodiesel whereas calcium oxide provides a slow reaction rate.[7] Barium oxide is not suitable because it is harmful and dissolves in methanol.[8-11]

In the work reported in this chapter, different types of metal oxides were explored to study their potential catalytic activity to produce biodiesel. Additionally, by using strontium oxide (SrO) as a solid base catalyst, experiments were designed to study the kinetics of the transesterification reaction which will help to optimise the reaction conditions. Strontium oxide is a well-known catalyst and can catalyse many chemical reactions such as oxidative coupling of methane, selective oxidation of propane, nitroaldol reactions and mixed Tishchenko reactions but not much work has been carried out using this catalyst in transesterification reactions. Strontium oxide due to it high basicity and insolubility in methanol, vegetable oils and fatty acid methyl esters has attracted attention as a heterogeneous catalyst.[12-15]

In addition, it has been found that glucosinolates are present in almost all plants of the order *Brassicales*. The glucosinolate can be adsorbed on the metal oxides. Considerable research has been conducted on the nutritional value of glucosinolate in crops, but not on the adsorption of catalysts.[16-18] The adsorption takes place by electrostatic interaction between the negatively charged glucosinolate (Figure 4A.1) and positive sites on the metal oxide catalyst.[19] It was hypothesised that the glucosinolate content in the rapeseed oil may deactivate the catalyst activity toward the conversion of triglycerides into ester content (%). Therefore, the effect of glucosinolate on the catalyst deactivation was studied.

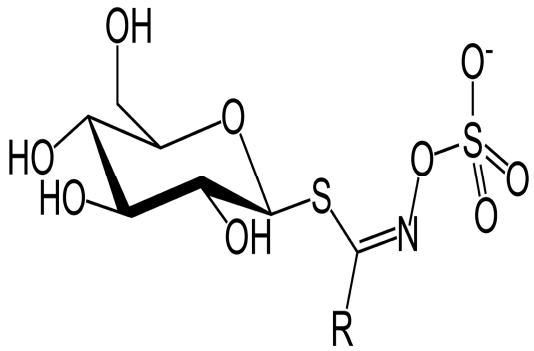

Figure 4A.1. Structure of glucosinolate, R group varies.

4A.2. EXPERIMENTAL AND INSTRUMENTATION

4A.2.1. Materials and Experimental Procedures

See Section, 2.1 for Materials and Sections 2.2.2-2.2.4, respectively, for Methods in Chapter 2.

4A.3. ANALYTICAL METHODS

4A.3.1. Determination of Ester/ and Linolenic Acid Methyl Ester Content and Free/ and Total Glycerol and Mono-, Di-, Triglyceride Contents

See Sections, 2.3.3 and 2.3.4, respectively, Methods in Chapter 2.

4A.4. RESULTS

4A.4.1. Transesterification of Unrefined Rapeseed Oil with Different Type of Metal Oxides

Experiments were conducted with different types of metal oxides in order to achieve the conversion of triglycerides into FAMEs. These metal oxides (Gd_2O_3, CeO_2, Y_2O_3, TiO_2, SnO_2, ZrO_2, La_2O_3 and SrO) were chosen according to their oxidation states as mentioned in Chapter 2, Section 2.2.3. The prediction prior to transesterification was that all the catalysts in Table 4A.1 would catalyse the transesterification reaction.

The results showed that there was no phase separation *i.e.* ester phase and glycerol phase, after the transesterification reaction was terminated and the separated the layers by centrifugation for all the catalysts used, except for strontium oxide. Moreover, with cerium oxide an emulsion was formed which was difficult to separate to continue further investigations on it.

Table 4A.1. Catalysts used for the transesterification reaction. (reaction conditions: 3% (w/w) catalyst, 6:1 CH_3OH: oil molar ratio, 120 min at 60 °C)

Catalysts	Prediction	Result
Gadolinium oxide(Gd_2O_3)	✓	No reaction
Cerium oxide (CeO_2)	✓	Emulsion formed
Yttrium oxide (Y_2O_3)	✓	No reaction
Titanium oxide (TiO_2)	✓	No reaction
Tin oxide (SnO_2)	✓	No reaction
Zirconium oxide (ZrO_2)	✓	No reaction
Lanthanum oxide (La_2O_3)	✓	No reaction
Strontium oxide (SrO)	✓	91.8% (w/w) ester content

Table 4A.2 showed that the ester content (%) obtained by using SrO catalyst was *ca.* 92% (w/w) which is below the limit set by the EN 14103 standard. Moreover, the mono- (*ca.* 2% w/w), di- (*ca.* 1% w/w) and tri-glycerides (*ca.* 3% w/w) content was higher than the limit specified by the EN 14105 standard. These results indicate that strontium oxide had effectively catalysed the transesterification reaction but the presence of intermediates represents the incompletion of the reaction. However, the glycerol content (%) was lower than the EN standard limit indicating that the glycerol formed during the reaction had been removed efficiently by centrifugation.

Table 4A.2. Properties of washed and dried biodiesel obtained from unrefined rapeseed oil *via* the transesterification reaction. (reaction conditions: 3% (w/w) SrO, 6:1 CH_3OH: oil molar ratio, 120 min at 60 °C)

Property	Value	Limits	Standard
Total FAME content (% w/w) [a]	91.8 ± 0.80	96.5 min	EN 14103
Monoglycerides (% w/w) [a]	2.09 ± 0.007	0.8 max	EN 14105
Diglycerides (% w/w) [a]	1.15 ± 0.001	0.2 max	EN 14105
Triglycerides (% w/w) [a]	2.97± 0.40	0.2 max	EN 14105
Free glycerol (% w/w) [a]	0.01± 0.011	0.020 max	EN 14105
Total glycerol (% w/w) [a]	1.02 ± 0.035	0.25 max	EN 14105

[a] Standard error calculated for three replicates.

4A.4.2. Transesterification Reaction Using SrO as a Catalyst

Taking the aforementioned results into account, optimisation of the transesterification reaction using SrO as a heterogeneous catalyst was required. Several experiments were conducted to study the kinetics of the transesterification reaction, as a function of time (60-420 min), and by varying the amount of strontium oxide (3%-7% w/w). The remaining parameters (6:1 CH_3OH: oil molar ratio, 60 °C, 600 rpm) were kept constant.

The total fatty acid composition of the unrefined rapeseed oil was calculated after the transesterification reaction was terminated at different time intervals by using 3

to 7% (w/w) SrO (Table 4A.3). There were no significant differences found for all the fatty acids. Therefore, it is believed that all the fatty acids are released at the same rate during the reaction irrespective of the amount of catalyst or reaction time. Table 4A.3 shows that the saturated fatty acid content was *ca.* 7% (w/w) whereas the unsaturated fatty acids content was *ca.* 93% (w/w) by using SrO catalyst.

Table 4A.3. Composition of fatty acids of unrefined rapeseed oil determined by using 3-7% (w/w) SrO at 60-420 min.

Chemical composition (% w/w)[a]	
Palmitic ester % (C16:0)	4.53 ± 0.17
Stearic ester % (C18:0)	1.37 ± 0.09
Oleic ester % (C 18:1)	63.20 ± 0.91
Linoleic ester % (C 18:2)	18.18 ± 0.62
Linolenic ester % (C 18:3)	9.43 ± 0.59
Arachidic ester % (C 20:0)	0.50 ± 0.05
Gadoleic ester % (C 20:1)	1.49 ± 0.07
Behenic ester % (C 22:0)	0.34 ± 0.04
Erucic ester% (C 22:1)	0.68 ± 0.11
Lignoceric ester % (C 24:0)	0.18 ± 0.07
Nervonic ester % (C 24:1)	0.09 ± 0.08
Average (% total esters)	282.01 ± 0.20

[a] Standard error calculated for 54 replicates.

Figure 4.2 shows that the ester content (%) at all the concentrations of SrO used increased from 60 to 120 min reaction time then decreased as the reaction proceeded beyond 120 min. Moreover, it was observed that by using higher concentrations (5% and 7% w/w) of SrO led to a mixing problem and therefore the ester content (%) was relatively lower than 3% (w/w) SrO. Therefore, it can be concluded that the increased concentration of SrO posed the mixing problem.

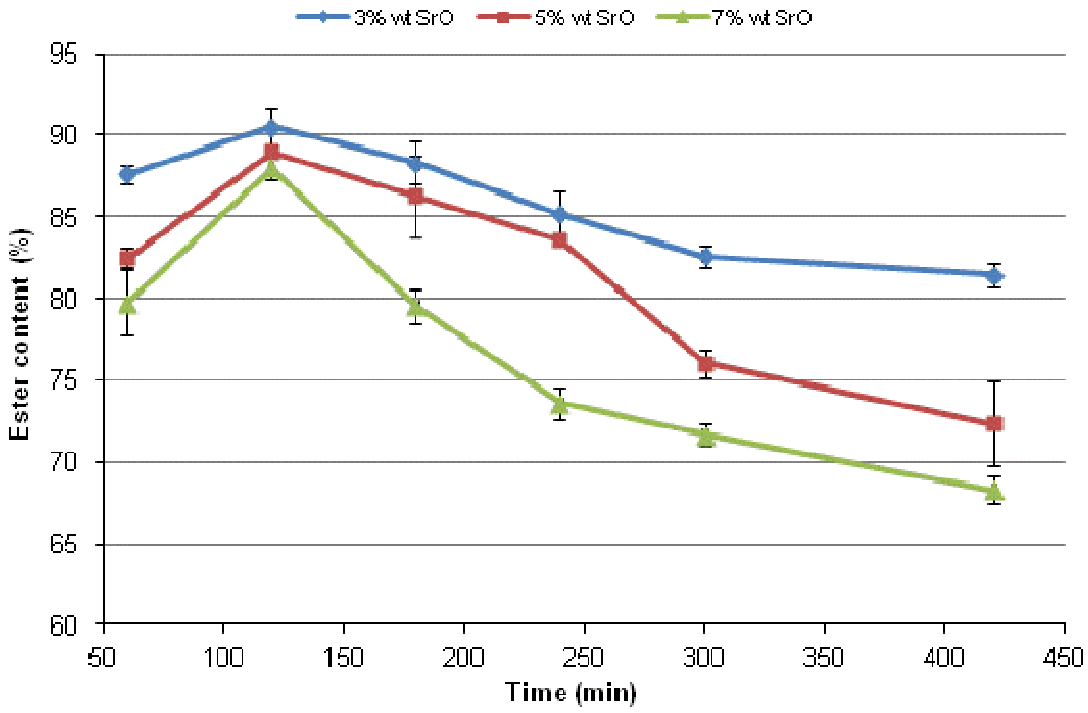

Figure 4A.2. Effect of variable amounts of SrO catalyst and reaction times on ester content (%). (reaction conditions: 6:1 CH_3OH: oil molar ratio, 60 ºC, 600 rpm.)

4A.4.3. Effect of Glucosinolate on Ester Content (%)

Figure 4A.3 shows the effect of glucosinolate on the transesterification reaction. The glucosinolate concentrations in the reaction were varied by adding 0.01% (w/w) or 0.10% (w/w) glucosinolate. The results showed that the ester content was 92.1% (w/w) in the absence of glucosinolate. The addition of 0.01% (w/w) glucosinolate in the reaction mixture decreases the ester content to 87.1% (w/w). A further decrease in ester content (80.1% w/w) was observed by adding 0.10% (w/w) glucosinolate.

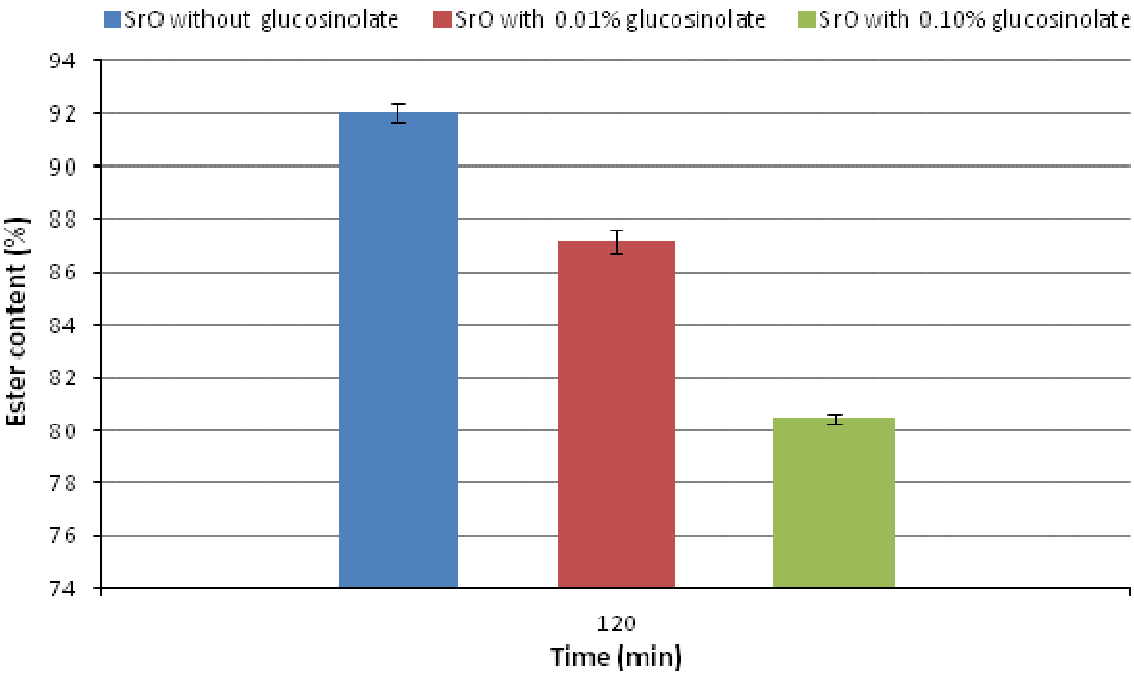

Figure 4A.3. Effect on ester content (%) by adding glucosinolate during the transesterification reaction. (reaction conditions: 6:1 CH_3OH: oil molar ratio, 60 ºC, 600 rpm.). Values are the mean of three replicates, error bars indicate standard deviations.

4A.5. DISCUSSION

The present analysis and optimisation study reveals that strontium oxide, amongst the heterogeneous catalysts used, is suitable for the transesterification of rapeseed oil. Except for SrO, the metal oxides tested used for the transesterification showed no phase separation after the reaction was terminated at 120 min. No reports exist of the use of Gd_2O_3, Y_2O_3 and CeO_2 metal oxide catalysts for the transesterification reaction. However, several reports suggest that the catalytic activity of ZrO_2, TiO_2, SnO_2 and La_2O_3 is enhanced when their surface contains anions such as sulphate and tungstate but this increases the production cost and moreover, is used for the esterification of fatty acids.[20-23] However, using SrO as a catalyst for the transesterification reaction has led to *ca.* 92% (w/w) esters that could be due to the higher alkalinity among alkaline earth metal oxides. The catalytic activities increased in

the order MgO<CaO<SrO<BaO.[24] Therefore, this suggests that the catalytic activities of metal oxides towards transesterification are associated with their alkalinities. The determination of glycerol and glycerides (%) content showed the presence of intermediates suggesting that either the reaction was incomplete or the reaction was reversed after attaining equilibrium.

To investigate this further, optimisation studies were carried out by varying the amount of catalyst and time. The result showed that at 120 min the highest ester content (%) was observed relative to other reaction times. As the equilibrium state was reached, the transesterification reaction may have started to revert to starting materials perhaps due to higher concentration of methyl esters and intermediates (mono- and di-glycerides) in the reaction mixture.[25,26] This idea is supported by the notion that at 120 min, intermediates were present as shown in the data presented in Table 4A.2; which shows that the reaction has been reversed because by increasing the time, the ester content (%) were proportionally lower. Even though the concentration of SrO and time was varied, no significant improvement was observed in ester content (%). The highest ester content was *ca.* 92% (w/w), obtained at 120 min, with 3% (w/w) SrO, a 6:1 CH_3OH: oil molar ratio and at 60 ºC.

By increasing the time of the reaction, the ester content (%) was decreased implying the reversibility of the reaction. However, an increase in SrO concentration decreased the ester content (%) due to mixing problems. A higher percentage of catalyst would be expected to result in a higher conversion of triglycerides to FAMEs because of the availability of more active sites. However, this may not always be true. Yang *et al*., and F.Qui *et al*., reported that by increasing the amount of loading catalyst, the slurry (mixture of catalysts and reactants) becomes too viscous to give rise to a problem of mixing, [27, 28] thus providing a limited surface area to the catalyst to react more efficiently. As oil and methanol are not completely miscible, the mixing efficiency can affect the course of the transesterification reaction. The reaction can only occur in the interfacial region between the methanol, oil and catalyst; the metal oxide catalysts are

essentially insoluble in the two phases.[29] Patil *et al.*, reported less than 65% (w/w) ester content for the transesterification of *Camelina Sativa* oil by using 2% (w/w) SrO, 6:1 CH_3OH: oil molar ratio, at 100 ºC for 3hrs. Nevertheless, by increasing the methanol concentration to 12:1 CH_3OH: oil molar ratio, the ester content (%) was increased to 85% (w/w).[30, 31] However, an excess of methanol can interfere in the separation of glycerol because of an increase in solubility, which in turn decreases the yield of biodiesel

The results obtained also highlight the fact that the presence of glucosinolate, in the reaction mixture, can affect the catalytic activity of SrO. This, presumably, could be one of the reasons for not achieving a ester content above 96.5% (EN 14103 standard). In the literature, no research has been reported on the interaction of glucosinolate with metal oxides.

In summary, these results showed that the ester content (%) was not achieved according to EN 14103 standard even by optimising the reaction conditions:

a) Glucosinolate present in rapeseed oil can inhibit the activity of metal oxides.[19] This could be the reason that the presence of glucosinolate can interfere with the activity of the catalyst, thus reducing the efficiency of the SrO to react at higher concentrations.

b) Heterogeneous catalysts are well-known for exhibiting slow reaction rates.[32] This is due to diffusion problems since the heterogeneous media behave as a three-phase system (oil/methanol/catalyst). Therefore, real-time monitoring of the transesterification reaction will help to understand the cause of incomplete reaction.

4A.6. CONCLUSIONS

In attempts to investigate the use of heterogeneous catalyst for biodiesel production, the results attained can be summarised as follows:

- The experimental results demonstrated strontium oxide to be an effective catalyst for the conversion of rapeseed oil to FAME. The ester content using 3% (w/w) SrO (90.5% w/w) showed higher conversion as compared to 5% (w/w) (89.0% w/w) or 7 % (w/w) (88.0% w/w) at 120 min. However, the ester content (%) was, comparatively, lower than the ester content (%) obtained with the homogeneous catalyst in Chapter 3 because heterogeneous catalysts are known for their slower reaction rates.

