UNIVERSITI TEKNOLOGI MARA

HYBRID IN/EX SITU HYDROLYSIS PRODUCING DIHYDROXYSTEARIC ACID FROM EPOXIDIZED NEEM OIL: SYNTHESIS & KINETIC STUDIES

ISMAIL BIN MD RASIB

PhD

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ISMAIL BIN MD RASIB

Thesis submitted in fulfilment
of the requirements for the degree of
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(Chemical Engineering)

Faculty of Chemical Engineering

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ABSTRACT

Depletion of oil reserve along with its volatility in terms of price and availability arise researchers concern on renewable resource for epoxidized material. Vegetable oils are commonly chosen as the alternative to petroleum resources. In this study, non edible oil, neem oil are chosen to be epoxidized by optimum level of process parameter obtained by Taguchi method. The optimum level of each process parameter involved, with its percentage contribution toward the epoxidation of neem oil is determined by S/N ratio and ANOVA analysis respectively by the Taguchi method. The results show most optimum levels for each process parameter are formic acid/neem oil molar ratio: 1:1, hydrogen peroxide/neem oil molar ratio: 4:1, catalyst loading: 1g, reaction temperature: 70 ^{0}C, and agitation speed: 300 rpm. Meanwhile, ANOVA states that catalyst loading was the most significant factor that affected the epoxidized neem oil (30.66%), followed by formic acid/neem oil molar ratio (A) at (29.77%) due to its role as an oxygen carrier compared to hydrogen peroxide, which acts as an oxygen donor and the third one is stirring speed (E) at (20.95%). The highest relative conversion to oxirane of 51.50% was achieved under optimized conditions. Both neem oil and its epoxide form are analysed by FTIR, where the formation of an oxirane ring is shown at a wavenumber of 1240 cm^{-1}. This work focuses on the production of dihydroxystearic acid (DHSA) through both in situ and ex situ hydrolysis, following the epoxidation of neem oil. The epoxidised intermediate was obtained using performic acid generated in situ, and hydrolysis conditions were systematically optimised using the Taguchi approach. Signal-to-noise (S/N) ratio analysis indicated the optimum parameters for achieving the highest hydroxyl value (129.4 mg KOH/g), namely: a water-to-neem oil molar ratio of 2:1, a water addition period of 90 minutes, and a reaction termination time of 120 minutes. Analysis of variance (ANOVA) further demonstrated that the parameters influenced the process in the order: reaction stop time > water addition duration > water/neem oil ratio. To support experimental findings, a kinetic model was constructed in MATLAB using the fourth-order Runge–Kutta algorithm in combination with simulated annealing for optimisation, enabling accurate representation of the system. Overall, the study demonstrates how neem oil can be converted into a high-value product, offering a sustainable alternative to petroleum-derived feedstocks and advancing understanding of the reaction kinetics critical are beneficial when it is supported more by kinetic intrepretation.

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LIST OF ABBREVIATIONS

Abbreviations

ANOVA	Analysis of Variance
AOCS	American Oil Chemist Society
DHSA	Dihydroxystearic acid
DOBI	Deteoriarity of Bleachability Index
DSC	Differential Scanning Calorimetry
FTIR	Fourier Transform Infrared
OOC	Oxirane Oxygen Content
RCO	Relative Conversion Oxirane

CHAPTER 1
INTRODUCTION

1.1 Background of the Study

In today's world, the depletion of oil reserves, combined with the rising cost of energy due to global economic growth, has made economic energy consumption an increasingly difficult goal to achieve [1]. Additionally, petroleum is a source for producing hydrocarbon compounds. Consequently, this issue has drawn researchers' attention to renewable resources for producing epoxides through the epoxidation of vegetable oils. Besides their renewability, vegetable oils are environmentally friendly and readily available at a lower cost, largely due to their high content of unsaturated fatty acids, such as oleic, linoleic, and linolenic acids [2].

Neem oil, also known as *Azadirachta indica*, is extracted from the neem tree and is commonly found in India. Moreover, it can also be found in other regions such as Africa and Asia. It is widely used as a pesticide due to the presence of active compounds like caryophyllene oxide, limonene, and organosulphur compounds [3]. In Ethiopia, the alternative medicine sector uses soybean oil to treat malaria and skin diseases [4]. This study focuses on epoxidising neem oil because of the limited research and data available from other studies. Neem oil is suitable for epoxidation as it contains fatty acids typical of vegetable oils, including oleic acid (50–60%), palmitic acid (13–15%), stearic acid (14–19%), linoleic acid (8–16%), and arachidic acid (1–3%) [3].

The most common epoxidation method is a chemical conversion process assisted by either a heterogeneous or homogeneous catalyst. Formic or acetic acid acts as an oxygen carrier, while hydrogen peroxide serves as an oxygen donor. Performic acid, which forms from formic acid and hydrogen peroxide, reacts with the double bonds of fatty acids in selected vegetable oils to produce epoxide groups. Heterogeneous catalysts are preferred in this study due to several advantages, such as easy separation from the mixture by filtration, which optimises purification costs. Additionally, the filtered catalysts can be reused in the same process. In contrast, the separation of homogeneous catalysts from the mixture is more complex and costly [1].

Amberlite IR-120 H was used in this study. It is a hydrogen-form cation exchange resin made from sulphonated polystyrene crosslinked with divinylbenzene.

This resin is commonly applied in processes such as water softening, deionisation, catalysis, and various organic reactions. Its widespread use is attributed to its strong mechanical strength, excellent ion-exchange efficiency, and resistance to heat and chemicals [2].