- The results showed that 60 min was not enough time for the transesterification of rapeseed oil to reach equilibrium. However, at 120 min all the concentrations of catalyst used reached maximum ester content (%). The reaction time beyond 120 min showed a decrease in ester content (%) for all the concentration of SrO used, which could be due to the fact that the reaction may have started to reverse due to the presence of increased amounts of methyl esters in the reaction mixture.

- The addition of glucosinolates in the reaction mixture proportionally lowered the ester content (%) and hence supported the hypothesis that metal oxide catalysts can interfere with glucosinolates. The poisoning of the catalyst is probably due to the presence of glucosinolate that is adsorbed on the surface of metal oxide.[19] However, detailed studies are required to establish this effect.

- Using heterogeneous catalysts, the transesterification reaction proceeds at a relatively slow rate compared to those conducted with homogeneous catalysts. It is believed that real-time monitoring of the transesterification study will provide

detail mapping of the reaction, which in turn will be helpful for reaction optimisation. In spite of this, the use of heterogeneous catalysts is advantageous because of their easy separation from the product and a potential reduction in environmental pollution.

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CHAPTER 4-B

KINETICS OF THE TRANSESTERIFICATION REACTION BY USING REFRACTOMETRY AND GC

4B.1. INTRODUCTION

In the work reported in this chapter, a kinetic study was conducted using refractometry, which can provide real-time monitoring of the reaction as compared to *e.g.*, gas chromatography. The refractive index differences are sufficient to give an indication of the conversion of triglycerides into methyl esters in the transesterification reaction.[1] Refractive index measurements can be used to get a quick and approximate measure of conversion provided the following conditions are met:

- the temperature is kept constant,
- the ester content (%) and refractive index of the reaction mixture at t=0 are known, and
- the ester conversion (%) from triglyceride and refractive index of the reaction mixture of the final sample are known.

Xie *et al.* reported a linear correlation between refractive indices and ester content (%) when using soybean oil. They found less than 4% (w/w) difference between the ester conversion determined by ^{1}H-NMR spectral data and those by refractometry (Table 4B.1). However, the ester content (%) estimation by the refractive index values were carried out at 30.15 ºC (303.15 K).[1]

Table 4B.1. Conversions of soybean oil determined by different analytical techniques.[1]

Entry	Refractive Index (RI)	Conversion (RI) (%)[a]	Conversion (^{1}H NMR) (%)[b]
1	1.4704	0	0
2	1.4660	23.2	22.6
3	1.4616	46.1	45.5
4	1.4599	55.4	52.9
5	1.4580	65.6	62.7
6	1.4560	76.1	73.4
7	1.4544	84.4	82.1
8	1.4515	100	100

[a] Conversion (RI), determined by refractometry.
[b] Conversion (^{1}H-NMR), determined by ^{1}H-NMR spectroscopy.

The possible components of the reaction mixture, *i.e.*, glycerol, MG, DG, TG, and methyl esters have different refractive indices. The refractive index of different fatty acid methyl esters show a difference at different temperatures (Figure 4B.1).[2] Therefore, in order to use this analytical technique a strict control of temperature is required.

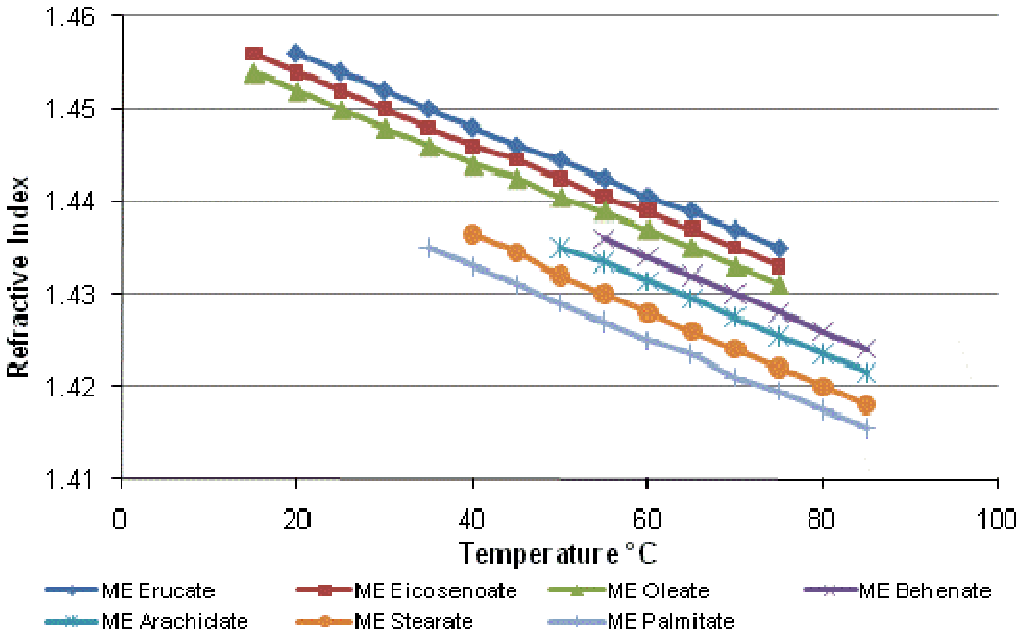

Figure 4B.1. Refractive indices of methyl esters of fatty acids at different temperatures.[2]

4B.2. EXPERIMENTAL AND INSTRUMENTATION

4B.2.1. Materials and Experimental Procedures

See Section, 2.1 for Materials and Section 2.2.5 for Methods in Chapter 2.

4B.3. ANALYTICAL METHODS

4B.3.1. Determination of Ester and Linolenic Acid Methyl Ester Content and Refractive Index

See Sections, 2.3.3 and 2.3.5, respectively, Methods in Chapter 2.

4B.4. RESULTS

4B.4.1. Use of Refractometry to Monitor the Rate of Transesterification Reaction

The starting refractive index for rapeseed oil and methanol was 1.4750±0.0012 and 1.3277±0.0010, respectively. Figure 4B.2 shows the change in refractive index on mixing oil with methanol at 60 °C in the absence of catalyst.

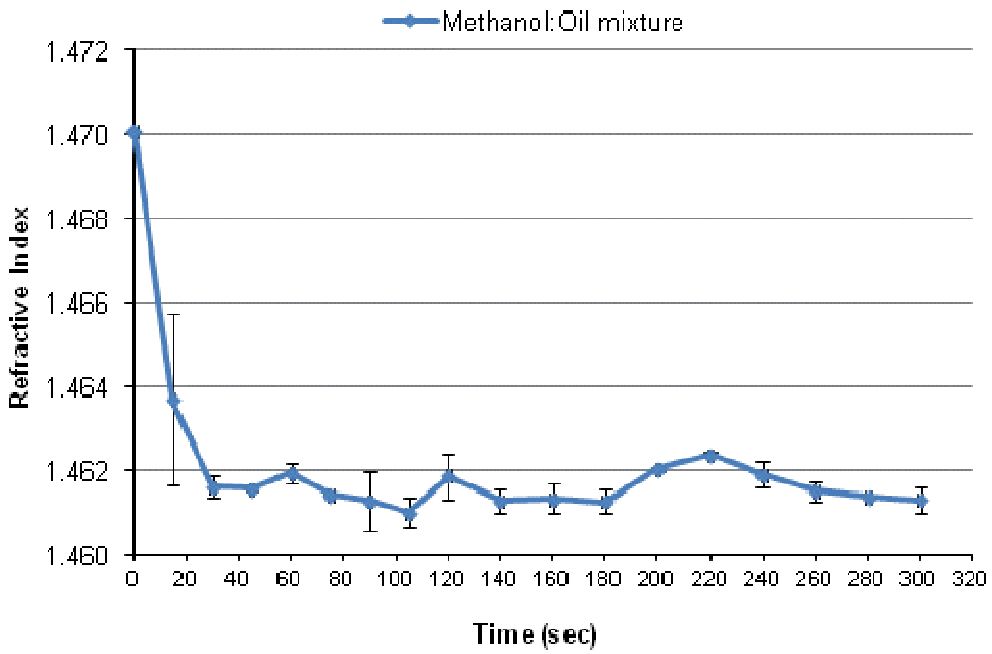

Figure 4B.2. Refractive index (RI) of a mixture of rapeseed oil and methanol with respect to time at 60 °C. Values are the mean of three replicates, error bars indicate standard deviations.

The data show that, as the mixing starts, there is a sharp decrease in the value of refractive index from 1.4705 to 1.4638. However, there was no significant change observed in refractive index values after 30 sec as it remained between 1.4610 and 1.4625.

Figure 4B.3 shows the relationship between the refractive indices and the ester content (%) measured using refractometry and GC for the transesterification of unrefined rapeseed oil with time. As the oil comes in contact with methanol and SrO, there is a decline in refractive index value from 1.4736 to 1.4641 in 30 sec corresponding to the mixing of the oil and methanol phases. The ester content of the oil, between 1 and 12 min was 0.3% (w/w) There was no significant change in refractive index values from 0-12 min (1.4639 -1.4648).

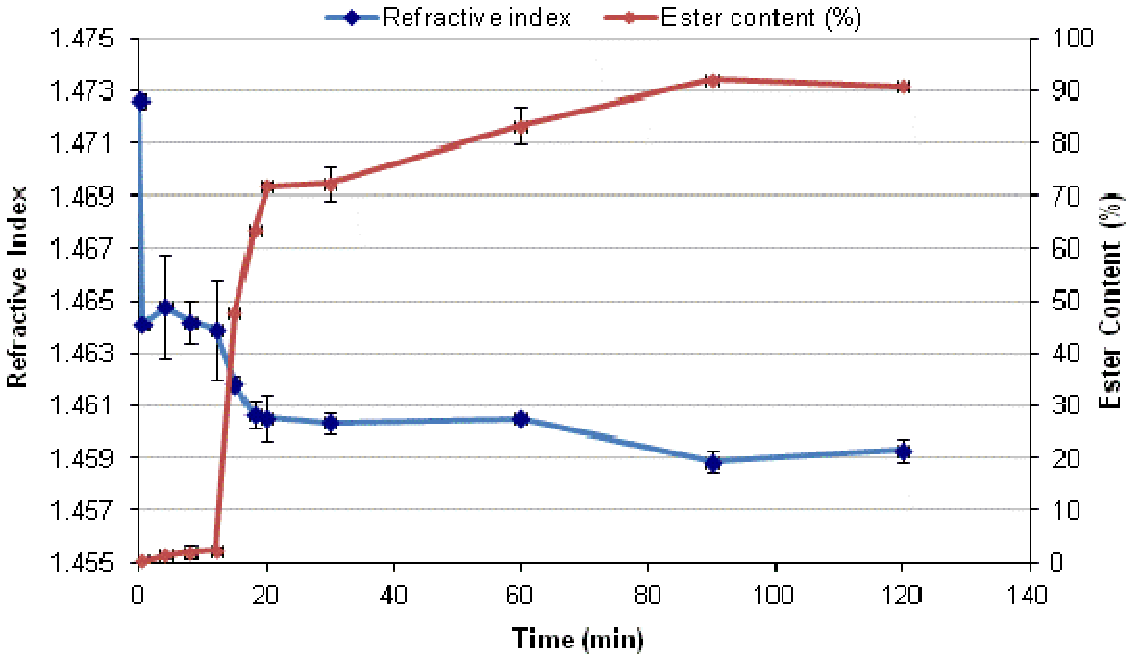

Figure 4B.3. Refractive index and ester content (%) measurement for the transesterification reaction with time. Values are the mean of three replicates, error bars indicate standard deviations.

There was a decrease in refractive index values after 12 min corresponding to an increase in the ester content (%). The ester content rapidly increased from 0.3 to 72%

(w/w) ester from 12 to 30 min. After that the ester content (*ca*. 92.0% w/w) continued to increase until 90 min and then plateau'd off with a further increase in time. Similarly, there was a decrease in refractive index value (1.4589) at 90 min before reaching a plateau at 120 min.

4B.5. DISCUSSION

The slow reaction rates exhibited by metal oxide catalysts in heterogeneous reaction media is due to the low solubility of oil in the methanol phase that slows down the transesterification reaction resulting in lower ester content and detected by using refractometry. The progression of the transesterification reaction can be categorised into three stages:

1. the oil comes in contact with methanol phase and catalyst.
2. the intersolubility of oil and methanol increases thus the concentration of FAMEs also gradually increases with respect to time.
3. the concentration of products *i.e*. FAMEs and glycerol is maximum in oil.

The start the reaction is delayed by 12 min, as the oil and methanol phases mix and is exposed to the full surface area of the catalyst. This conclusion is verified by the GC results as the ester content (%) were negligible in the oil until 12 min, assuming that no catalytic activity occurred. This is because oil and methanol are immiscible due to the differences in their polarity, as clearly shown in Figure 4B.2.

After 12 min, the RI showed a decrease with an increase in ester content (%). The result highlights the fact that the inter-solubility of oil-CH_3OH gradually increases with increasing concentration of FAMEs. Formation of methyl esters in the reaction mixture acts as a co-solvent for the transesterification reaction. Therefore, the decrease in refractive index values at this point represents the concentration of FAMEs in the reaction mixture. Once the single phase mixture is formed, the inter-phase mass transfer limitation is removed and the reaction rate increases dramatically.[3] Hence, the ester

content (%), in the reaction mixture after 12 min, showed a rapid progression until 30 min.

The rate of conversion of triglycerides into FAMEs slowed down due to the presence of glycerol (a co-product of the reaction) after 30 min. There was also no significant change in refractive index. The foregoing also indicates the presence of glycerol in the reaction mixture with respect to time. It can be observed from the data shown in Figure 4B.3, that the highest ester content (%) was obtained at 90 min. When the reaction was carried out beyond 90 min there was no significant improvement in the ester content (%). Therefore, to achieve the highest ester content (%) 90 min was enough reaction time instead of 120 min.

Thus, a real-time kinetic study of the transesterification reaction with refractometry supports the data obtained by GC and helped to improve the optimum reaction conditions used earlier in Chapter 4-A. It also highlights the fact that the presence of methyl esters in the reaction mixture promotes the reaction towards completion. However, the formation of glycerol reverses the transesterification reaction.

4B.6. CONCLUSIONS

In attempts to investigate the use of refractometry as an analytical tool for tester determination, the following conclusions can be drawn:

- The experimental results show that refractometry, as an analytical tool, can be used to monitor the completion of the transesterification. Compared with existing chromatographic methods (as well as other methods), this technique of monitoring the transesterification of vegetable oils with methanol is rapid, simple and inexpensive and is especially suitable for process control purposes.

- The application of refractometry allowed real-time kinetic studies of the transesterification reaction. For cross-checking purposes, ester content (%) was also monitored by gas chromatography which helped to establish this analytical method for future studies.

- A kinetic study of the transesterification reaction, using a heterogeneous catalyst, by refractometry helped to optimize the reaction time used in Chapter 4-A. It is concluded that the reaction could be terminated at 90 min rather than continuing until 120 min.

- The slow reaction rates are due to diffusion problems since the heterogeneous media behave as a three-phase system (oil/methanol/catalyst).[4] It is believed that a phase solubility study is required to promote oil/methanol miscibility, which in turn, will accelerate the transesterification reaction by enhancing the contact of reactants with the solid catalyst.

4B.7. REFERENCES

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CHAPTER 5

PHASE SOLUBILTY

5.1. INTRODUCTION

The kinetic studies reported in the previous chapter raised the problem of complex phase behaviour in respect of the reactants *i.e.* methanol, oil and heterogeneous catalyst. Low solubility of the reactants is a likely cause of slow reaction rates as detailed earlier in Chapter 4-B. The phase behaviour of the oil and methanol and the distribution of the catalyst between these respective liquid phases can significantly affect reaction rates.[1] As oils and alcohols are immiscible, the initial reaction system comprises two phases–an alcohol phase and an oil phase.[2] Gunvachai *et al.* reported that transesterification occurs in the methanol phase.[3] Therefore, the challenge is to obtain a single phase comprising all three components in order to increase the reaction rate. However, the problem is that neither oil nor the catalyst is soluble in the methanol phase. If the solubility of the oil is increased in the methanol phase then the catalyst will presumably have only one phase in which to react. Therefore, the reaction rate for transesterification very much depends on the solubility of oil in methanol.

Co-solvents have been used to overcome slow reaction rates. They typically show amphoteric properties dissolving both in hydrophobic and hydrophilic phases enabling the close proximity of oil and methanol. The disadvantage of using a co-solvent is that when the reaction is completed the solvent needs to be removed from the reaction mixture. Such co-solvents include dimethyl ether,[4] tetrahydrofuran (THF) and 1,4-dioxane.[5] On the other hand, FAMEs is also a co-solvent with amphoteric property. The advantage of using FAME is it does not need to be removed from the reaction mixture. Therefore, to avoid further processing, addition of FAMEs is also an efficient method for producing a single phase. Zhou *et al.* measured the inter-solubities of *Jatropha curcas L.* oil, methanol and FAME and concluded that the reaction system

changes with the oil sources.[6] In this chapter, the solubility of multi-component systems, using rapeseed oil, methanol and FAMEs is reported. A ternary phase diagram was plotted in order to study the transesterification reaction in the miscible region of the phase diagram based on the solubility data.

Other experiments were designed to study the kinetics of the transesterification reaction by mixing the FAME in the oil-methanol phase in order to obtain a homogeneous phase.

Refractive index measurements were used to monitor the phase changes during the transesterification reaction in Chapter 4-B and a good correspondence was found. Therefore, it can provide a marker in order to probe the progress of the transesterification reaction.

5.2. EXPERIMENTAL AND INSTRUMENTATION

5.2.1. Material and Experimental Procedures

See section, 2.1 for Materials and 2.2.6 to 2.2.11 for Methods in Chapter 2.

5.3. ANALYTICAL METHODS

5.3.1. Determination of Ester /and Linolenic Acid Methyl Ester Content, Free/ and Total Glycerol and Mono-, Di-, Triglyceride Contents and Refractive Index.

See sections, 2.3.3 to 2.3.6 in Chapter 2, respectively.

5.4. RESULTS

5.4.1. Phase Diagram

Figure 5.1 shows the solubility system for the rapeseed oil–methanol–FAME system plotted as a ternary phase diagram (concentrations given in volume percent). The results were plotted with the 100% compositional points for oil, methanol and FAME located at the bottom left, bottom right, and the upper apex, respectively, on the

plot. Based on turbidimetric measurements, two zones of miscibility were identified. A gradient of miscibility was achieved due to the amphoteric nature of the FAME. The shaded area represents the immiscibility of oil and methanol in the presence of FAME. The un-shaded area represents the zone of miscibility between the solvents. In the shaded area, two liquid phases coexists, while only one liquid phase occurs in the un-shaded area.

For example, if the starting mixture has a composition of 20 vol. % oil and 60 vol. % of methanol and 20 vol. % of FAME (Figure 5.1, shown in arrow), the composition of all these reactants (oil, methanol and FAME) have to be changed to 10 vol. % of oil, 50 vol. % of methanol and 40 vol. % of FAME before a liquid homogeneous system is obtained.

At higher a concentration of oil (up to 40%) and lower concentrations of methanol (up to 10 %) with FAME concentration (up to 50%), immiscibility was increased to such an extent that it was difficult to judge the endpoint (turbidity). Therefore, only one data point is plotted on the phase diagram. The miscibility of oil and methanol gradually increases with increasing concentration of FAMEs; when the concentration of FAMEs in the mixture reaches above 52 vol. %, the two-phase mixture becomes homogeneous. This ternary phase diagram will be used to investigate whether it can be used to optimise reaction conditions and the effect of working in a one phase system. Selecting any concentration of rapeseed oil, methanol and FAMEs from the miscible region should reduce the immiscibility problem.

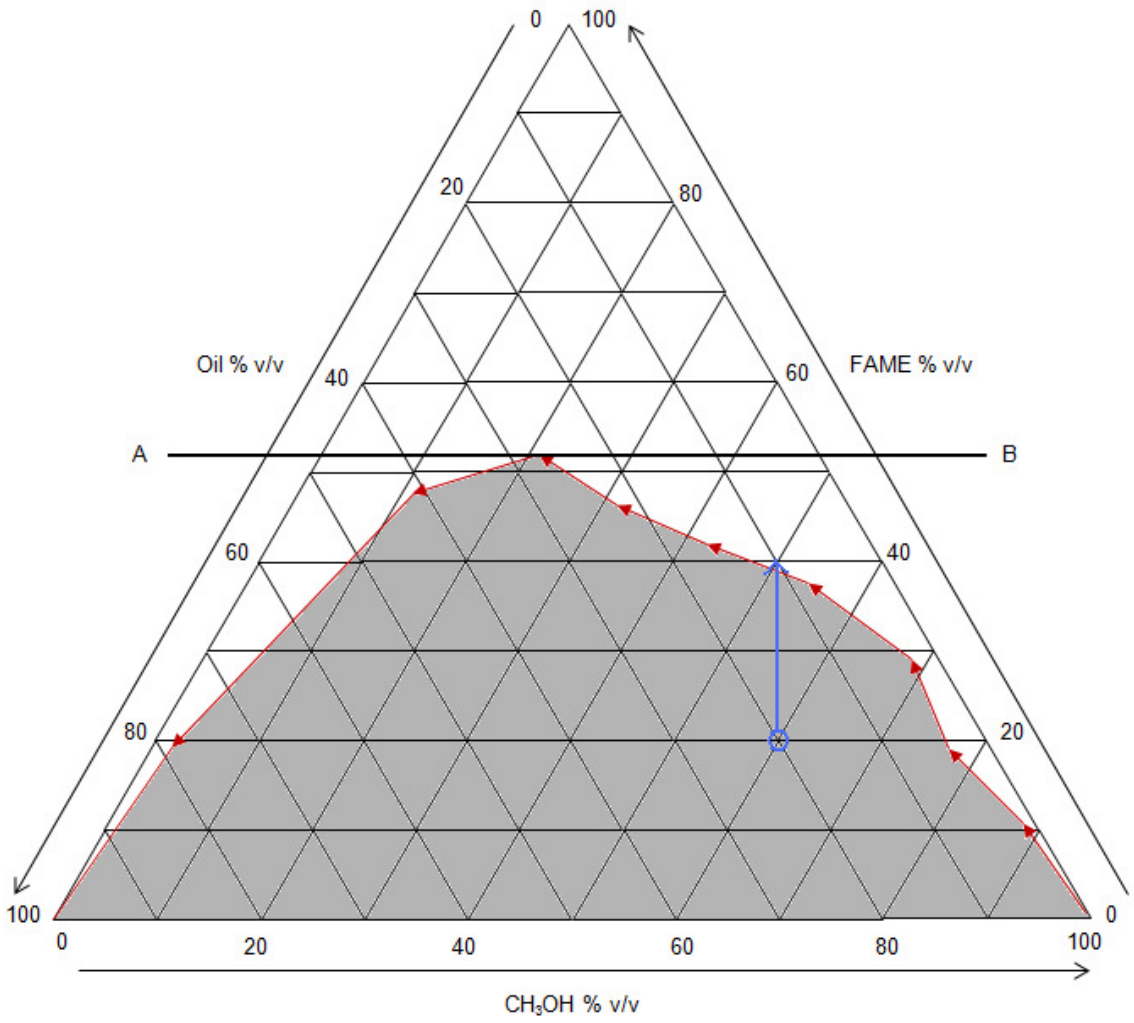

Figure 5.1. Ternary phase diagram showing the miscibility properties of rapeseed oil–methanol–FAME at 60 °C. Rapeseed oil–methanol–FAME was titrated to the point of miscibility, by turbidimetric analysis using titration. The starting point of arrow (⟶) represents a composition of 20 vol. % oil, 60 vol. % methanol and 20 vol. % FAME and the endpoint of arrow is 10 vol. % oil, 50 vol. % methanol and 40 vol. % FAME. Line A to B represents the point of miscibility. The shaded area is the immiscible region and the un-shaded area is the miscible region.

The data points for oil: methanol: FAME (right to left) plotted in Figure 5.1 are shown in Table 5.1.

Table 5.1. Volume (%) ratio of oil, methanol and FAME.

Data Entry	Oil (vol %)	Methanol (vol %)	FAME (vol %)
1	01	89	10
2	04	77	19
3	03	68	29
4	08	55	37
5	16	42	42
6	23	31	46
7	26	22	52
8	42	10	48
9	78	02	20

5.4.2. Transesterification Reaction Conducted in the Miscible Region

Reactant quantities were selected based on the output described by the phase diagram (Figure 5.1). In experiments **1**-**4**, the vol. % of the reactants used were 60 mL FAME: 33 mL oil: 7 mL CH_3OH with addition of 3% (w/w) SrO. Detailed reactions conditions are given in Section 2.2.7, Chapter 2.

The sample of FAME used in experiment **1** was not pure; it had an ester concentration of 88.6% (w/w) determined by GC. Therefore, the final concentration of esters was calculated to be 53.1 mL ester/60 mL of FAME (v/v) *i.e.* 46.24 g of ester/52.2 g of FAME (w/w) in the reaction mixture. As the concentration of esters in the FAME sample was 88.6% (w/w) the remaining 12.4% (w/w) was assumed to be composed of the total/free glycerol and unreacted mono-, di-, and triglycerides. The particular reaction based on the purity of FAME sample was conducted by taking the reactant quantities (in vol. %) as shown on the phase diagram (Figure 5.2).

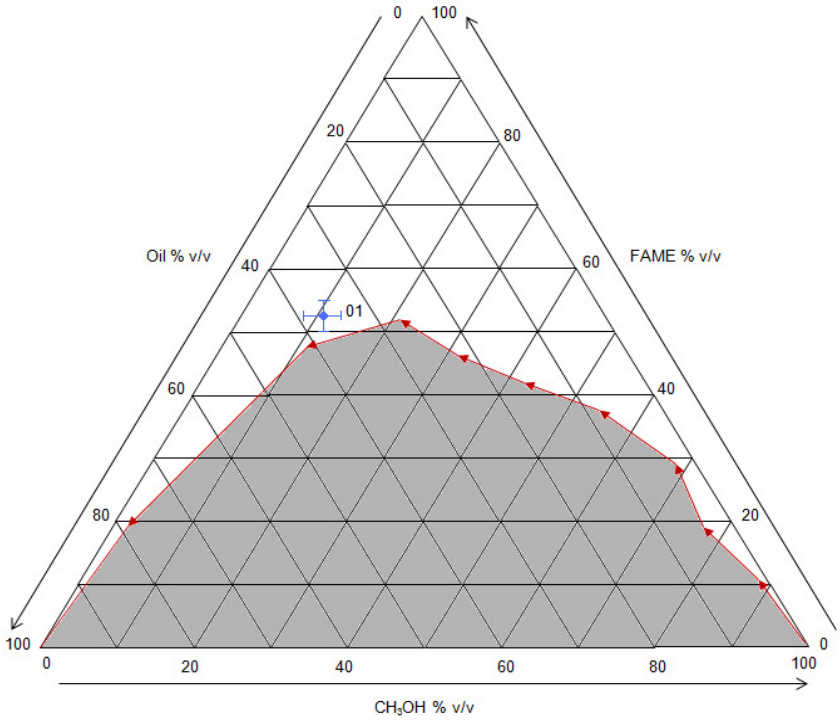

Figure 5.2. Ternary phase diagram showing the data point for experiment **1**.

The reaction was monitored at different time intervals by refractometry and two reaction samples were collected at 45 and 60 min for ester content (%) determination by GC. The ester content at 45 min was 54.7% (w/w) (Expt. **1**) and it increased to 56.7% (w/w) when the reaction was stopped at 60 min (Figure 5.3). Therefore, the ester content (%) at 45 and 60 min showed an increase of 8.46% (w/w) and 10.46% (w/w), respectively from the starting concentration of esters in the FAME sample (reactant) used.

In respect of refractive index measurements, the refractive index value was 1.45835 at 0 min, representing the mixture of methanol, oil and FAME sample. There was an initial drop in the values of refractive index indicating the physical process of mixing of phases. Once the mixing was settled, there was a rapid increase in refractive index values. The rate of increase in refractive index value from 5 to 10 min was 0.000124/min. The rate of change in refractive index value was, relatively, less *i.e.* 0.000109/min from 20 to 60 min. This decrease in rate of change in refractive index

values as the reaction proceeds is due to the gradual consumption of methanol and production of methyl ester and glycerol.

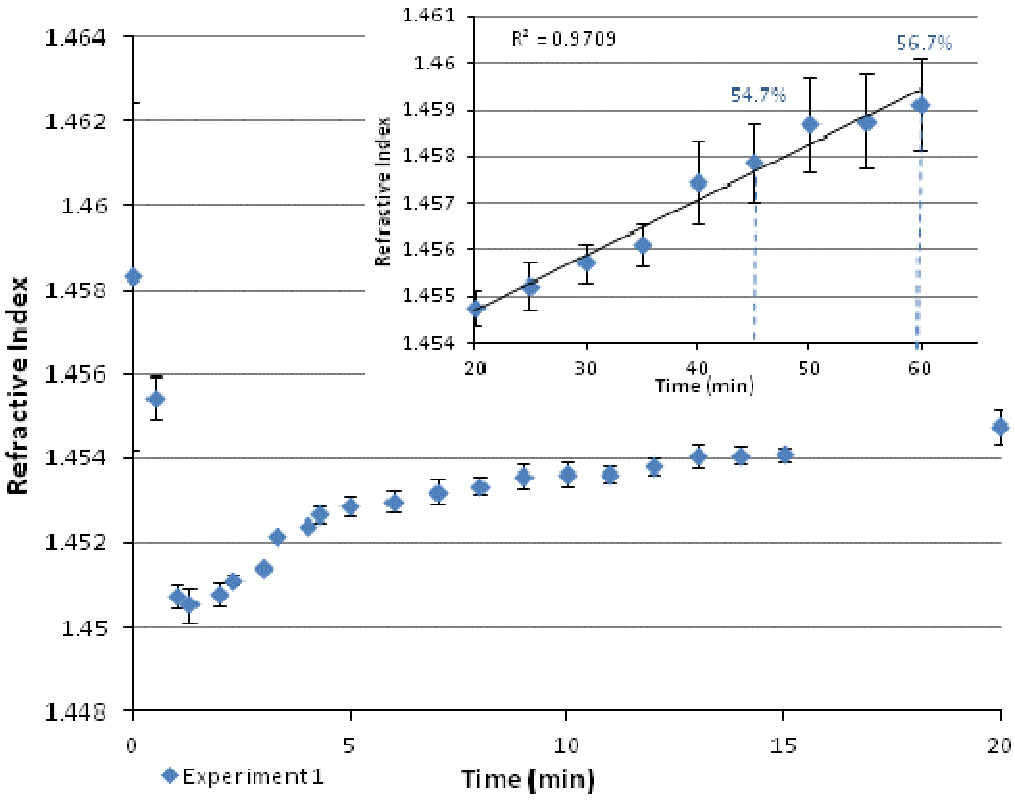

Figure 5.3. Refractive indices and ester content (%) of experiment **1**. (Expt.**1**: 60 mL FAME: 33 mL oil: 7 mL CH_3OH, 3% (w/w) SrO at 60 °C for 45 min and 60 min). Dotted line represents the ester content (%) at that point determined by GC. Values are the mean of three replicates, error bars indicate standard deviations.

In the next set of experiments, the effect of methanol and SrO on the transesterification reaction was investigated. Figures 5.6 and 5.7 show the refractive indices and ester content (%) for experiments **2** and **3**. These experiments were carried out by using similar vol. % of FAME, oil and CH_3OH as for experiment **1**. The FAME sample (reactant) used for experiment **2** and **3** had an ester concentration of 93.6% (w/w) and 95.4% (w/w), respectively, as determined by GC. Therefore, the concentration of esters in the FAME sample was calculated prior to each experiment. For experiment **2**, the concentration of esters in the FAME sample was calculated to be 56.1 mL ester/60 mL of FAME (v/v) *i.e.* 47.7 g of ester/51 g of FAME (w/w) and for

experiment **3** was 57.24 mL ester/60 mL of FAME (v/v) *i.e.* 46.93 g of ester/49.2 g of FAME (w/w). Based on these calculations the points for experiment **2** and **3** on the phase diagram were plotted (Figures 5.4 and 5.5).

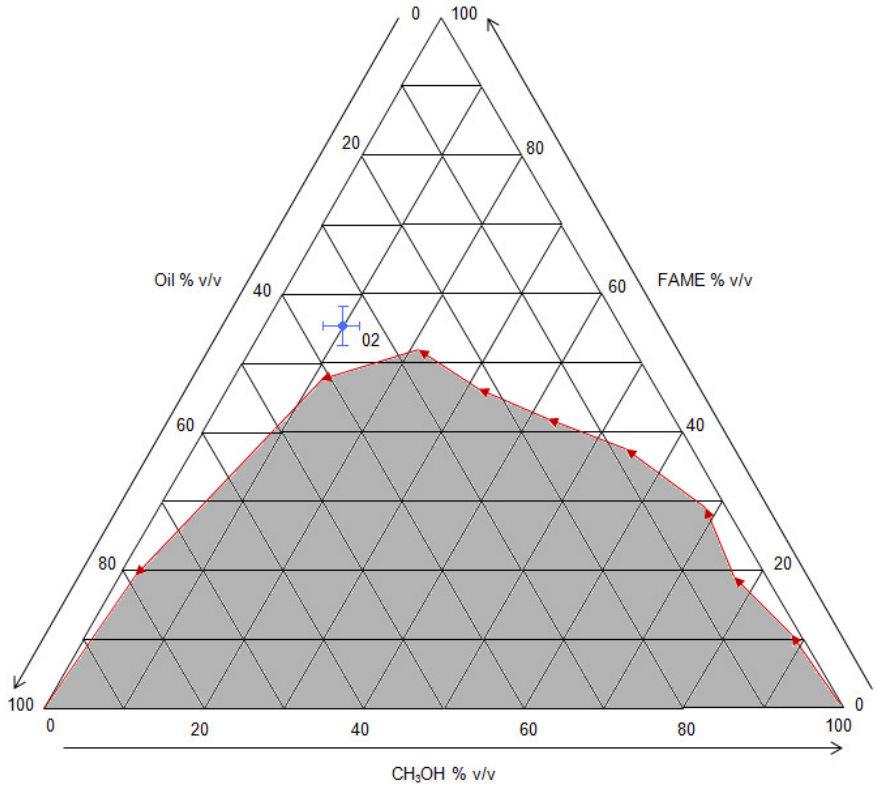

Figure 5.4. Ternary phase diagram showing the data point for experiment **2**.

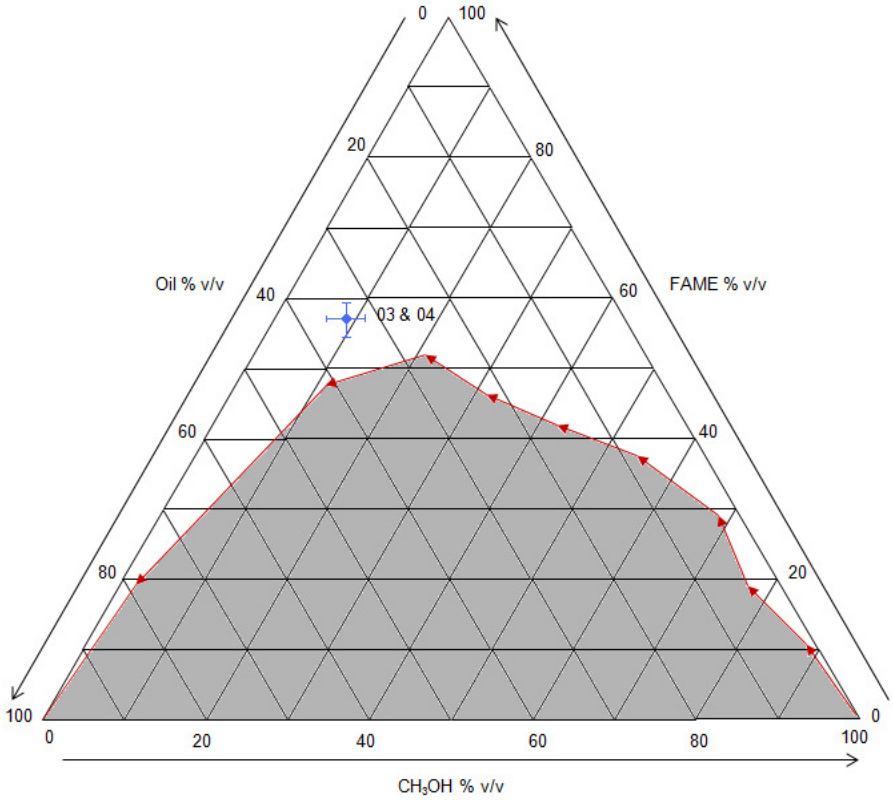

Figure 5.5. Ternary phase diagram showing data point for experiments **3** and **4**.

Figure 5.6 shows that as the transesterification reaction proceeded, the ester content at 50 min was 66.4% (w/w), as determined by GC. The ester content reached 86.0% (w/w) when the reaction was terminated at 90 min. There was an increase in esters from the starting concentration of esters by 18.7% (w/w) and 38.3% (w/w) at 50 min and 90 min, respectively. At the beginning of the reaction, the refractive index value was 1.4493 and increased to 1.4552 at 50 min. Addition of SrO to the reaction mixture at 50 min showed no further increase in the rate of change in refractive index suggesting that methanol is a limiting reactant. However, addition of methanol at 65 min decreased the refractive index value, as expected, to 1.44726. However, the refractive indices started to increase again as the reaction proceeded till 90 min.