The oxirane ring, also known as an epoxide, is a three-membered cyclic ether characterised by significant ring strain and pronounced reactivity, making it valuable in both synthetic and industrial chemistry. However, this same reactivity can lead to undesirable side reactions, especially when reaction conditions are not well controlled or when reactive species such as acids, bases, or nucleophiles are present. Understanding these reactions is crucial for improving the yield, purity, and selectivity of processes involving epoxides [3],[4]. A predominant side reaction associated with epoxides is non-selective ring opening, particularly under acidic or basic conditions. The inherent ring strain in epoxides makes them highly reactive, leading to non-selective nucleophilic attacks, especially under acidic or basic conditions [5].

Due to the high reactivity of the oxirane ring, epoxides can also act as raw materials for various chemicals, such as glycols, carbonyl compounds, and polymers like polyesters, polyurethanes, and epoxy resins [2]. Dihydroxystearic acid (DHSA) is a product that can be obtained from the opening of the oxirane ring. It is produced by the addition of a hydroxyl group through water, which is a by-product of peracid formation earlier during epoxidation. Furthermore, DHSA possesses properties that make it suitable for further use in the cosmetic industry [5]. DHSA, a high-value hydroxylated fatty acid, is commonly derived from oleic acid found in palm oil, due to its high percentage of unsaturated oleic acid. The common method involves two steps: epoxidation to produce the highly reactive epoxide, or known as the oxirane ring, followed by hydrolysis to yield the valuable DHSA end product [6]. The most common approach to producing DHSA involves epoxidation of oleic acid, followed by hydrolysis of the epoxidized oleic acid. During epoxidation, the carbon-carbon double bond (unsaturated bond) of oleic acid in vegetable oils, typically palm oil, is converted to a saturated three-membered cyclic epoxide ring using performic acid, which is generated in situ by mixing formic acid and hydrogen peroxide prior to the reaction [7]. Epoxidation can also be catalysed by heterogeneous catalysts, commonly ion-exchange resin or calcium chloride. Key parameters such as the molar ratios of formic acid to oleic acid and hydrogen peroxide to oleic acid, reaction temperature, and stirring speed must be optimised to maximise the yield of epoxidized oleic acid, which directly

influences DHSA production [6]. Next, the epoxidized oleic acid is hydrolysed to open the three-membered cyclic carbon. The main reactants are hydrogen donors, typically water, although some researchers use alcohols. Catalysts such as sulphuric acid or Amberlite are employed to enhance the reaction, resulting in shorter reaction times and higher yields [6].

1.2 Problem Statement

Concerns about renewable resources as precursors for hydrocarbon materials are increasing worldwide due to price volatility, non-renewability, and environmental pollution caused by petroleum sources. Using vegetable oil as a starting material, epoxidation can increase its value by offering numerous advantages, such as low toxicity and lower cost [6]. Epoxides derived from vegetable oils can be used as plasticisers in plastic or rubber materials, which help make them softer and more flexible, increase their plasticity, and reduce viscosity and friction during handling in the manufacturing process [2]. There is a lack of research on the epoxidation of non-edible vegetable oils. Most studies focus on epoxidation of edible vegetable oils, such as corn, sunflower, palm, cottonseed, and soybean [1], [6][7]. Both types of vegetable oils likely contain free fatty acids, which are crucial for epoxidation, as their double bonds are attacked by nucleophiles to produce epoxidized vegetable oil or oxirane rings. In this study, the renewable resource neem oil is chosen for epoxidation using performic acid with Amberlite IR-120 H as a heterogeneous catalyst to replace homogeneous catalysts, such as sulphuric acid, since the use of homogeneous catalysts can cause corrosion in industrial-scale reactors. Formic acid is chosen to produce performic acid by mixing it with hydrogen peroxide due to the instability of peracetic acid [8], which results from its mild acidity compared to acetic acid. Additionally, formic acid is readily available and low in cost, which further justifies its selection [9].

Researchers focus mainly on non-edible oils as industrial feedstocks to replace non-renewable petroleum resources. Due to the limited research on the epoxidation of neem oil, the non-optimum levels of each parameter were established to study their effects and compare the resulting epoxidation rates, hence providing a data set for other researchers. The parameters investigated in this study include formic acid/neem oil molar ratio (A), hydrogen peroxide/neem oil molar ratio (B), catalyst loading (C), reaction temperature (D), and stirring speed (E). Each parameter was tested at four

levels in terms of concentration, weight, temperature, and speed. A large number of experiments are required because the formation of the oxirane ring, which indicates successful epoxidation, can only be confirmed during the reaction. Taguchi's design of experiment (DoE) methodology was implemented to determine the optimum number of experiments with a higher possibility for oxirane ring formation. This approach reduces the number of necessary experiments that need to be repeated, thereby lowering costs and improving time efficiency. Analysis of variance (ANOVA) and signal-to-noise (S/N) ratio were used to identify the optimum parameters and their levels based on the relative conversion to oxirane obtained from the previous DoE.

Due to the high reactivity of the oxirane ring in epoxidized neem oil, the formation of DHSA, a hydroxyl fatty acid containing three functional groups, made up of one carboxyl and two hydroxyl functional groups, is to be synthesised [9]. To achieve a high hydroxyl value suitable for industrial applications, transesterification of vegetable oils is performed prior to epoxidation. According to Arniza M et al., transesterification of palm olein with glycerol enhances functionality by introducing an additional hydroxyl group into the triglyceride structure before proceeding with a ring-opening reaction using performic acid [10]. However, this method is time-consuming, with the transesterification step alone requiring 6 h to complete. Moreover, a lot of resources are needed, as each step involves different chemicals and separation processes before epoxidation and ring opening can proceed. Other studies by Holser R. indicate that oxirane ring values remain unchanged during transesterification [11]. Nevertheless, DHSA produced after a 2-h epoxidation process without transesterification contains a low quantity of hydroxyl groups, possibly due to insufficient by-product water acting as an oxygen donor. Hence, the formation of DHSA via continuous hydrolysis of epoxidized neem oil was investigated, where the degradation of the oxirane ring after it reached the highest oxirane ring content for epoxidation was continued until 2 h. Formic acid, generated from performic acid formation, along with water, a by-product of the same reaction, introduces the hydroxyl group through ring opening, producing DHSA. This method requires less time as it involves two processes (epoxidation and hydrolysis) in an in situ system without the need for additional chemicals. Producing DHSA from neem oil aims to maximise its market potential beyond its current uses as a pesticide and alternative medicine [4].

DHSA is a compound of significant industrial value, with applications across various sectors due to its functional hydroxyl groups. These groups make it ideal for

roles requiring thickening, plasticisation, or bio-based alternative chemicals. Furthermore, DHSA is extensively used in lubricants, cosmetics, and polymer synthesis [8]. Current DHSA production methods are hindered by inefficiencies in catalytic epoxidation and hydrolysis, leading to low epoxide selectivity, undesirable by-products, and reduced yields. These processes rely on costly catalysts, high energy inputs, and harsh chemicals, resulting in environmental challenges and scalability issues. Additionally, hydrolysis may be inefficient if the oil substrate lacks optimal epoxide content, further complicating cost-effectiveness and production feasibility. Thus, this study employs a hybrid approach of in situ and ex situ hydrolysis, where water is added after the degradation of the oxirane ring. The optimum parameters in DHSA formation, namely water/neem oil ratio (F), water addition time (G), and reaction stop time (H) with their corresponding levels, are determined using the one-factor-at-a-time (OFAT) method.

The unique properties of neem oil make it a strong candidate for biopolymer and resin production. Its high oxidative stability, attributed to azadirachtin and triterpenoids, reduces the need for stabilisers, thereby lowering costs and extending product lifespan. Unlike soybean oil, the fatty acid profile of neem oil provides flexibility and compatibility in resin formulations, while offering better durability than linseed oil. However, there are no studies on the kinetics of epoxidising neem oil using performic acid. The kinetic study is important to determine the degree of conversion and to ensure that DHSA produce in this research are supported by kinetic itrepretation. Conducting kinetic research provides valuable insights into the reaction rates, reaction mechanisms, and factors influencing the conversion of neem oil to epoxidized neem oil.

1.3 Objectives of the Study

The objectives for this study are as follows:

1. To optimise the process parameters for the epoxidation of neem oil using Amberlite IR-120 H via the Taguchi method and to investigate the effect of each parameter's level relative to other parameters at their respective optimum levels on the epoxidation rate of neem seed oil.
2. To elucidate and evaluate the performance of DHSA production by introducing a hybrid approach of in situ and ex situ hydrolysis towards epoxidized neem oil.

3. To develop a single-phase kinetic model of epoxidized neem oil and DHSA from epoxidized neem oil in order to determine reaction rate constant.

1.4 Scope of the Study

Epoxidized neem oil was produced through two main steps: (1) the reaction between formic acid and hydrogen peroxide to produce performic acid, and (2) the reaction of performic acid with neem oil to produce epoxidized neem oil. A heterogeneous catalyst, Amberlite IR-120 H, accelerates the reaction rate.

Taguchi's DoE was employed prior to conducting a series of experiments for the epoxidation of neem oil, aiming to achieve a promising oxirane oxygen content (OOC). The parameters considered include formic acid/neem oil molar ratio (A), hydrogen peroxide/neem oil molar ratio (B), catalyst loading (C), reaction temperature (D), and stirring speed (E). The OOC results obtained from the epoxidation of neem oil using the DoE determined by the Taguchi method were further analysed using ANOVA and S/N ratio analysis to determine the optimum parameters and their levels for producing epoxidized neem oil via epoxidation with performic acid. A sample exhibiting the highest oxirane ring conversion was then analysed by Fourier transform infrared spectroscopy to confirm oxirane ring formation. Subsequently, the epoxidation rate of neem oil under the optimum conditions was investigated by conducting experiments in which each parameter was individually varied from its optimal level while keeping the others constant. Finally, the physicochemical properties of the produced epoxidized neem oil, such as acid value, deterioration of bleachability index, iodine value, saponification value, and moisture content, were determined.

DHSA from epoxidized neem oil was produced through a hybrid approach of in situ and ex situ hydrolysis of epoxidized neem oil. This was achieved by prolonging the epoxidation of neem oil for up to 5 h. This extension is necessary because, after obtaining the highest OOC, oxidation of the oxirane ring occurs via hydrolysis with water, which is present as a by-product during performic acid formation. However, additional water is needed to increase the hydroxyl group content of the DHSA produced. Another set of parameters was optimised in this step: water/neem oil molar ratio (F), water addition time (G), and reaction stop time (H). The OFAT method was used to determine the optimum parameters for DHSA formation from the combined in situ and ex situ hydrolysis of epoxidized neem oil. The same experimental setup for

DHSA production from neem oil was repeated to validate the proposed method. However, additional water was introduced following the ex situ method, where water was added during the highest oxirane ring and before the opening of the oxirane ring. Meanwhile, in the in situ method, water was not added to the system until the end of the experiment. The hydroxyl group content of all DHSA samples was analysed and compared.