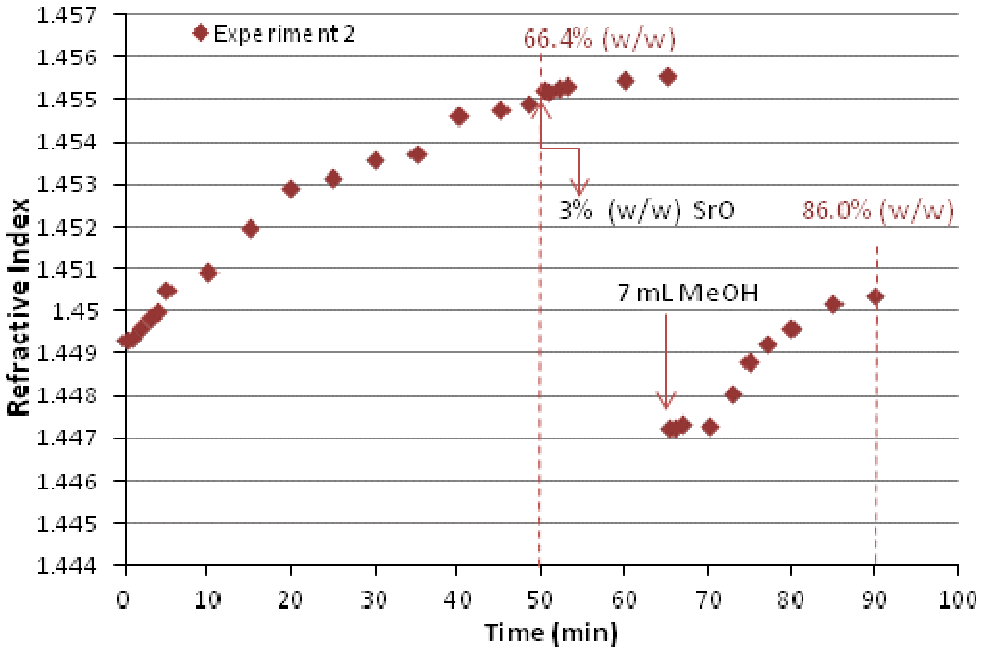

Figure 5.6. Refractive indices and ester content (%) for experiment **2**. (Expt. **2**: 60 mL FAME: 33 mL oil: 7 mL CH_3OH, 3% (w/w) SrO at 60 °C for 90 min. Arrow represents the addition of 3% (w/w) SrO at 50 min and addition of CH_3OH at 60 min. Dotted line shows the ester content (%) with respect to time.

For experiment **3** at 60 min, the ester content was 75.0% (w/w), determined by GC. Addition of SrO to the reaction mixture at 60 min showed no significant difference in ester content (Figure 5.7). The ester content was 75.4% (w/w) at 80 min. Therefore, there was an increase of 0.4% (w/w) esters in 20 min. However, addition of methanol at 80 min showed a rapid increase in ester content (%). There was an increase of 15.6% (w/w) esters from 80 to 120 min. The increase in concentration of esters from the starting concentration of esters at 120 min was 44.0% (w/w).

There was no significant change in the rate of refractive index values with the addition of SrO to the reaction mixture after 60 min; similar to that observed in experiment **2**. The addition of methanol at 80 min showed a rapid decrease in the refractive indices from 1.4538 to 1.4444 corresponding to the fact that methanol was added. However, the refractive index values started to increase again to 1.4509. This trend is similar to that observed earlier in experiment **2**.

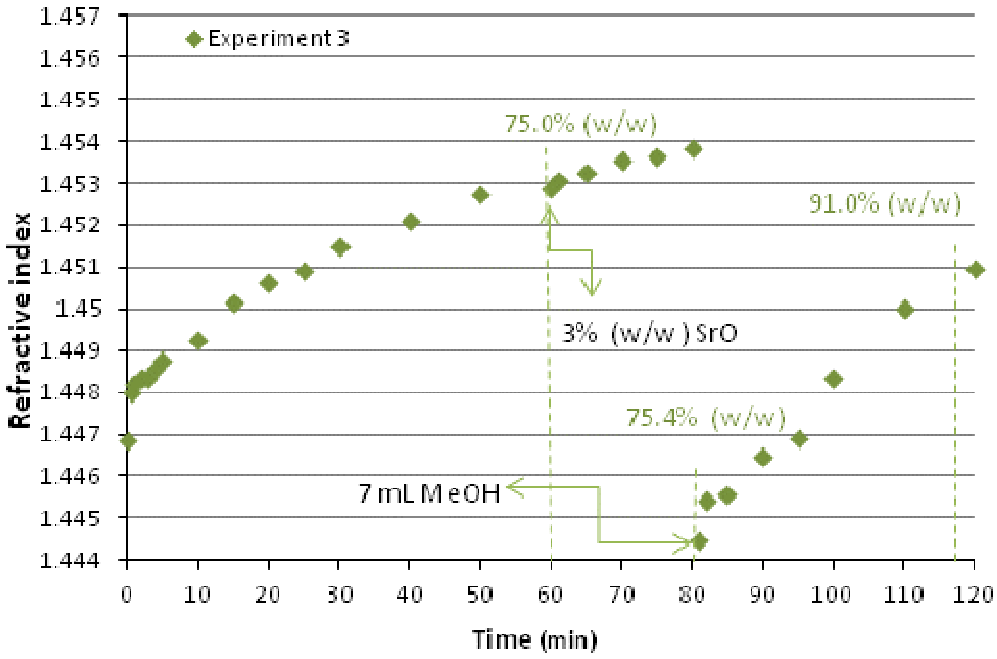

Figure 5.7. Refractive indices and ester content (%) for experiment **3**. (Expt. **3**: 60 mL FAME: 33 mL oil: 7 mL CH_3OH, 3% (w/w) SrO at 60 °C for 120 min. Arrow represents the addition of 3% (w/w) SrO at 60 min and addition of CH_3OH at 80 min. Dotted line shows the ester content (%) with respect to time.

In experiment **4**, the reaction was started by using the same vol. % of methanol, oil and FAME as for earlier experiments. The FAME sample (reactant) used was 95.4% (w/w) *i.e.*, as for experiment **3**. Therefore, the final concentration of esters in the reaction mixture will be same and conducted at the same position shown on the phase diagram (Figure 5.5). The only difference between experiments **3** and **4** is that the SrO was added after the addition of methanol for the latter experiment.

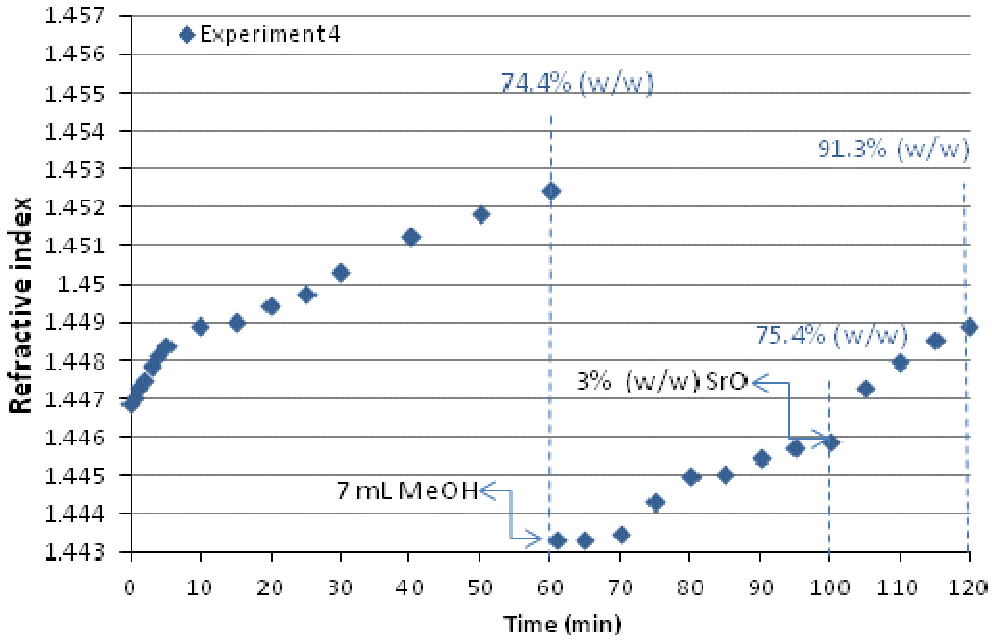

Figure 5.8. Refractive indices and ester content (%) for experiment **4**. (Expt. **4**: 60 mL FAME: 33 mL oil: 7 mL CH_3OH, 3% (w/w) SrO at 60 °C for 120 min. Arrow represents the addition of CH_3OH at 60 min and addition of 3% (w/w) SrO at 100 min. Dotted line shows the ester content (%) with respect to time.

The ester content was 74.4% (w/w) at 60 min (Figure 5.8) which was similar to the ester content obtained for experiment **3** (Figure 5.7). With the addition of methanol the ester content increased by 1% (w/w) after another 40 min. However, addition of SrO at 100 min increased the ester content from 75.4% to 91.3% (w/w). Therefore, the difference in total esters formed at the end of the reaction (120 min) was 43.6% (w/w). This increase in ester concentration at 120 min was similar to that achieved for experiment **3**. This result highlights that either the addition of SrO or methanol alone does not show a significant difference in ester content (%). However, when methanol or SrO was added earlier or later a remarkable difference in ester content at the end of the reaction was observed.

The refractive index values were similar to those for experiment **3** till 60 min because the reactants used for this experiment were the same as for experiment **3**.

However, after 60 min the refractive index values dropped due to the addition of methanol and started to increase gradually till 120 min. The addition of SrO at 100 min still showed an increase in refractive index because methanol was added at 60 min. At 120 min, the difference in refractive indices for experiment **3** and **4** was 0.0020.

5.4.3. Comparative Study of the Effect of Methanol Addition in Miscibility and Non-Miscibility Experiments

In the next experiments (experiments **5** to **8**) the effect of SrO on the transesterification of oil with methanol in the absence of added FAME sample under conditions of immiscibility was investigated and compared with that for FAME added to ensure full miscibility of oil and methanol.

Experiments **5** and **7** were carried out by using standard method/conditions used for the transesterification reaction (Chapter 4-A). No FAME sample was added to the reaction mixture of experiments **5** and **7**. The reactants used were 100 g oil, 6:1 CH_3OH: oil molar ratio and 3% (w/w) SrO. The other reaction conditions are given in Section 2.2.8, Chapter 2.

Experiments **6** and **8** were carried out in a miscible region of the phase diagram. The data point selected from the phase diagram was 60 mL FAME: 33 mL oil: 7 mL CH_3OH (Figure 2.1, Chapter 2). However, the FAMEs used for experiments **6** and **8** were 95.4% (w/w) and 96.1% (w/w) ester content, respectively determined by GC. Therefore, the actual concentration of esters in experiment **6** was calculated to be 57.24 mL ester/60 mL of FAME (v/v) *i.e.* 46.93 g of ester/49.2 g of FAME (w/w) and in experiment **8** the corresponding values were 57.66 mL ester/60 mL of FAME (v/v) *i.e.* 46.7 g of ester/48.6 g of FAME (w/w).

The ester content (%) for experiments **5** and **6** (Figure 5.9) was approximately the same at 60 min under both conditions. Similarly, the refractive indices for

experiment **5** and **6** were the same at 60 min, irrespective of using miscibility or immiscibility of oil to methanol phases. Methanol addition had a significant effect on the reaction carried out in the miscible phase (Expt. **6**) compared to the standard experiment (Expt. **5**).

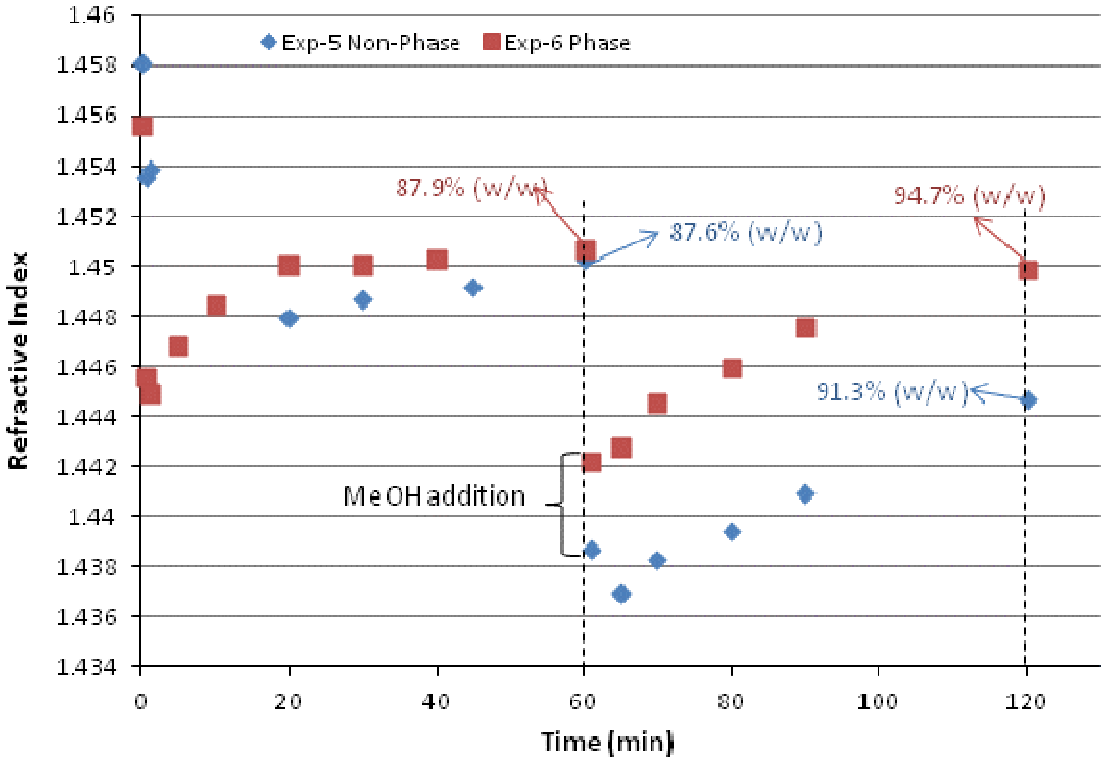

Figure 5.9. Refractive indices and ester content (%) for experiments **5** and **6**. (Expt. **5**: 100 g oil, 3% (w/w) SrO, methanol/oil molar ratio 6:1 at 60 °C for 120 min. Expt. **6**: 60 mL FAME: 33 mL oil: 7 mL CH_3OH, 3% (w/w) SrO at 60 °C for 120 min). At 60 min, methanol was added in both experiments. In Expts. **5** and **6**, 27.5 mL and 7 mL of methanol were added, respectively. Arrows represent the ester content (%) at that point. The dotted lines represent the ester content (%) noted with respect to time.

For the standard experiment in the non-miscible region (Expt. **5**), the methanol was added at a 6:1 CH_3OH: oil molar ratio or 27.5 mL whereas for the experiment (Expt. **6**) in the miscible region 7 mL of methanol was added. After 60 min, the refractive index values decreased in both experiments on addition of methanol. Since, more methanol was added in experiment **5** (27.5 mL) than experiment **6** (7 mL) so the

refractive indices were proportionally altered. At 120 min, experiment **6** yielded higher ester content (94.7% w/w) than experiment **5** (93.4% w/w).

In contrast to experiments **5** and **6**, no methanol was added in experiment **7** and **8**. As expected there was no sudden drop in refractive indices at 60 min as compared to experiments **5** and **6**. The ester content for experiment **7** (87.3% w/w) was lower than in experiment **8** (90.1% w/w) at 60 min. Then in both experiments, the ester content (%) started to increase and at 120 min, the ester content was 91.3% (w/w) and 92.4% (w/w), respectively. This shows that the experiment conducted in the miscible region of the phase diagram yielded higher ester content (%) compared to the experiment carried out in the non-miscible region. This trend was also observed in the case of experiments **5** and **6**.

The experiments conducted in the non-miscible region (Expt. **5** in Figure 5.9 and Expt. **7** in Figure 5.10) showed that the ester content (%) and refractive indices were similar at 60 min. However, at 60 min the addition of methanol in experiment **5** (1.4447) decreased the refractive indices compared to experiment **7** (1.4515). At 120 min the refractive indices for experiments **5** and **7** were different but the ester content (%) was same. This result illustrates the fact that the extra addition of methanol in the non-miscible experiments does not contributes to the higher ester content (%).

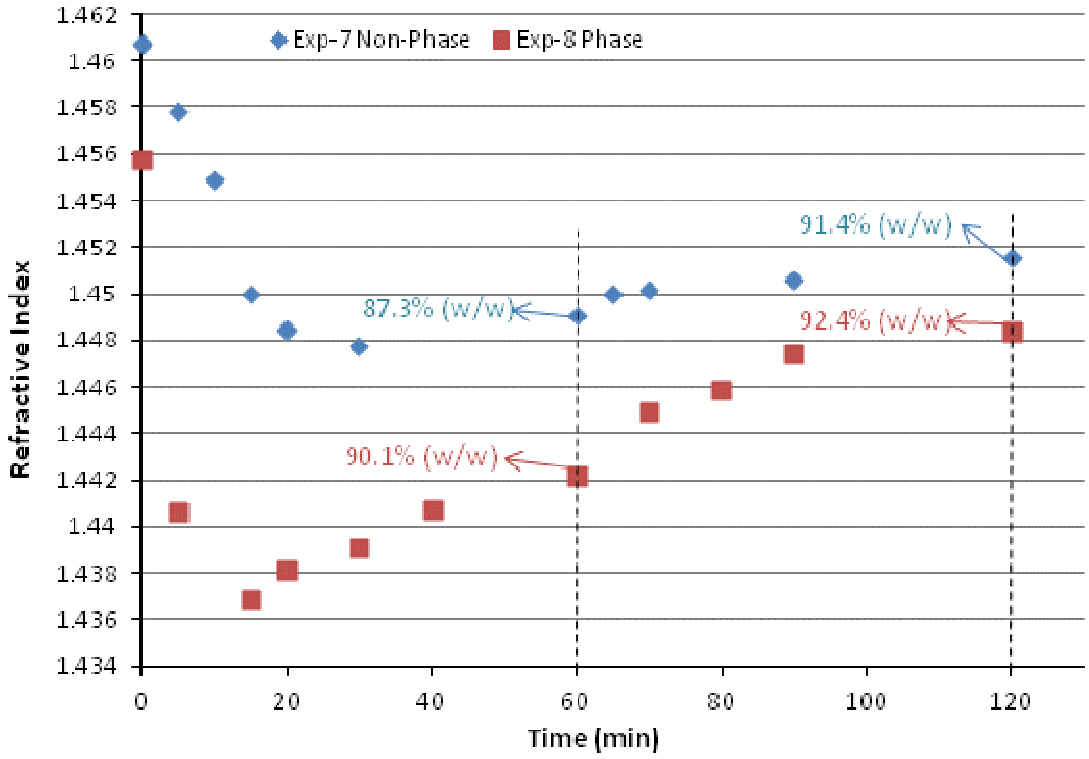

Figure 5.10. Refractive indices and ester content (%) of experiments **7** and **8**. (Expt. **7**: 100 g oil, 3% (w/w) SrO, methanol/oil molar ratio 6:1 at 60 °C for 120 min. Expt. **8**: 60 mL FAME: 33 mL oil: 7 mL CH_3OH, 3% (w/w) SrO at 60 °C for 120 min). Arrows represent the ester content (%) at that point. The dotted lines represent the ester content (%) noted with respect to time.