This study aims to develop and validate a comprehensive kinetic model for the epoxidation of neem oil and its subsequent hydrolysis to produce DHSA. Neem oil, a renewable resource rich in unsaturated fatty acids, offers significant potential for producing high-value epoxides through environmentally friendly routes. Understanding the reaction kinetics involved in its transformation is essential in order to make sure it is supported by kinetic interpretation.

The scope of this research is divided into two main phases. The first phase focuses on the epoxidation of neem oil using performic acid generated in situ from formic acid and hydrogen peroxide. The system is treated as a single-phase homogeneous reaction to eliminate complexities due to interfacial mass transfer. A kinetic model was developed based on the elementary reactions, and rate equations were derived for each species involved, including formic acid, hydrogen peroxide, performic acid, neem oil, and the resulting epoxide. MATLAB R2021b was employed to numerically solve these equations using the fourth-order Runge-Kutta method. Kinetic parameters were estimated by minimising the error between simulated and experimental epoxy concentrations, with genetic algorithms applied to enhance optimisation accuracy. The reliability of the model was validated through statistical error analysis and comparison with experimental results.

The second phase extends the kinetic model to include the hydrolysis of epoxidized neem oil to DHSA. The reaction mechanism was updated to incorporate an additional irreversible reaction, in which the epoxide reacts with water to form DHSA. Intermediate species, such as performic acid and formic acid, were assumed to be in a steady state, based on experimental observations. The model was simultaneously fitted to experimental data for both epoxidized neem oil decay and DHSA formation, with further optimisation performed using a genetic algorithm framework. The model was validated by assessing the accuracy of simulation predictions against experimental results using error metrics, such as mean absolute error and root mean square error.

Experimental work includes the preparation of neem oil epoxidation and hydrolysis under varying conditions of temperature and reactant ratios. Concentrations of key species were measured at regular time intervals using appropriate analytical techniques. These data form the foundation for model calibration and validation.

This thesis is expected to produce validated kinetic models for both reaction stages, supported by MATLAB simulation files, cleaned datasets, optimised kinetic parameters, and graphical validation outputs. The results will offer insights into optimal operating conditions for maximum yield and efficient reaction conversion. A comprehensive 24-week timeline was structured, beginning with a literature review and experimental setup, followed by phased experimental work, model development, validation, and final reporting. The outcomes of this study are expected to provide a strong technical foundation for future process intensification, design, and further study of neem oil-based epoxide and DHSA production.

1.5 Main Contribution of the Study

The main contributions of this study are the significant improvement in epoxidation of neem oil process and formation of DHSA by due to oxirane ring opening. Other contributions of this study include:

1. By introducing the hybrid method of in situ and ex situ hydrolysis, higher quality DHSA is expected to be produce because this method are believed to enhance the degradation of oxirane ring of epoxidized neem oil along with providing excess of OH group for producing DHSA.
2. Would beneficial to other researchers in future in terms of finding physicochemical properties of raw and epoxidized neem oil since this study would provide it, along with the effect on epoxidation rate when there is a difference in level for each parameter while maintaining other at optimum level. The thermodynamic properties obtain by kinetic model would also beneficial to see whether the DHSA produce are supported by kinetic interpretation.
3. Optimum production of DHSA with high hydroxyl value content with minimal step of procedure would be produce at the end of this study, along with its purification, DHSA from neem oil epoxidation can be used in industry, hence

varies the usage of neem oil from conventionally used as an alternative medicine and pesticide only.

1.6 Concluding Remarks

In summary, neem oil presents itself as a promising renewable feedstock for epoxidation due to its high unsaturated fatty acid content and underexplored potential compared to other vegetable oils such as palm oil and and sunflower oil. By utilising heterogeneous catalysts such as Amberlite IR-120 H and optimising reaction conditions, the production of epoxides and their subsequent conversion into high-value compounds like DHSA can be made more efficient, sustainable, and industrially viable. This study therefore aims to bridge the existing research gap, offering both scientific insights and practical pathways for advancing bio-based alternatives to petroleum-derived chemicals.

1.7 Outline of Thesis

This thesis investigates the catalytic epoxidation of neem oil using Amberlite IR 120 H and the subsequent conversion of oxirane rings into DHSA. The study is motivated by the need for renewable alternatives to petroleum derived epoxides amid resource depletion and price volatility. Neem oil, a non-edible and widely available vegetable oil rich in unsaturated fatty acids, is selected due to its suitability for epoxidation. Epoxidation is carried out via in situ generated performic acid, which converts carbon–carbon double bonds into oxirane rings. The use of Amberlite IR 120 H as a heterogeneous catalyst is emphasised for its reusability and operational advantages over conventional homogeneous acid catalysts.

Previous studies have focused mainly on the epoxidation of edible oils, while non-edible oils remain underexplored. Literature indicates that acid concentration and temperature strongly affect oxirane yield and ring stability, with excessive acid promoting unwanted hydrolysis. In this study, formic acid is selected as the peracid precursor due to its lower cost and availability, highlighting the need to optimise reaction conditions to maximise epoxide formation and minimise side reactions. The work also addresses limitations of conventional DHSA synthesis by proposing a hybrid in situ/ex situ hydrolysis that integrates epoxidation and ring opening using by-product

water. The objectives are to optimise neem oil epoxidation using Taguchi experimental design, assess the efficiency of the hybrid hydrolysis for DHSA production, and develop a kinetic simulation which validates the proposed reaction mechanism by accurately reproducing the experimental trends.

However, This study is limited to laboratory-scale experiments and kinetic simulations; therefore, large-scale industrial validation and scale-up analysis are not addressed. The applicability of neem oil is also constrained by its limited acceptance and commercial use in Malaysia. In addition, the absence of established industrial case studies in existing literature restricts direct benchmarking of the proposed process against real-world applications.

The thesis will conclude with a synthesis of the main findings. It is expected that the Taguchi-based optimisation will achieve high oxirane yields while keeping reaction times and side reactions low. The hybrid hydrolysis is anticipated to produce DHSA efficiently without the need for separate transesterification, and the kinetic model should provide guidance for scaling up the process. Overall, this work aims to expand the body of knowledge on neem oil epoxidation, demonstrating how a non-edible oil can be converted into value added products using a recyclable solid catalyst.

CHAPTER 2
LITERATURE REVIEW

2.1 Neem Oil

2.1.1 Introduction to Neem Oil

Neem oil is pressed directly from the seed of neem, which majorly can be found in India but can also be found in Southeast Asian and African countries. Its physical characteristic are brown in colour, has bitter taste and a garlic/sulphur smell. Main application for neem oil are insect repellent, insect growth regulator by affecting hormone of insects during an early age, which altering its development hence cause its death[4]. Azadirachtin, which are the most dominant component in neem oil can also interfere the mitosis stage of an insect, and directly interfere with its guts, epithelial cells and muscle tissue, hence decreasing their self protection. It also classified as biochemical pesticide due to its apparent non toxic mode of action and occur naturally in the environment [4],[12].

In developing countries such as Ethiopia, rural people use neem leaves as for an alternative medicine to cure malaria and diabetes. Ayurvedic medicine that is originated from India 7000 years ago also use neem oil to treat cardiovascular disease, ulcer and skin conditions. However, the use of neem seed oil as an antimicrobial and food preservatives raise concern since several report are made about the side effect of synthetic oils [12].

The main component found in the neem oil are azadirachtin which mainly found in their seed. Other parts of neem plant contain less of it. Its chemical structure as shown in Figure 2.1 [13].

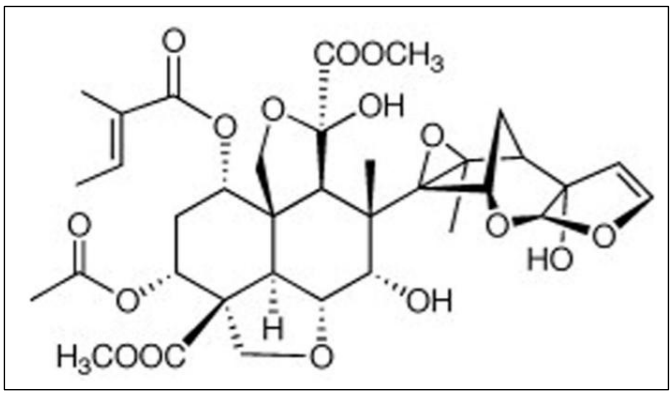


Figure 2.1 Chemical Structure of Azadirachtin [13]

2.1.2 Extraction of Neem Oil

Soxhlet extraction are simple and effective method discovered and practice for over a century for an extraction for a wide range of samples like soils, sediments and animal fat. Solvents like dichloromethane, pure or mixed with acetone or hexane and acetone-hexane mixture can be used while the use of nonpolar solvents only is not recommended. According to Beyecha et al, soxhlet extraction process are chosen for extraction of neem oil due to its low operating cost, higher yield oil and low turbidity compared to other methods. Solvent that mostly chosen are n-hexane due to its higher boiling point, stability, low corrosiveness and high oil yield [4]

As for the procedure of neem oil extraction by Beyecha et al, neem seeds were collected, repeatedly wash, and dried in an oven at 50^0C for 24 hours to remove the moisture and mill are used to further reduce their size. Soxhlet extraction process is chosen and the bottom flask are filled with organic solvent n-hexane. Parameter are varied as temperature at 50^0C - 80^0C, particle size of 0.15-2mm and a time of 60-180 min. At the time interval, oil was collected in volumetric flask then centrifuged to separate the solid apart from the solution and evaporated by rotary evaporator to get solvent free oil. Workflow illustration of soxhlet as in Figure 2.2 [4].

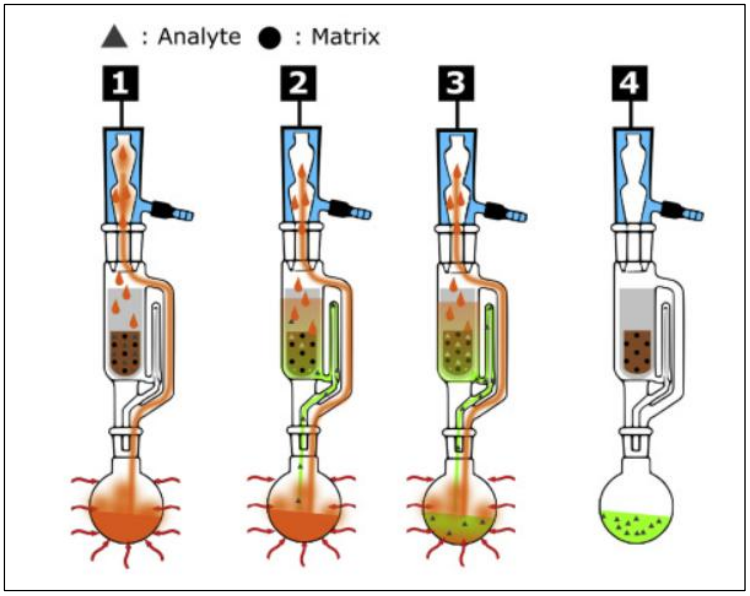


Figure 2.2 Workflow Illustration of Soxhlet [4]

Yield of oil was determined using Equation 2.1, where W_1 is sample weight initially placed in thimble and W_2 is sample weight after dried in an oven.

$$\text{Oil yield (\%)} = \frac{W_1\text{-}W_2}{W_1*100} \tag{2.1}$$

Based on the most optimum parameter chosen by Box-Behnken experimental design, 44.14% oil yield can be obtain with 132 min reaction time, 64^0C of extraction temperature, and 0.21mm of particle size[4].