The experiments carried out in the miscible region (Expts. **6** and **8**) of the phase diagram showed that at 60 min, the ester content was lower in experiment **6** (87.9% w/w) than in experiment **8** (90.1% w/w). This is because the starting concentration of esters in the FAMEs sample differed, as discussed earlier. In experiment **6**, there was 46.93 g of ester/49.2 g of FAME (w/w) and in experiment **8** there was 46.7 g of ester/48.6 g of FAME (w/w). The ester content (%) yield at 60 min showed an increase in esters of 41% (w/w) and 43.4% (w/w) for experiments **6** and **8**, respectively. In experiment **6**, methanol was added therefore, the ester content (%) was higher compared to experiment **8** with no methanol addition. Therefore, this result shows that methanol addition in the miscible region of the phase diagram drives the transesterification reaction faster than compared to no addition of methanol in miscible region.

The determination of free glycerol and glyceride content (%) was also carried out on biodiesel samples obtained from experiments **5**-**8** (Table 5.2). The results showed that the free glycerol content (%) met the EN specification for samples of experiment **5** at 60 min and experiment **7** at 60 and 120 min. However, the free glycerol content (%) was higher in the case of experiments conducted in the miscible region (Expts. **6** and **8**) or where extra methanol was added to the reaction. The mono-glyceride content (%) was higher in those cases where additional methanol was added. All the experiments at 60 min showed the tri-glyceride content (%) to be higher as compared to 120 min.

Table 5.2. Percentage glycerol and glycerides content for experiments **5** to **8**.

Biodiesel samples		Ester content %[a]	Free glycerol %[a]	Mono-	Di-	Tri-	% Total glycerol[a]
				Glycerides %[a]			
Exp **5**	60 min	87.6	0.02	0.90	3.34	8.10	12.36
	120 min	93.4	0.40	1.25	1.65	3.39	6.69
Exp **6**	60 min	87.9	0.35	1.43	1.64	8.22	11.64
	120 min	94.7	0.43	0.59	0.65	3.43	5.10
Exp **7**	60 min	87.3	0.02	0.94	2.95	8.31	12.22
	120 min	91.3	0.02	0.67	0.65	6.78	8.12
Exp **8**	60 min	90.1	0.35	1.45	1.65	6.35	9.80
	120 min	92.4	0.35	2.33	1.32	3.56	7.56

[a] Values are the mean of three replicates.

5.4.4. Transesterification Reaction in the Miscible Region of the Phase Diagram

Experiments **9** to **14** were carried out by using different vol. % of FAME, methanol and oil in the miscible region of the phase diagram (see Chapter 2, Figure 2.2). The FAME (reactant) used for these experiments have an ester concentration of 94.0% (w/w), as determined by GC.

In experiment **9**, the concentration of esters in the FAME sample was 65.8 mL ester/70 mL of FAME (v/v) *i.e.* 53.9 g of ester/57.4 g of FAME (w/w). As the transesterification reaction proceeded, the ester content was 98.2% (w/w) at 24 min (determined by GC; Figure 5.11) meeting the EN 14103 standard set for biodiesel. At

30 min, the ester content increased to 99.0% (w/w) but after that there was a gradual decline in the ester content (%) characterising the reaction as reversible. The rate of increase in ester content from 20 to 30 min was 0.1%/min. The ester content decreased to 89.9% (w/w) at 105 min. The refractive index values started to rise sharply from 1.43917 to 1.45211 till 30 min. The refractive indices values plateau'd after 30 min.

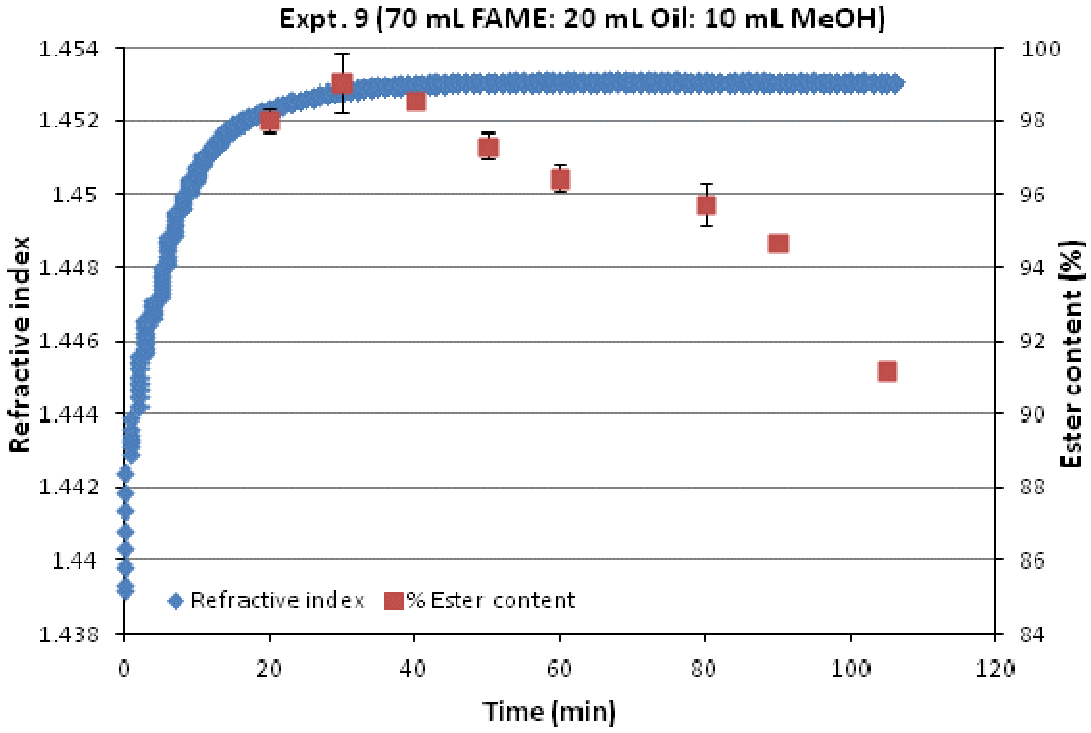

Figure 5.11. Refractive indices and ester content (%) for experiment **9**. (Expt. **9**: 70 mL FAME: 20 mL oil: 10 mL CH_3OH, 3% (w/w) SrO with respect to triglycerides at 60 °C for 105 min. FAME purity: 94.0% (w/w) determined by GC). Values are the mean of three replicates, error bars indicate standard deviations.

The reversible nature of the transesterification reaction was confirmed by the analysis of glycerol (free and total) and glycerides (mono-, di-, and tri-) whose content increased after 30 min. Figure 5.12 shows the glycerol (free and total) and glycerides (mono-, di-, and tri-) for experiment **9** during the reaction. The mono- and di- glycerides content (%) met the EN specification for all the samples collected at different time intervals (Figure 5.12). However, the triglyceride content (%) was higher in all the

samples indicating the presence of unreacted triglyceride in the reaction mixture except when the ester content was 99.0% (w/w).

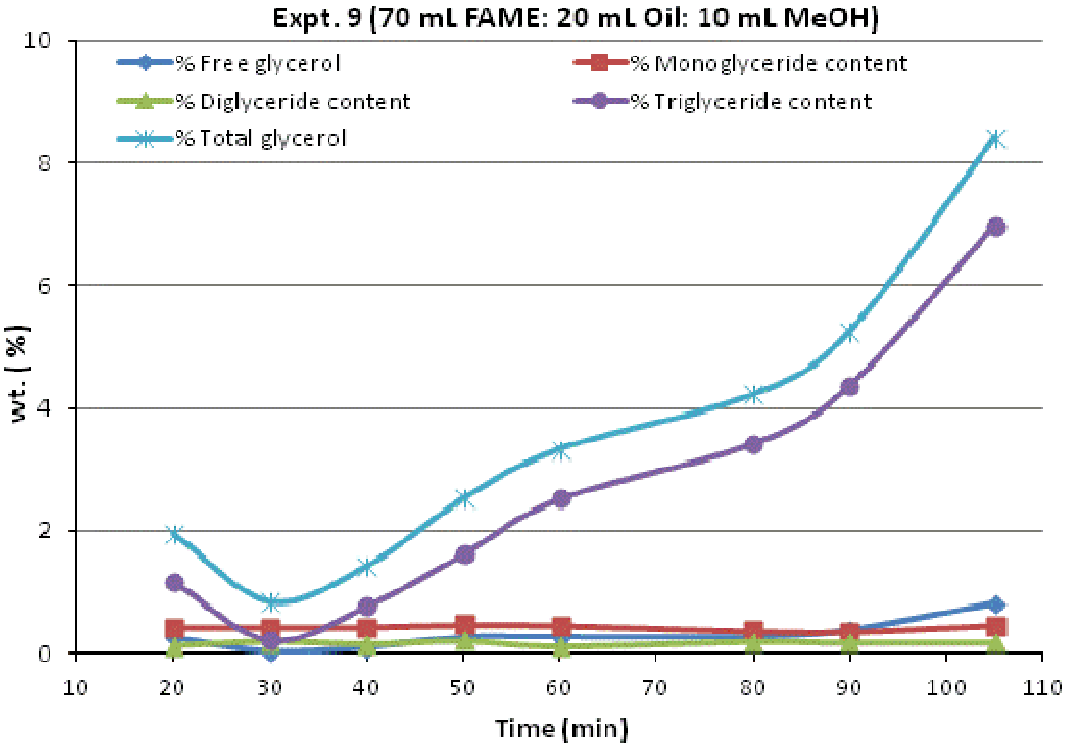

Figure 5.12. Results for the glycerol (free and total) and glycerides (mono-, di-, and tri-glycerides) in experiment **9**. The data points represent the mean of three experiments, where the corresponding standard deviation was lower than 0.05 and therefore the variability among the readings was insignificant.

Figure 5.13 shows the ester content (%) and values of refractive index determined for experiment **10** at different time intervals. As the vol. % of FAME used in this experiment was 80% (w/w), the concentration of esters in the FAME sample, based on the purity of FAME, was 75.2 mL ester/80 mL of FAME (v/v) *i.e.* 61.6 g of ester/65.6 g of FAME (w/w). The concentration of esters was higher at the start of the reaction for this experiment compared to experiment **9**.

The ester content (%) formed from 20 to 60 min was higher than EN standard (96.5% w/w) and then decreased to 90.8% (w/w) at 90 min (Figure 5.13). The rate of increase in ester content (%) from 20 to 30 min was the same as for experiment **9**. The

refractive indices plateau'd after 30 min, similar to that observed for experiment **9**. However, the refractive indices were relatively lower for experiment **10** from 30 to 90 min in the range of 1.4502 to 1.4510 than compared to experiment **9** (1.4528 to 1.4530). This is because the vol. % ratio of FAME used was higher in experiment **10** than experiment **9** which is responsible for the decrease in the refractive indices.

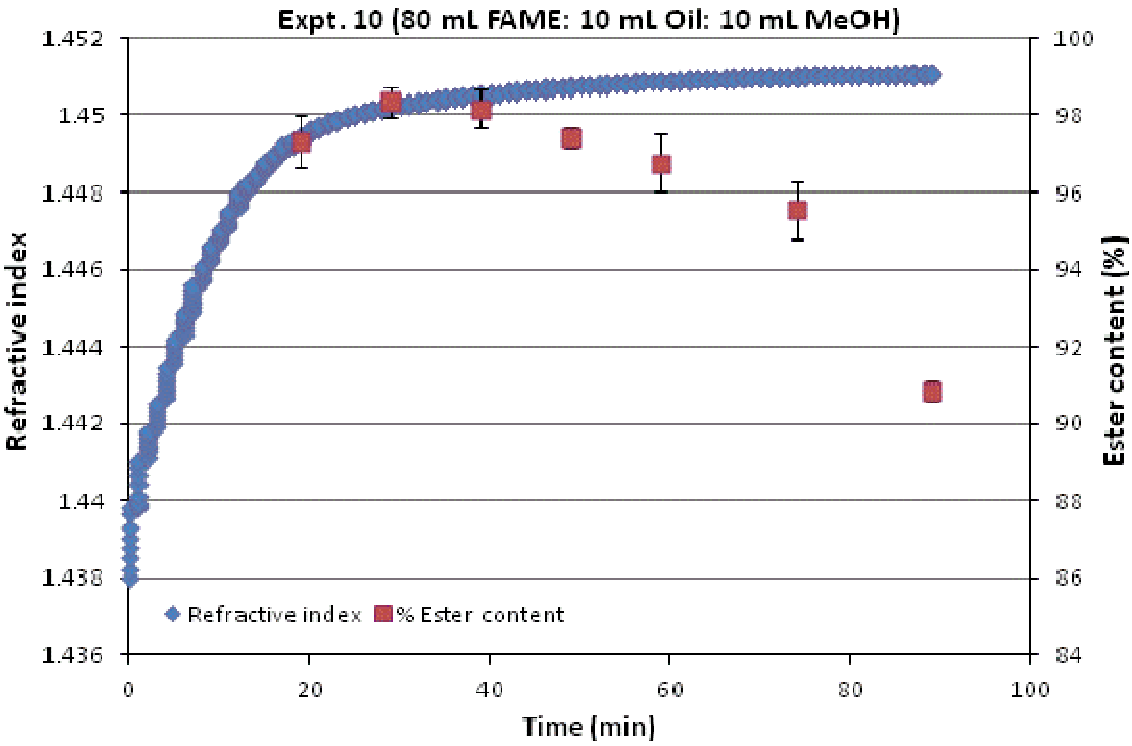

Figure 5.13. Refractive indices and ester content (%) for experiment **10**. (Expt. **10**: 80 mL FAME: 10 mL oil: 10 mL CH_3OH, 3% (w/w) SrO with respect to triglycerides at 60 °C for 90 min. FAME purity: 94.0% (w/w) determined by GC). Values are the mean of three replicates, error bars indicate standard deviations.

Figure 5.14 shows the glycerol and glycerides content (%) for experiment **10**. The triglyceride content (%) was higher than EN 14105 standard at 20 min but after that decreased to 0.16% (w/w). However, by increasing the reaction time the triglyceride started to increase in the reaction mixture due to the reversibility of the transesterification reaction. This supports the data pertaining to the ester content (%) in

Figure 5.13. Moreover, the mono-, and di- glyceride content (%) was in the range specified by EN 14105 standard.

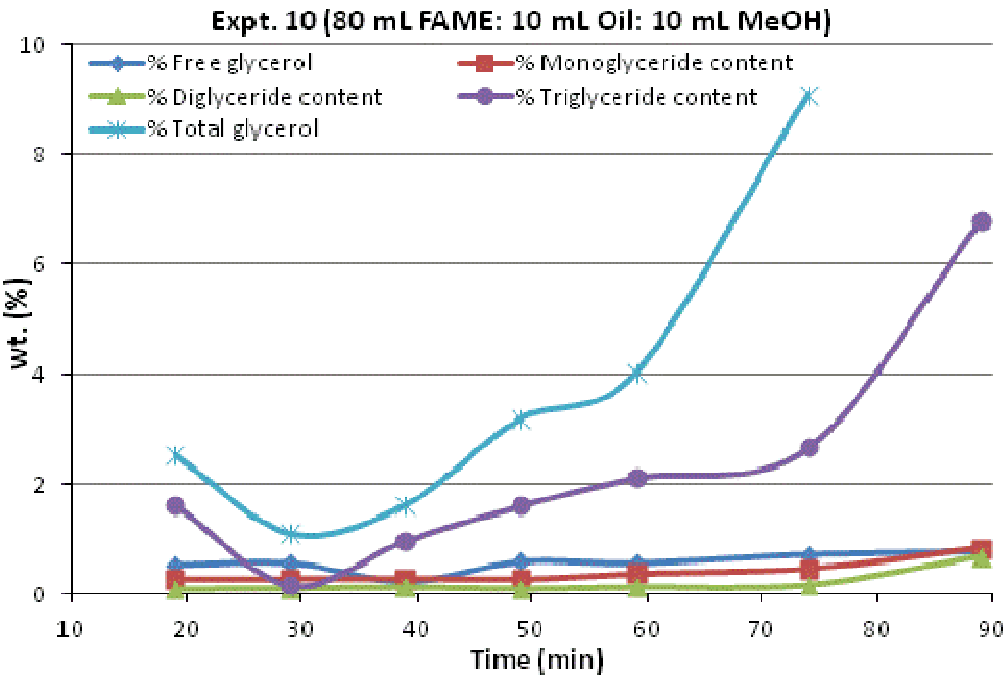

Figure 5.14. Results for the glycerol (free and total) and glycerides (mono-, di-, and tri-glycerides) in experiment **10**. The data points represent the mean of three experiments, where the corresponding standard deviation was lower than 0.05 and therefore the variability among the readings was insignificant.

Experiment **11** was conducted by using a vol. % ratio of 60 FAME: 20 oil: 20 CH_3OH (Figure 5.15). Based on the purity (94.0% w/w) of FAME (reactant) used, the final ester concentration (%) in the FAME sample was 56.4 mL ester/60 mL of FAME (v/v) *i.e.* 46.2 g of ester/49.2 g of FAME (w/w). The ester concentration (%) at the start of the reaction was less as compared to experiments **9** and **10**. Therefore, it is expected that the ester content (%) obtained during the reaction at different time intervals were proportionally affected.

The result showed that the ester content was 95.0% (w/w) at 20 min and increased to 97.5% (w/w) at 40 min. After that, a decrease in ester content (%) was observed. The rate of increase in ester was 0.19%/min from 20 to 30 min that is comparatively high compared to the results obtained in experiments **9** and **10** showing

that the ester content was still increasing in the reaction. The ester content (%) for all the data points determined with respect to time (Figure 5.15) were, relatively, lower as compared to experiments **9** and **10**. This was expected because the final ester content (%) was dependent on the amount and purity of the FAME (reactant) used at the start of the reaction. However, the ester content (%) for this experiment met the EN standard from 30 to 50 min.

The refractive index values plateau'd after 20 min and ranged between 1.4530 and 1.4540. The refractive indices were relatively high as compared to those reported in experiments **9** and **10**. This is because the vol. % of FAME in the experiment was lower than that in experiments **9** and **10**.