2.1.3 Properties of Neem Oil

Neem oil is a mixture of several terpenoids which consist of five carbon skeleton for simple terpenoid or between 20 to 40 carbon atoms for complex terpenoid. Azadirachtin, the most complex terpenoids are an active ingredient for pesticide. However, neem oil also contain steroids, fatty acid and a number of essential oils that have potential for other usages [14]. Physiochemical property of neem oil were analysed according to AOAC (1990) and ASTM standard. Specific gravity for neem oil is 0.91, which in standard limit of non edible oil and important factor to be concern if neem oil are use for biodiesel production to allow optimal air to flow ratio of fuel combustion and particulate matter emission. Iodine value is an indication of the unsaturation of fat

and oil. The greater iodine value shows a higher degree of unsaturation of oil and fats. Iodine value of oil above 120$\frac{mg\ I_2}{g}$ also known as drying oil, where it can be used in cosmetic industry, since they absorb into skin within second of application. Physicochemical properties of neem oil are tabulated in Table 2.1 [15].

Table 2.1
Physiochemical Properties of Neem Oil [15]

Components	**Value**	**ASTM 763 standard**
Density (g/cm^3)	0.875	0.8-0.9
Moisture content (%)	4.61	0.3-0.6
Ash content (%)	2.31	1.5-4.5
Viscosity (mm^2/s)	33.5	25-35.5
Iodine value (mg I_2 per 100g)	122.5	120
Saponification value (mgKOH/g)	206.7	200-220
Acid value (mg KOH/g)	1.81	1.5-2.4

Fatty acid composition are analyse with gas chromatography mass spectrometry method. It was revealed that considerable amount of unsaturated fatty acid in the neem oil and presented in Table 2.2.[15]

Table 2.2
Fatty Acid Composition of Neem Oil [1]

Fatty acid type	**Formula**	**Composition (%)**
Saturated fatty acid		
Caprylic acid	$C_8H_{16}O_2$	16.621
Myristic acid	$C_{14}H_{28}O_2$	0.0058
Palmitic acid	$C_{16}H_{32}O_2$	45
Stearic acid	$C_{18}H_{36}O_2$	0.0062
Total		39.076
Unsaturated fatty acid		
Oleic acid	$C_{18}H_{34}O_2$	40

Fatty acid type	Formula	Composition (%)
Linoleic acid		11
Total		51

2.1.4 Previous Study on Neem Oil

The global interest in natural products and their derivatives for disease prevention and treatment is growing, primarily due to their minimal side effects. Neem and its compounds have long been recognized for their therapeutic value, especially in traditional medicine systems of the Indian Subcontinent. Clinical research has demonstrated that neem plays a significant role in preventing various diseases. One of it are antimicrobial effect.[9] A study evaluated 21 strains of foodborne pathogens and found that guava and neem extracts contain bioactive compounds with antibacterial properties, indicating their potential for controlling foodborne pathogens and spoilage microorganisms [10].

In dentistry, neem oil is believed to have same efficiency in reducing the periodontal indices as chlorhexidine due to its antigingivitis effect and it is proven by Chatterjee et al [11]. Another study done by Lekshmi et al [12] prove that organic extract of neem show strong antimicrobial properties against bacterial strain causing dental caries, even chewing the dried sticks of neem shows maximum antibacterial activities against S.Mutans.[9]

2.2 Epoxidation Reaction

The mechanism of epoxidation of vegetable oils is a chemical reaction that occurs at the double bonds of the triglycerides of oil used [6]. Epoxidation is the crucial process for functionalization of the double bonds. Epoxidized vegetable oils or known as oxirane ring are highly reactive that are beneficial as a precursor for alcohols, polyols, glycol mono esters, glycol diesters, carbonyl compound and epoxy polymers [16]. Until now, a few methods of epoxidation had been established, such as Prilezhaev method and enzymatic way. Commonly chosen method are Prilezhaev. This method involve in situ production of performic or peracid with presence of catalyst. Both performic acid (HCO_3OH) and peracetic acid (CH_3CO_3OH) function as electrophilic oxygen donors in

the Prileschajew reaction, facilitating the formation of an epoxide ring by transferring an oxygen atom to the alkene. This process typically occurs in situ, with hydrogen peroxide (H_2O_2) reacting with formic acid or acetic acid in the presence of a catalyst, such as sulfuric acid or ion-exchange resins [13]. Both performic and peracetic acid have the same weightage in order to act as reactant for epoxidation of vegetable oils in terms of mild operation temperature, environmental friendly, and both are suitable to be used with ion exchange catalyst. However, safety concern are extra needed for peracetic acid due to its exothermic reaction since it is more oxidizing and unstable compared to performic acid [14]

Epoxides, which also known as oxirane are rings with three members, two of which are carbon vertices and one of which is an oxygen. Because of the tension that the three-membered ring creates, epoxides behave differently from other ethers as figure below.[17]