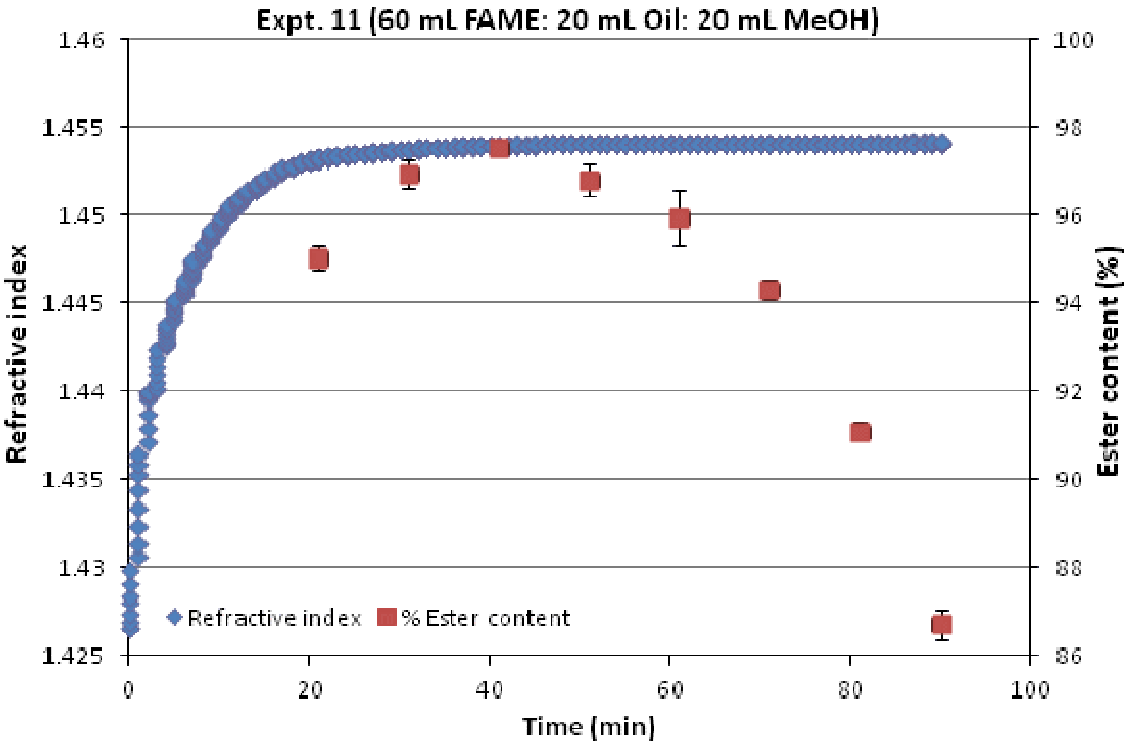

Figure 5.15. Refractive indices and ester content (%) for experiment **11**. (Expt.**11**: 60 mL FAME: 20 mL oil: 20 mL CH_3OH, 3% (w/w) SrO with respect to triglycerides at 60 °C for 90 min. FAME purity: 94.0% (w/w), determined by GC). Values are the mean of three replicates, error bars indicate standard deviations.

Figure 5.16 shows the glycerol and glyceride content (%) for experiment **11**. The triglyceride content (%) was higher at 20 min and then started decreasing till 40 min, after which an increase in triglyceride was observed. This trend is similar to earlier experiments in that as the ester content (%) reached the highest point, the reaction starts to reverse. Although, the di- and mono- glyceride content (%) were lower than the EN standard from 20 to 80 min at 90 min, an increase in di- and mono- glycerides (%) was observed indicating the formation of intermediates in the reaction mixture.

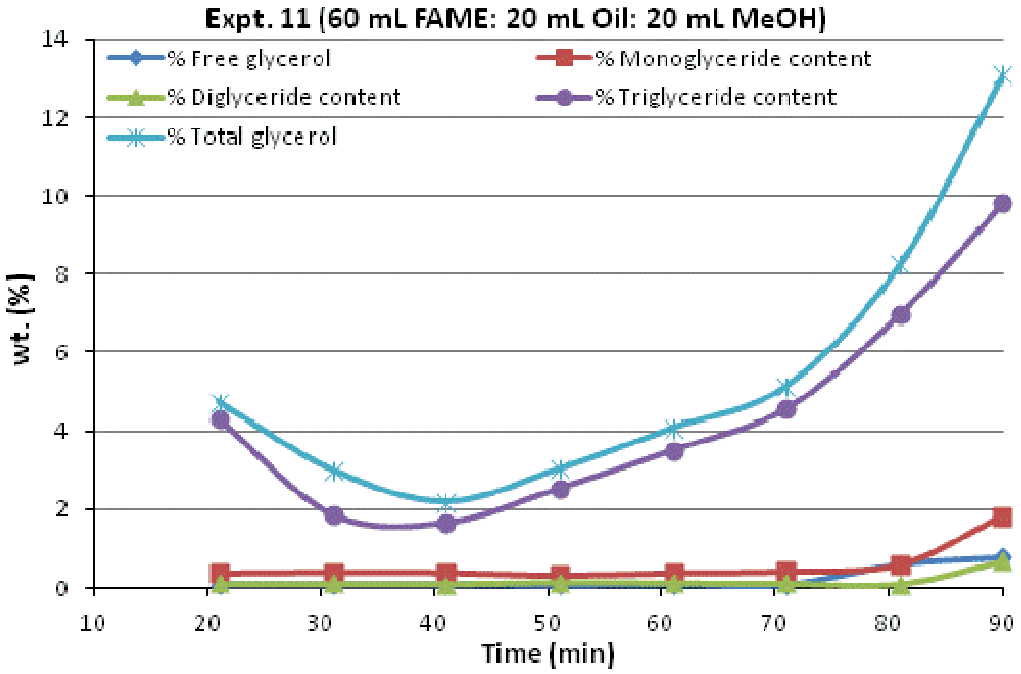

Figure 5.16. Results for the glycerol (free and total) and glycerides (mono-, di-, and tri-glycerides) in experiment **11**. The data points represent the mean of three experiments, where the corresponding standard deviations were lower than 0.05 and therefore the variability among the readings was insignificant.

In experiment **12**, the vol. % of FAME used was the same as in experiment **11**. The difference between both experiments was the vol. % of oil and methanol. The vol. % of oil was higher in this experiment; therefore, it is expected that the ester content (%) will be, relatively, lower than that in experiment **11**. Figure 5.17 show the ester content was 91.5% (w/w) at 20 min, which is comparatively less as compared to experiment **11**. After 20 min, the ester content (%) increased but still lower than in

experiment **11**. At 60 min, the ester content formed (*ca.* 98.0% w/w) in this experiment is similar to that observed in experiment **11** at 40 min. Therefore, this shows that by using a higher vol. % of methanol than oil, the highest ester content (%) in the reaction was attained at 40 min in experiment **11** whereas in this experiment it has shifted to 60 min. However, the ester content (%) achieved at 50 to 60 min met the EN standard specifications. There was a decrease in % ester content to 92.0% (w/w) at 90 min. This trend is similar to earlier experiments in that after achieving the highest concentration, the transesterification reaction tends to reverse due to the presence of reaction intermediates. The refractive indices plateau'd after 40 min and ranged in value from1.4540 to 1.4544 which is similar to the values obtained in experiment **11**.

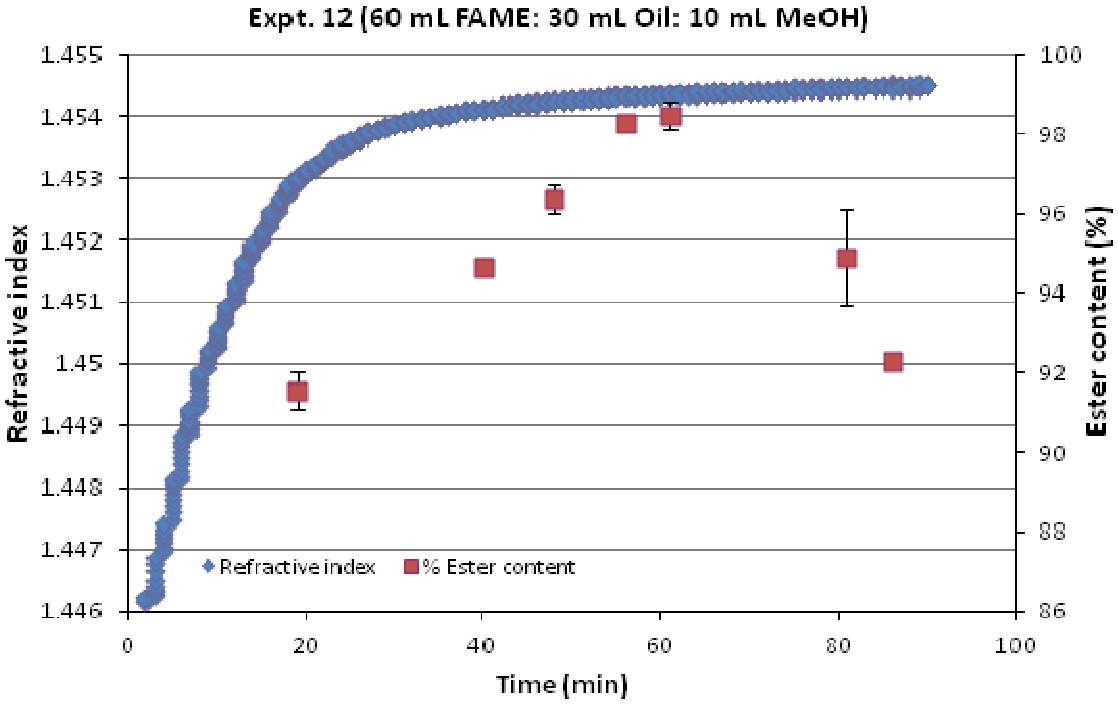

Figure 5.17. Refractive indices and ester content (%) for experiment **12**. (Expt.**12**: 60 mL FAME: 30 mL oil: 10 mL CH_3OH, 3% (w/w) SrO with respect to triglycerides at 60 °C for 90 min. FAME purity: 94.0% (w/w), determined by GC). Values are the mean of three replicates, error bars indicate standard deviations.

The data in Figure 5.18 show that the triglyceride content (%) was higher at the start of the reaction compared to earlier experiments. This is due to the higher amount

of oil used in this experiment than earlier experiments. There was a decrease in triglyceride content (%) at 60 min corresponding to the ester content (%) formed at that point in Figure 5.17. The monoglyceride content increased to 0.85 % (w/w), slightly above the EN standard limit at the end of the reaction.

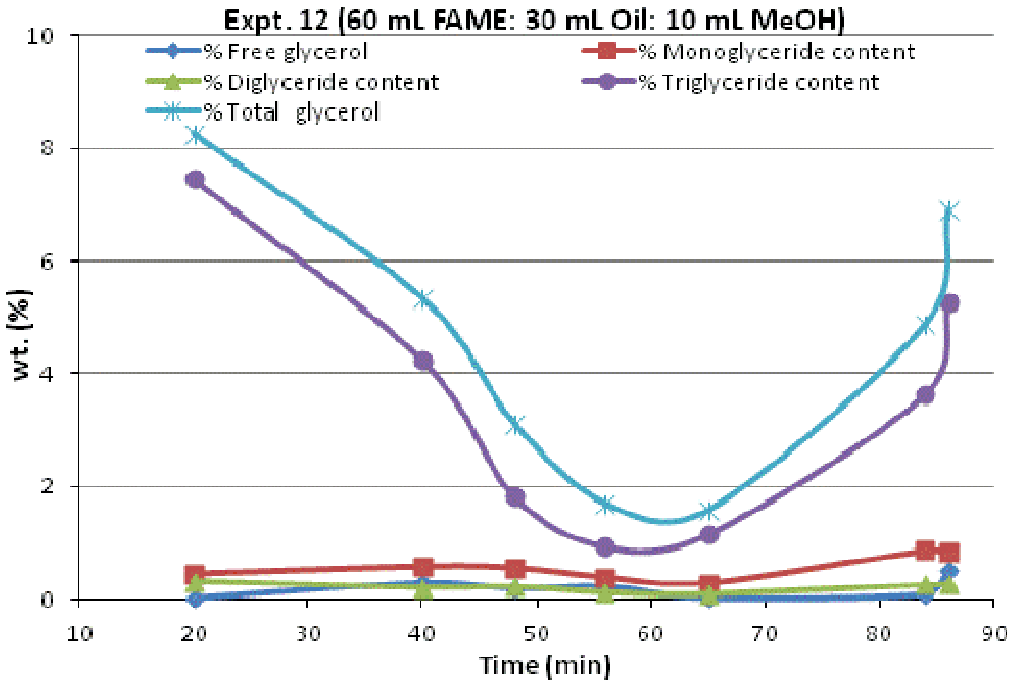

Figure 5.18. Results for the glycerol (free and total) and glycerides (mono-, di-, and tri-glycerides) in experiment **12**. The data points represent the mean of three experiments, where the corresponding standard deviation was lower than 0.05 and therefore the variability among the readings was insignificant.

Experiment **13** (Figure 5.19) shows a similar ester concentration (%) using 70 mL FAME as for experiment **9**. The only difference in experiment **9** was that less vol. % of methanol was used than in experiment **13**. The trend observed in this experiment is similar to earlier experiments, **9**-**12**. The ester starts to increase and then at 40 min the ester content (%) peaked and after that there was a gradual decrease. The refractive indices also showed the same trend *i.e.* plateau'd after 40 min. The refractive indices, after 40 min, were in the range of 1.4506 to 1.4513, which is lower than that observed in experiment **9**. The data in Figure 5.20 shows the drop in triglycerides and total glycerol at 40 min corresponding to higher ester formed.

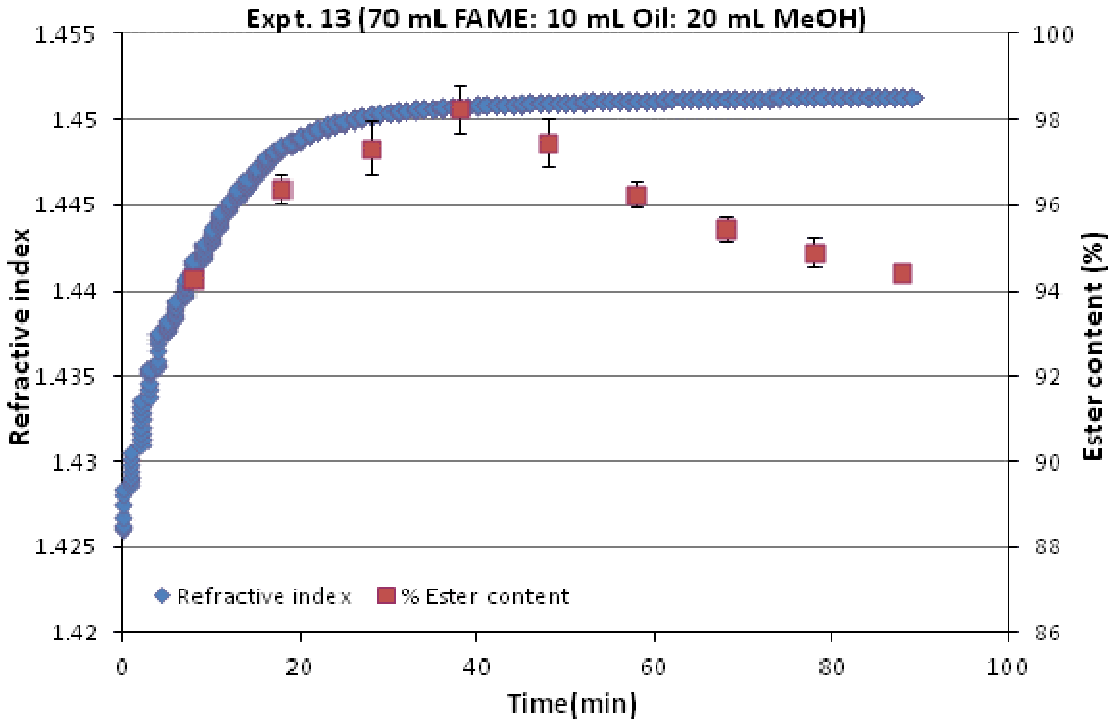

Figure 5.19. Refractive indices and ester content (%) for experiment **13**. (Expt.**13**: 70 mL FAME: 10 mL oil: 20 mL CH_3OH, 3% (w/w) SrO with respect to triglycerides at 60 °C for 105 min. FAME purity: 94.0% (w/w), determined by GC). Values are the mean of three replicates, error bars indicate standard deviations.

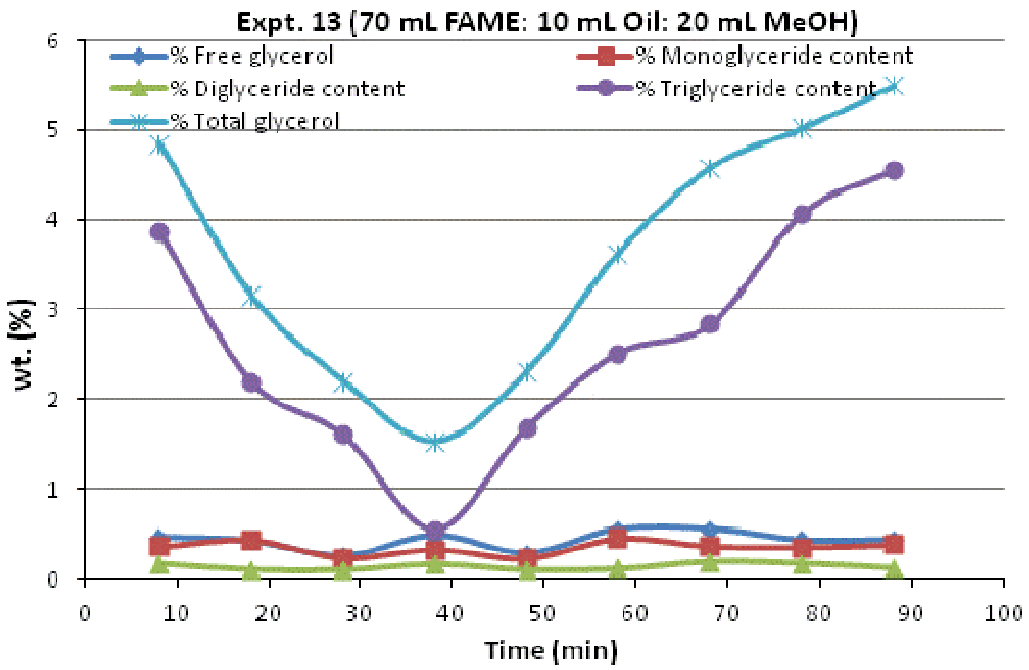

Figure 5.20. Results for the glycerol (free and total) and glycerides (mono-, di-, and tri-glycerides) in experiment **13**. The data points represent the mean of three experiments, where the corresponding standard deviation was lower than 0.05 and therefore the variability among the readings was insignificant.

In experiment **14**, the vol. % of FAME was similar (60 mL) to experiments **11** and **12**. Therefore, the ester concentration (%) at the start of the reaction will be same. In this experiment, a higher methanol vol. % was used compared to experiments **11** and **12**. Figure 5.21 shows the ester content as being 98.1% (w/w) at 40 min, which is similar to that achieved in earlier experiments. However, a large drop in ester content to 70 % (w/w) observed at 90 min that was not observed in experiments **9**-**13**. Similarly, the refractive index values showed fluctuating readings (Figure 5.21). This might be due to excess methanol in the reaction mixture that tends to lower the refractive indices. The data in Figure 5.22 shows that there was a drop in triglyceride and total glycerol content (%) at 40 min which complements the data obtained for ester content (%) (Figure 5.21).

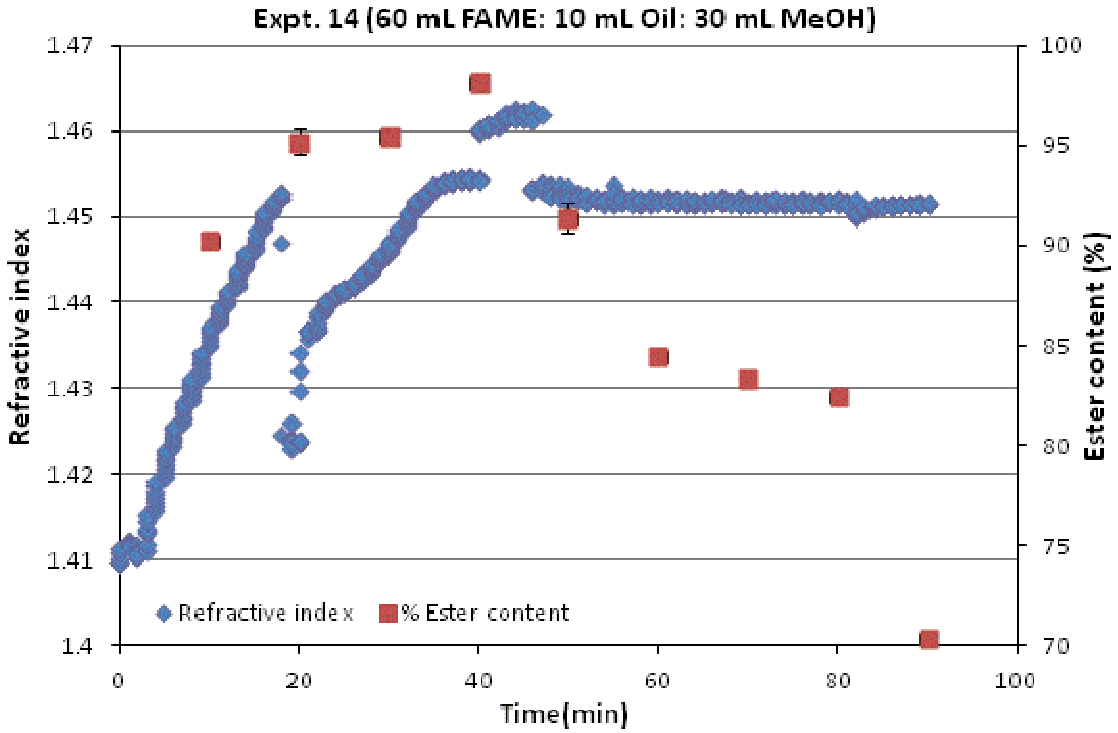

Figure 5.21. Refractive indices and ester content (%) for experiment **14**. (Expt. **14**: 60 mL FAME: 10 mL oil: 30 mL CH_3OH, 3% (w/w) SrO with respect to triglycerides at 60 °C for 90 min. FAME purity: 94.0% (w/w), determined by GC). Values are the mean of three replicates, error bars indicate standard deviations.

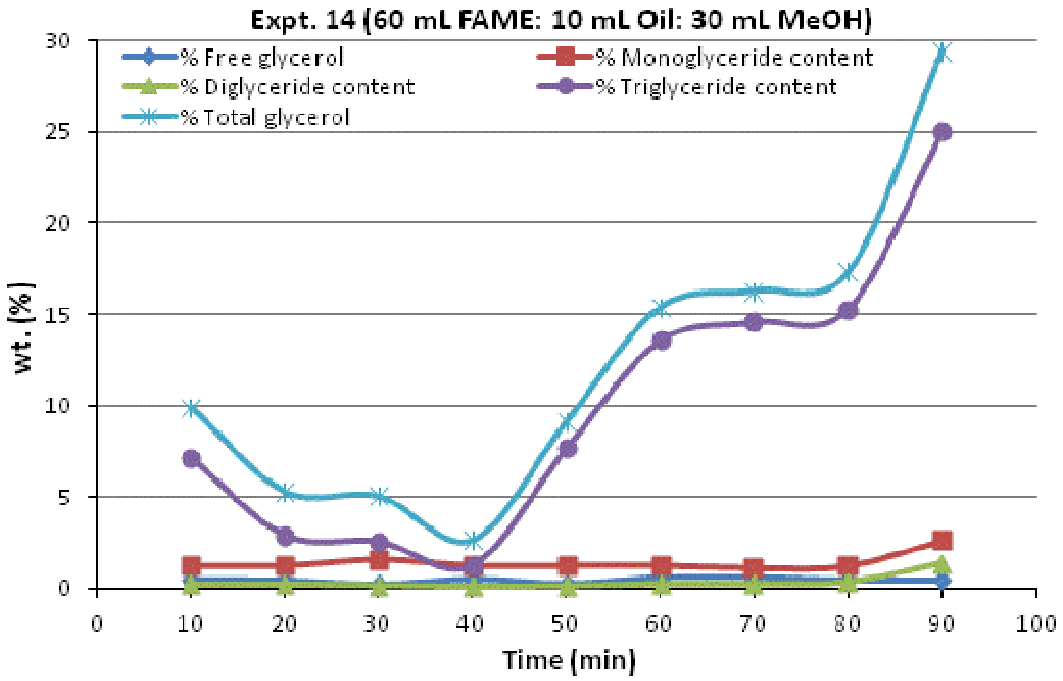

Figure 5.22. Results for the glycerol (free and total) and glycerides (mono-, di-, and tri-glycerides) in experiment **14**. The data points represent the mean of three experiments, where the corresponding standard deviation was lower than 0.05 and therefore the variability among the readings was insignificant.

5.4.5. Investigations of the Effects of Using Different Metal Oxides

Several metal oxide catalysts (CeO_2, Gd_2O_3, La_2O_3, ZrO_2, SnO_2, Y_2O_3, TiO_2, SrO) were selected for the transesterification reaction in the miscible region (70 mL FAME: 20 mL oil: 10 mL CH_3OH) of the ternary phase diagram (Figure 5.1). The reaction conditions are detailed in Section 2.2.10, Chapter 2.

Figure 5.23 show the change in refractive indices during the transesterification reaction with various metal oxides. It is clear from the data that the reaction using SrO showed higher refractive index values than the other metal oxides. The values of refractive indices were stable for all the experiments after 30 min. No catalytic activity was observed for any of the metal oxides except strontium oxide. The data for the strontium oxide was the same as for experiment **9** (Figure 5.12). The values of refractive indices of the reaction mixture containing Gd_2O_3, ZrO_2 and CeO_2 were lower

with respect to other metal oxides. These data highlight the dilemma of using refractive index measurements.

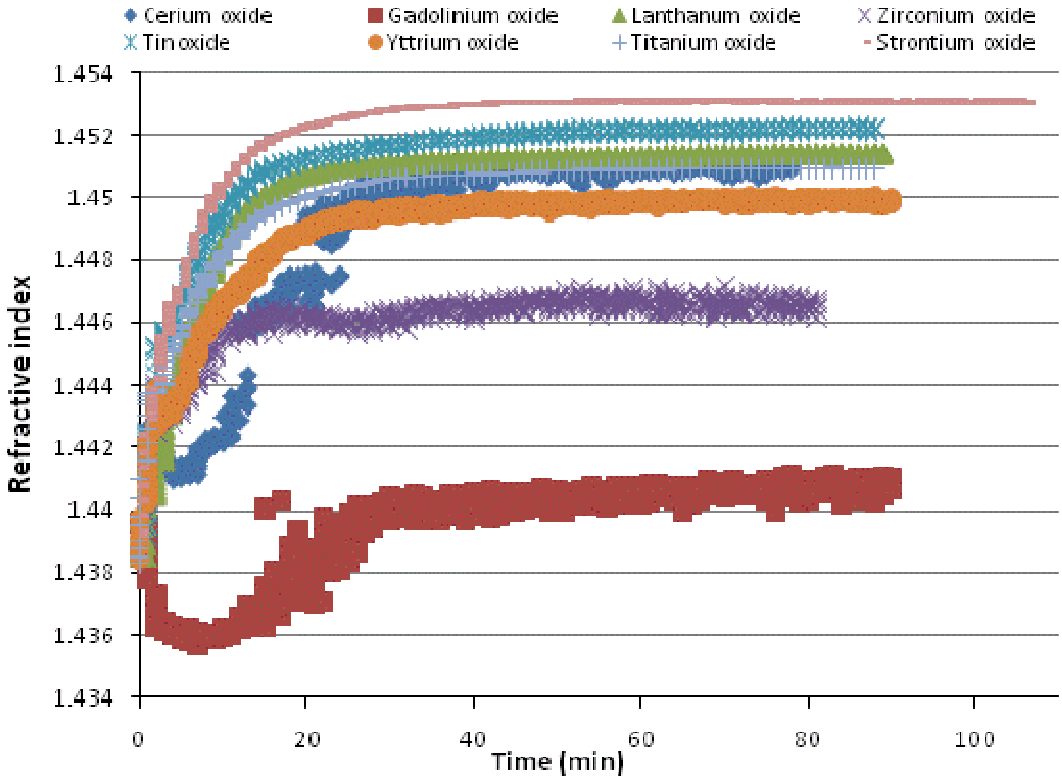

Figure 5.23. Refractive indices for various metal oxide catalysts. (reaction conditions: 70 mL FAME: 20 mL oil: 10 mL CH_3OH, 3% (w/w) SrO with respect to triglycerides at 60 °C for 105 min. FAME purity: 94.0% (w/w), determined by GC).

5.4.6. Effect of Adding Glucosinolate to the Transesterification Reaction

The effect of adding glucosinolate to the transesterification reaction was investigated under conditions of full miscibility. The data in Figure 5.24 show a comparison of refractive indices and ester content (%) achieved by the addition of glucosinolate and control (without glucosinolate) for the transesterification reaction. The procedure to carry out these experiments is described in Chapter 2, Section 2.2.11.

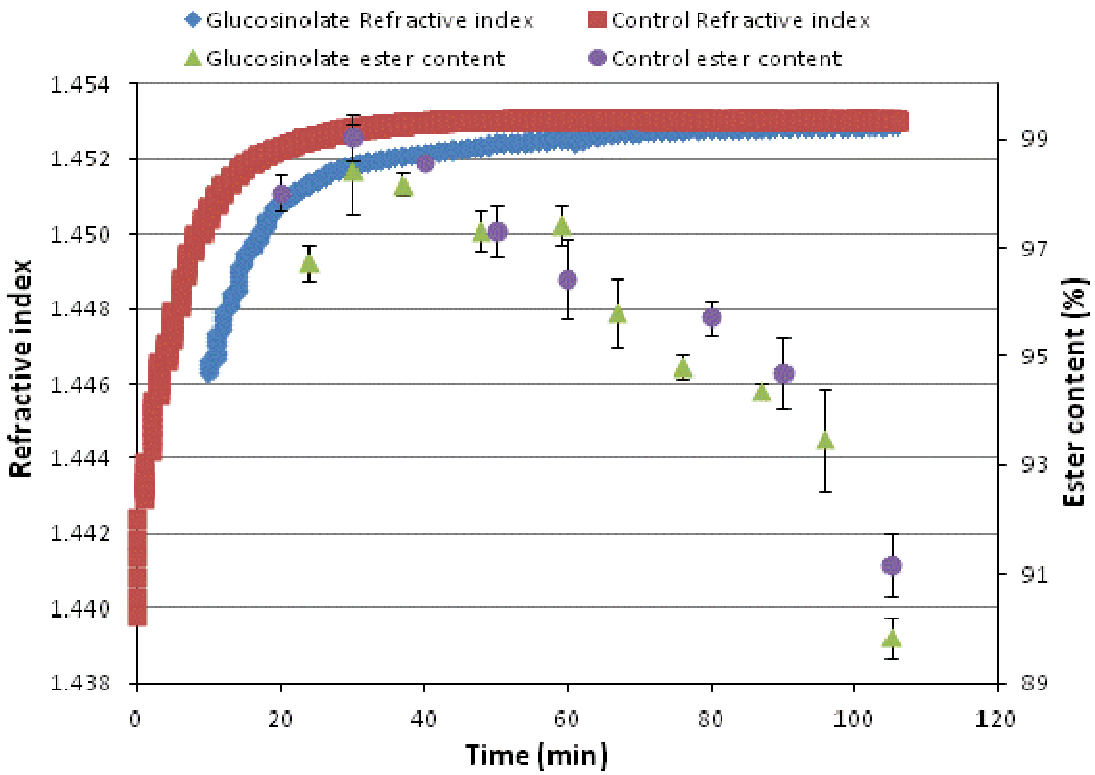

Figure 5.24. Comparison of refractive indices and ester content (%) for the transesterification reaction in the presence and absence of glucosinolate. (Glucosinolate experiment: 70 mL FAME: 20 mL oil: 10 mL CH_3OH, 3% (w/w) SrO with respect to triglycerides and 0.10 % glucosinolate at 60 °C for 105 min. FAME purity: 94.0% (w/w). Control experiment: similar conditions as for glucosinolate addition experiment except for the absence of glucosinolate addition). Values are the mean of three replicates, error bars indicate standard deviations.

The ester content (%) obtained were similar in the presence and absence of glucosinolate. For example, at 20 min the ester content for the control experiment was 99.0% (w/w) whereas in the presence of glucosinolate the value was 98.4% (w/w) at 24 min. However, at 105 min the ester content for control and glucosinolate added reaction was 91.1% (w/w) and 89.8% (w/w), respectively.

In the presence of glucosinolate, the refractive indices were lower than the control experiments until 60 min. After 60 min, no significant change in refractive indices was observed.

A comparison of glycerol (free and total) and glyceride (mono-, di-, and tri-glycerides) content (%) was also obtained in the presence and absence of glucosinolate (Figure 5.25). The free glycerol and triglyceride content (%) were higher than the EN 14105 specification in all the samples of both experiments except at 30 min for the control experiment. The mono- and di-glyceride content (%) of control and glucosinolate experiments were relatively lower and in accord with the EN standard in the first 60 min. However, after 60 min the mono- and di-glyceride content (%) were higher in the presence of glucosinolate only.

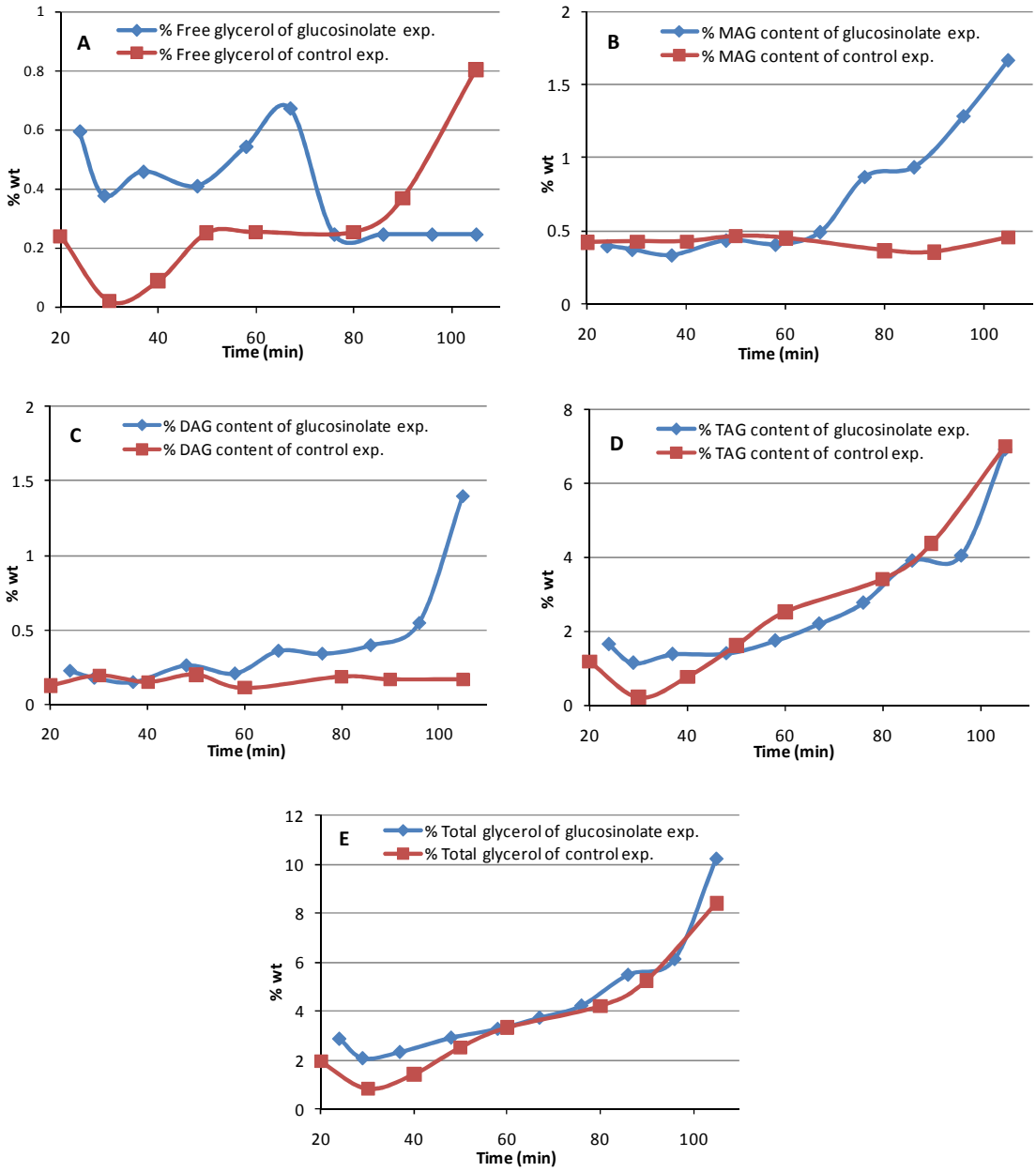

Figure 5.25. Results for the glycerol (free and total) and glycerides (MAG, DAG and TAG) in control and glucosinolate added experiments. **A:** free glycerol content, (%) **B:** monoglyceride (MAG) content, (%) **C:** diglyceride (DAG) content, (%) **D:** triglyceride (TAG) content (%) and **E:** total glycerol (%).