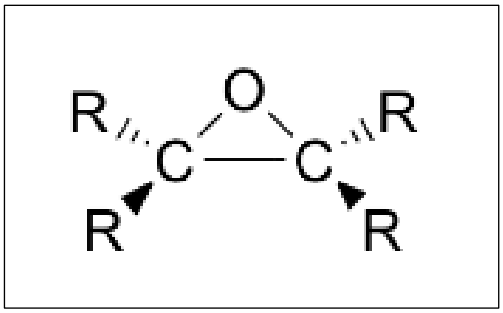


Figure 2.3 Oxirane Ring [17]

2.2.1 In-Situ Epoxidation

In situ epoxidation involve the formation of performic acid by mixing of both oxygen donor and oxygen carrier, which are hydrogen peroxide and formic acid respectively[18]. The word “in-situ” refers to all the reaction happen in a system. Formic acid is more preferable as oxygen donor compared to acetic acid due to its higher reactivity, thus no catalyst needed for performic acid formation. However, according to Derawi et al, molar ratio of performic acid form would affect the rate of epoxidation. Other parameters that need to be consider are the stirring speed, type of catalyst used and reaction temperature [16].

Epoxide vegetable oil form or known as oxirane ring are very unstable in its form especially in acidic condition due to performic acid used in earlier process [19].

Due to the high reactivity and instability, oxirane ring formed can also act as raw material for synthesis of variety of other chemicals. In this study, we focus on formation of DHSA.

For epoxidation of vegetable oil, two major steps are involve. Firstly, the formation of performic acid that act as an oxidizing agent towards the double bond in vegetable oils where hydrogen peroxide acts as an oxygen donor while formic acid as an oxygen carrier and would be regenerate after epoxidation had occur. Other than involve in formation of oxirane ring, formic acid also act as the reactant in the degeneration of oxirane ring along with water that formed by earlier process to produce DHSA[7]. The performic acid reaction as at Equation 2.2.

$$HCOOH + H_2O_2 \longleftrightarrow HCOOOH + H_2O \tag{2.2}$$

Peroxyacid or performic acid form transfer the furthest oxygen atom in their structure to the alkene of the vegetable oils through one-step mechanism without intermediates reaction. Due to electrophilic at oxygen, has an extra oxygen atom in their structure and its acidic hydrogen, performic acid are chosen as an oxidizing agent whereby it can displace carboxylate which is a good leaving group. The mechanism is essentially an electrophilic attack, with proton being transferred from epoxide oxygen to the carboxylic acid by product. Firstly, the nucleophilic π bond donates its electrons to the oxygen, breaking the O-O bond to form a new carbonyl bond. The electrons from the old O-H bond form a new C-O bond and the original carbonyl group uses its electrons to pick up the proton. The transition state of the reaction makes the bond formation and bond breaking processes much clearer. The whole epoxidation process involving the mechanism of electrophilic addition as shown in Figure 2.4.[7]

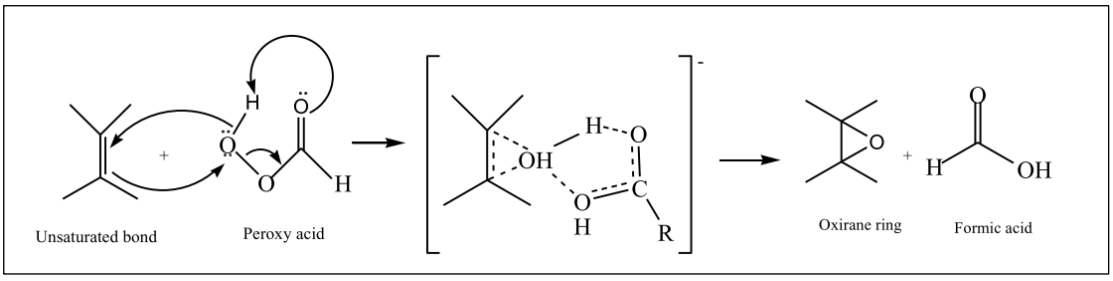


Figure 2.4 Mechanism of In-Situ Epoxidation Reaction

Epoxidation by in situ method are the most common method chosen by other researchers. The differences is usually type of oil used, catalyst either homogeneous or heterogenous. Finding of other researchers are tabulated in Table 2.3

Table 2.3
Finding of Other Researchers on Epoxidation

Author	Type of oil	Oxidizing agent	Catalyst	Finding
Turco R, et al [20]	Linseed and soybean	Performic acid	Phosporic acid	Ring opening for epoxidized linseed oil is four times higher compared to soybean oil due to reciprocal inductive effects exerted by the vicinal oxirane groups.
Nurazira et al [7]	Palm oil	Performic acid	Formic acid	86% epoxide yield are obtain. Oxidative stability, flash point and viscosity index increase for epoxidized palm oil, hence suitable for use in as industrial fluid.
Fatemah et al [21]	Soapstock	Hydrogen peroxide	c. regusa lipase	RCO at 7.86% show FFA content in soapstock have potential to be the cheapest raw material for epoxides. However, hydrogen peroxide inhibition are major limiting factor for higher epoxidation rate. Micro channel bioreactor able to speed up the reaction by increase the mass transport and decreasing biocatalyst deactivation.
Adriana et al [22]	Oleic acid	Peracetic acid	Dowex 50x8	Catalyst effect is more dominance for epoxidation rate compared to conventional and microwave heating. It is because the solid acid catalyst enhanced the slow step of the process, which is perhydrolysis of acetic acid.