5.5. DISCUSSION

The work reported in this chapter details the effects of inter-solubility of FAME, methanol and oil on ester content (%) and refractive indices at 60 °C. There are two phases at the start of the reaction, the methanol phase and an oil phase. This is the reason that it takes time to start the reaction even if catalyst is present (see Chapter 4B, Section 4.4.1). However, as the ester content increases at the end of the transesterification reaction it helps the system to become homogeneous. This is because the esters in the reaction mixture act as solubilising agents. The rate of reaction increases with the solubility of oil in the methanol phase as a function of conversion.[7-9]

Multiphase, mass-transfer controlled reactions are strongly influenced by removing phase boundaries,[10, 11] and has the potential to accelerate the methanolysis.[12] Methanol is completely soluble in both FAME and glycerol but is not soluble in oil. With an increase in the mass fraction of FAME, the solubility of methanol in the oil and FAME phases increases.

The data in Figure 5.1 shows that when the FAME content increases up to *ca.* 52%, the oil–methanol–FAME mixture becomes a homogeneous phase. Glycerol has low solubility in both oil and FAME, and hence, can be easily separated from the final biodiesel product. The data points plotted in the phase diagram were similar to the results reported by Gunvanchai *et al.* However, in the aforementioned study the phase diagrams for oil-methanol-FAME at 20 °C, 40 °C and 60 °C were examined and it was found that the intersolubilities between rapeseed oil and methanol increase with increasing temperature.[3]

The inter-solubility between glycerol-methanol-FAME was not determined in the current study because the purpose was to investigate the kinetics at the initial stages of the transesterification reaction. The concentrations of the reaction products, which are produced in the methanol phase, are relatively low. Therefore, it is reasonable to

assume that all the products remain in the methanol phase without diffusing to the oil phase.

By selecting the concentrations of oil, methanol and FAME the problem of miscibility of the reactants was overcome. It is hypothesised that the transesterification reaction in the miscible region of the phase diagram (Figure 5.1) will yield higher ester content (%) in less time. Therefore, for this purpose several experiments were designed, using heterogeneous catalysts (mainly strontium oxide as it was the only catalyst which displayed good catalytic activity; Chapter 4A).

The results for the initial experiments (**1**-**4**) showed that the ester content (%) was never higher than 96.5% (w/w), set by the EN 14103 standard, even though, the transesterification reaction was carried out in the miscible region (60 mL FAME: 33 mL oil: 7 mL CH_3OH) of the phase diagram. The reason for this result could be the sample of FAME used as the reactant did not have 100% (w/w) esters as determined by GC. Therefore, the ester content (%) in FAME sample (reactant) was calculated depending on the volume of FAME used in a particular reaction mixture. The purity of FAME (reactant) had a proportional affect on the ester content (%) determined at the end of each experiment. For example, in experiment **1**, the FAME sample used had 88.6% (w/w) esters as determined by GC therefore, by using 60 mL of FAME sample in experiment **1** calculated to 53.1 mL ester (v/v) or 46.24 g of ester/52.2 g of FAME (w/w) in the reaction mixture. From the phase diagram 60 mL FAME was selected but actually due to less ester concentration in FAME it has decreased to 53.1 mL (Figure 5.2). Therefore, by examining the phase diagram (Figure 5.1), *ca.* 53 mL of FAME volume % is near to the immiscible phase boundary. This is a significant reason for not achieving ester content higher than 96.5% (w/w) till 60 min because the reactant FAME falls in an immiscible region. Although the ester concentration in FAME (reactant) samples calculated for experiments **2**-**4** were comparatively higher than experiment **1** but they followed the same trend as shown by experiment **1**.

Moreover, the refractive indices were also dependent on the sample of FAME (reactant) purity selected for experiments **1**-**4** *i.e.* the FAME (reactant) used for experiment **3** was 95.4% (w/w) and showed a lower refractive index value than experiment **2** (93.6% w/w). This is because the value of refractive index depends on the density of the measured sample. Typically, refractive index values decrease with decreasing density (increasing temperature). Hence, by adding a higher ester concentration/FAME sample the density of the reaction mixture is reduced. In the case of additional methanol and catalyst used during the reactions in experiments **2** and **3**, the refractive indices showed a insignificant change with the addition of strontium oxide. However, the addition of methanol has a significant effect on refractive indices. Experiments **3** and **4** were conducted in order to assess the effect of adding the methanol or catalyst during the reaction on the ester content (%). The results showed that using either catalyst or methanol alone does not show a significant difference in ester content (%). It also suggests that methanol was a limiting reactant. However, when both the catalyst and methanol were added during the reaction showed the higher ester content (%) as given in Figure 5.7 and 5.8. Therefore, the extra addition of both methanol and catalyst favours the transesterification reaction towards completion.

Taking into account the points made above, comparative studies were carried out between the reactions in the miscible region of the phase diagram using standard experimental conditions from Chapter 4-A (non-miscible region). Furthermore, the effect of methanol addition during the reaction was also studied. The ester content was the same for experiments **5** (non-phase) and **6** (miscible phase) at 60 min. In both experiments, when methanol was added during the reaction the higher ester content (%) was observed for experiment **6** only**.** This is because experiment **6** was conducted in the miscible phase, which increased the inter-solubility of the reactants, hence the increase in the ester content (%). When no additional methanol was added to the reaction (experiment **7** and **8**), the ester content (%) was lower than in experiments **5** and **6** at 120 min. This clearly shows that addition of methanol favours the increase in ester content (%). However, in experiments **5** and **6** where excess methanol was used a higher

free glycerol and monoglyceride content (%) was found in the reaction mixture. Similarly, the experiments carried out in miscible region also showed a higher monoglyceride content (%) in the reaction mixture (Table 5.2). This observation can be explained by the fact that even though excess methanol favours the conversion of triglycerides into monoglycerides, monoglycerides enhance the solubility of glycerol in biodiesel production and this result in glycerolysis. Glycerolysis is a reaction whereby ester/biodiesel reacts with glycerol to form monoglycerides and therefore causes a drop in ester content (%).[13, 14]

This study also showed that the addition of excess methanol, during the reaction, changes the vol. % of reactants in the phase solubility diagram. Therefore, next step was that no additional methanol or SrO will be added during the reaction. Moreover, the data point indicating the reactant quantities on the phase diagram will be selected from the middle of miscible region in a phase diagram. This is to avoid the possibility of reactants % vol. to fall in an immiscible region of the phase diagram due to low esters concentration in FAME sample. Therefore, several experiments **9**-**14** were carried out in the miscible region (Figure 2.2, Chapter 2) but with varying vol. % ratio of oil, FAME and methanol. In experiments **9**-**14**, the FAME/biodiesel sample used as a reactant had 94.0% (w/w) ester and 6% (w/w) glycerol/glyceride content. Therefore, extra catalyst balanced the amount of triglycerides present in the reaction mixture. The results showed that the ester content (%) could be achieved according to the EN 14103 specification for experiments **9, 10** and **14** in 20 min whereas, for experiments **11** and **13** this was achieved in 30 min. In the case of experiment **12,** the ester content was above 96.5% (w/w) at 50 min. This was due to the higher % vol. ratio of oil used in contrast to other experiments. These results also demonstrate that the higher % vol. ratio of FAME (reactant) helps in solubilising the reaction mixture thus increasing the ester content (%). In the case of experiment **14**, the use of higher % vol. ratio of methanol showed fluctuating refractive indices (Figure 5.21). This is because the prism quality dropped to 20 on the scale whereas for all other experiments it was above 60 at the end of the reaction. In all the experiments, the glycerol and glycerides content (%) also correlates

with the ester content (%) data. The useful information obtained from these results showed that after attaining the highest ester content (%), the transesterification reaction was reversed as intermediates were formed in the reaction mixture.

These results proved the hypothesis that the transesterification reaction carried out in a miscible region of the phase diagram can overcome the phase solubility issues and hence the reaction can proceed faster towards completion. Earlier in Chapter 4-A, different metal oxide catalysts were studied for the transesterification reaction, which showed no catalytic activity, and therefore no esters were formed. Using the same metal oxide catalysts, the reaction was carried out in a miscible region in order to monitor the refractive indices and ester content (%). However, no esters were found but the results obtained by the measurement of refractive indices are plotted in Figure 5.23. The refractive indices of all the reactions carried out with metal oxide catalysts were lower except with SrO. They showed a plateau around 20- 30 min. This shows that after this time no increase/decrease in the reactants or products changed as the refractive indices were stable.

The effect of glucosinolate on the ester content (%) was also studied in Chapter 4-A, which showed that the catalytic activity of SrO was proportionally lowered by adding glucosinolate to the oil. Therefore, to prove this effect further studies were necessary. For this purpose, experiments using glucosinolate were carried out in the miscible region of a phase diagram. The reaction conditions for experiment **9** were selected as a control experiment, since strontium oxide had shown a promising result and good catalytic activity due to the existence of one-phase. The actual experiment (glucosinolate experiment) was also designed to match the reaction conditions of experiment **9** but with 0.10% (w/w) glucosinolate addition (Figure 5.24). The results showed that the ester content (%) in the control experiment was approximately the same as for the glucosinolate experiment. The ester content was above 96.5% (EN standard) at 24 min even with the addition of glucosinolate. According to the results obtained, it is obvious that if the transesterification takes place in the miscible region, limitations

associated with the catalytic activity of strontium oxide could be overcome. As a result, glucosinolate showed a slight effect on the ester content (%) in the miscible region as compared to the non-miscible region (as shown in Chapter 4A, Section 4A.4.3). The mono- and di-glyceride content (%) in the glucosinolate experiment were higher than control experiment after 60 min, showing the reversibility of the transesterification reaction.

5.6. CONCLUSIONS

The objective of this research was to characterise the inter-solubility of oil-methanol-FAME and to use these results for multi-component biodiesel production. Based on the experiments carried out the following conclusions can be drawn:

- Intersolubility of methanol and oil increases with increasing concentration of FAME and when the concentration of FAMEs reached *ca*. 52 vol. %, the oil–methanol–FAME mixture becomes a homogeneous phase.

- To reduce the problem of immiscibility of the phases, the transesterification reaction was conducted in the miscible region of the ternary phase diagram. This aids the reaction in proceeding at a faster rate and provides a greater surface area for the catalyst to react.

- The presence of both additional methanol and strontium oxide favoured the transesterification reaction carried out in the miscible region but affected the solubilities of the reaction components.

- The use of refractometry for reaction monitoring is limited to catalyst screening and will only give semi-quantitative answers as to whether a catalyst is active. There was not enough sensitivity at higher conversions of methyl esters to compare different reaction conditions.

- All the reactions carried out in the miscible region of the phase diagram, without changing the reactant quantities between the reactions, yielded ester content (%) according to the EN 14103 Standard in approximately 20-30 min.

- Glucosinolate addition showed the similar ester content (%) to the control experiment (without glucosinolate) due to the miscibility of the phases.

- These results can be very helpful in designing a continuous flow process for biodiesel production in a single phase using either homogeneous or heterogeneous catalysts.

5.7. REFERENCES

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CHAPTER 6

SUMMARY AND FUTURE STUDIES

6.1. SUMMARY

In order to establish the reproducibility of the methodologies, for the synthesis of biodiesel, a transesterification reaction was carried out using glycerol trioleate by following those published reference methods. Contrary to expectation, the result showed that the ester content (%) did not meet the required specification set by EN 14103 standard for biodiesel. The presence of water and FFAs at the end of the reaction showed that competing reactions *i.e.* saponification and hydrolysis were ongoing at the outset of the transesterification reaction. Therefore, to understand the anomalies of this result and to optimise the reaction conditions, the transesterification reaction was extended to unrefined rapeseed oil. For optimisation of the transesterification reaction, various concentrations of homogeneous catalyst (NaOH) and methanol were explored. Results showed that increased concentrations of NaOH did not improve the ester content (%); instead the saponification reaction was favoured. Unrefined rapeseed oil has a higher FFA content (1.05 mg KOH/g) at the start of the reaction, therefore NaOH was consumed to neutralise the FFAs and increased the formation of soap. This in turn, increased the concentration of water in the reaction which caused the hydrolysis of triglyceride thereby reversing the direction of the reaction towards the formation of diglyceride and FFAs. On the other hand, low concentrations of NaOH caused an incomplete transesterification reaction at a given reaction time. The optimum concentration of catalyst required was 0.015 mol NaOH with 6:1 CH_3OH: oil molar ratio, which yielded an ester content of 93.3% (w/w). The results obtained by varying the concentration of methanol showed that there was no reaction at 3:1 CH_3OH/oil molar ratio at a given reaction time. Higher concentrations of CH_3OH/oil than 6:1 molar ratio complicated the recovery of esters.

The results obtained after optimising the reaction conditions for transesterification were still unsatisfactory as they failed to meet the EN standard specifications. Therefore, a survey using refined vegetable oils obtained from different sources was carried out with under similar reaction conditions used in the optimisation studies. Refined oils were used because they have low FFA content at the start of the reaction, compared to unrefined rapeseed oil. The results showed that the specifications for the EN 14103 standard were not met and this could be due to several reasons. Using NaOH as a catalyst the competing reactions accompanying the transesterification cannot be avoided. This is because when NaOH dissolves in methanol, water molecules are generated in the reaction mixture which in turn drives the chain of competing reactions. It was not possible to study the detailed kinetics of the transesterification reaction by using homogeneous catalysts. The FFAs and water interfere with the transesterification reaction and moreover, the catalyst has to be removed from the reaction mixture using several washing steps. For the purpose of studying the kinetics of transesterification reaction, heterogeneous catalysts were preferred over homogeneous catalysts due to ease of separation from the reaction medium.

Different metal oxide heterogeneous catalysts were tested for use in the transesterification reaction but only strontium oxide (SrO) showed promising results. The reaction conditions were optimised using SrO with respect to time. The results showed a decrease in ester content (%), at all concentrations of SrO used, when the reaction time was greater than 120 min. Moreover, a decrease in ester content (%) was also found when higher concentrations of SrO were used at a given reaction time. From these set of experiments, the optimum reaction conditions using a heterogeneous catalyst were found to be 3% (w/w) SrO, 6:1 CH_3OH/oil at120 min; even though by using the heterogeneous catalyst the EN 14103 standard specification was not met. This was not due to competing reactions, as was the case when using homogeneous catalysts. In this case, the reason that the EN standard specification was not met was due either to the catalyst being poisoned or because of slower reaction rates. One of the major problems associated with heterogeneous catalysts is the formation of three phases with

alcohol and oil. It was postulated that the slow transesterification rate was mainly due to slow mass transfer between polar methanol/glycerol and the non-polar oil phases.

In terms of the poisoning of catalyst it was hypothesised that glucosinolate in the rapeseed oil interacts with the metal oxide catalyst and decreases the ester content (%). The results of these investigations showed that the ester content (%) was proportionally affected by the addition of glucosinolate in the reaction.

Moreover, in order to study the slow reaction rates for the transesterification reaction in the presence of heterogeneous catalyst, real-time kinetic studies using SrO were conducted. For such studies, refractometry was employed to monitor reaction progress, as it is a quick and easy analytical method relative to gas chromatography. However, the ester content (%) obtained after the transesterification reaction were also determined by gas chromatography thus validating the results obtained by refractometry. The results obtained from such experiments helped to ascertain the optimum reaction conditions mentioned earlier for the transesterification reaction using strontium oxide.

Data obtained by measuring refractive indices of the reaction mixtures during the transesterification reactions also showed a delay in the instigation of the reactions. It was assumed that due to solubility issues (related to oil and methanol) at the initial stages of the reaction, the ester content (%) obtained did not meet the EN standard. This issue was further investigated and a ternary phase diagram was plotted on the basis of solubility data obtained from rapeseed oil, FAME and methanol. The phase-solubility diagram displayed two regions; the miscible and immiscible regions. In order to avoid the solubility issue, the transesterification reaction was conducted in the miscible region of the phase diagram. The results explain the fact that the ester content (%) achieved in the miscible region depends on the purity of esters/FAME sample. The earlier experiments conducted in the miscible region did not show the ester content (%) expected. This was due to the lower ester/FAME contents of the samples being used,

which caused the data points for the reactants to fall in the immiscible region of the phase diagram. Further experiments were conducted to compare the addition of methanol and SrO during the transesterification reaction. The results from refractive index and GC measurements showed that the addition of methanol, SrO or both during the reaction in the miscible region caused a problem in relation to the phase diagram such that and it can no longer remains the same reactant quantities in vol. % ratio as selected before the start of the reaction. Finally, experiments was conducted by using the data points *i.e.* vol. % ratio of reactants from the middle of the miscible region of the phase diagram. The ester content obtained was *ca.* 98.0% (w/w) in 24-30 min of the reaction time for all the experiments. These results confirmed that if the transesterification reaction is carried out in the miscible region of the phase diagram, the phase limitation problems caused by the heterogeneous catalysis could be avoided. In addition it has been found that the use of refractive index for reaction monitoring was helpful in identifying the slower reaction rates but limited to catalyst screening and could only provide semi-quantitative results as to whether a catalyst is active or not. Additionally, there was not enough sensitivity using refractometry at higher conversion rates of esters to compare different reaction conditions.

6.2. FUTURE STUDIES

The following research is aimed at providing recommendations for further work, based on the observations made during the experiments reported herein:

1. The data obtained from the experiments conducted in the miscible region of the phase diagram will be helpful as it allows the design of continuous flow processes for the production of biodiesel at the industrial scale. The FAME produced in this way can be used again for the transesterification reaction in the miscible region. However, this method is limited to heterogeneous catalysts as they can be easily removed from the reaction system.

2. The experimental results showed that SrO acts as an excellent, stable catalyst in the miscible region of the phase diagram. In order, to decrease the cost of the catalyst for the production of biodiesel, the catalyst can be recycled and reused. SrO has the ability to sustain its catalytic activity even after 10 transesterification cycles. Therefore, it will be worthwhile to study the stability and catalyst lifetime from an economic, scale-up point of view.

3. Other heterogeneous catalysts need to be tested for the transesterification reaction. Heterogeneous catalysts that need support on *e.g.* zeolites, alumina or silica to increase their catalytic activity and to minimise the mass transfer limitations can be used without supports in the miscible region. Thus, the phase limitation problem associated with heterogeneous media could be overcome and thereby increase the reaction rate for transesterification.

4. Another possibility is to try to enhance the catalysed transesterification reaction rates by microwave irradiation. In microwave technology, boiling is a kinetic as well as a thermodynamic process and therefore solvents heated under microwave conditions often boil at elevated temperatures even at 1 atm pressure. For example, methanol has a conventional boiling point of 64.7 °C; however when heated using dielectric microwave procedures it boils at 84 °C. This temperature rise can lead to an enhancement of ~10^2 in the reaction rate. Therefore, transesterification reactions conducted in the miscible region of the phase diagram reported in the current study can be examined using microwave-assisted synthesis in order to reduce reaction times.