2.2.2 Ex-Situ Epoxidation

Ex situ epoxidation refers to processes where the epoxidising agent or intermediate is formed and/or handled separately from the primary reaction environment to improve control over reaction conditions and product outcomes. In vegetable oil epoxidation, generating or isolating peracids outside the main reaction

phase allows better regulation of temperature, oxidant concentration, and reaction time, which can lead to higher oxirane yields and reduced side reactions such as ring opening or hydrolysis. Conventional epoxidation methods often involve peracid formation followed by transfer to the oil phase, and separating these stages can improve selectivity and ease of catalyst or reagent management compared to fully integrated processes. However, ***ex situ*** approaches tend to require additional steps, which increases complexity, processing time, and operational costs relative to direct methods. These trade-offs have driven interest in hybrid strategies that retain the selectivity benefits of ***ex situ*** control while reducing process steps and waste[2].

2.2.3 Hybrid In-Situ and Ex-Situ Hydrolysis

Hybrid hydrolysis integrates in situ and ex situ approaches to enhance the conversion of epoxidized vegetable oils into value-added hydroxylated products such as dihydroxystearic acid (DHSA). In this method, water is introduced during or after epoxidation to facilitate oxirane ring opening, combining the rapid reactivity of in situ hydrolysis with controlled optimization typical of separate ex situ processes. Studies on neem oil have shown that hybrid hydrolysis can significantly increase hydroxyl values compared to in situ hydrolysis alone, with parameters such as water addition timing, water-to-oil ratio, and reaction duration strongly affecting DHSA yield; optimal hybrid conditions achieved hydroxyl values up to 129.4 mg KOH/g, indicating more effective ring opening and product formation [3].

Previous research similarly highlights that integrating hydrolysis steps can improve process efficiency by utilising by-product water from peracid formation, reducing the need for additional reagents, and shortening overall processing time compared to traditional separate epoxidation and hydrolysis routes. Despite these advances, hybrid methods still require careful control of reaction conditions to balance epoxidation and hydrolysis without promoting undesired side reactions, underscoring the need for further optimisation in different vegetable oil systems[3].

2.2.4 Other Epoxidation Method: Transesterification

Transesterification is a chemical reaction where the alkoxy group in an ester is replaced by an alcohol. This process is typically facilitated by an acid or base catalyst.

It's a key step in the production of biodiesel from biological lipids [15]. Alkali-catalyzed transesterification are the most preferable method to produce biodiesel which consist of long fatty acid chain and considered as best alternative for a clean, renewable fuel. Transesterification enhanced by alkali where it react with free fatty acid and water which readily available in the raw materials, either vegetable oils or animal fats producing undesirable reaction. However, this can be reduce by pre treatment of the free fatty acid by esterification process with alcohol such as methanol [16].

2.2.5 Homogenous Catalyst

Homogeneous catalysts-those that operate in the same phase as the reactants, usually in solution-have played a crucial role in advancing synthetic chemistry. Initially used in acid/base reactions, the field expanded significantly with the introduction of transition metal complexes as found by [17] and palladium-based systems for carbon–carbon bond formation [18].

Because they interact at the molecular level, homogeneous catalysts offer exceptional selectivity and efficiency. The ability to fine-tune their behavior through ligand design enables precise control over reaction mechanisms [19]. These qualities make them particularly useful for asymmetric synthesis and intricate organic transformations. According to Swarup et al, mixture of acetic acid with with hydrogen peroxide produce the homogenous reactant in order to produce epoxide of oleifin with 1:1 molar ratio. Cyclopentene, on of compound other oleifin group also turns to its epoxide by using the same molar ratio 1:1 using acetic acid and hydrogen peroxide reactant [20]. Another study by Maxim et al conclude that bismulth salt are one of the homogenous catalyst that promise the conversion of olefin epoxide by reacting it with hydrogen peroxide along with bismuth salts by non radical epoxidation, where this pathway transfer the oxygen atom from hydroperoxide ligand to the olefin, hence replace the unsaturated bond which lead to epoxide formation. This method are favourable since it is low in toxicity, non corrosive and functional well in aqueous solution [21].

2.2.6 Heterogenous Catalyst

Heterogeneous catalysis is crucial in the chemical industry because of its practical benefits, including easy separation from reaction mixtures, reusability, and compatibility with continuous operations. In contrast to homogeneous catalysts, heterogeneous catalysts are typically in a different phase than the reactants, most often solid catalysts interacting with liquid or gaseous reactants. For heterogeneous catalysis, reactions generally occur at active sites located on the surface of a solid catalyst, where reactant molecules are adsorbed and undergo transformation. The effectiveness of the catalyst is largely influenced by factors such as its surface area, porosity, and the specific characteristics of its active sites [22].

Recent studies have concentrated on nanostructured catalysts, single-atom catalysts, and metal-organic frameworks to improve performance by achieving greater precision in controlling active sites and catalyst structure. Heterogenous catalyst play an important role in various industry such as petrochemical engineering, environmental applications, renewable energy and biomass conversion [23]. Titanium silicate -1 which is titanium substituted silica zeolite are one of the catalyst that convert alkene to its epoxide by using hydrogen peroxide as its oxidant. The advantage are commonly such as high selectivity, can react in mild temperature, (30 to 70^0C) and the most interesting parts is its economical features where it can be recycle and stable [24].

In automotive sector, heterogenous catalyst plays an important role where platinum (Pt) being a critical component in catalytic converters which designed to reduce harmful emission as a product from engine combustion by facilitate the oxidation of carbon monoxide and hydrocarbons and the reduction of nitrogen oxides in exhaust gases which take place on the surface of (Pt). Usage of platinum in the converter surely give an impact towards reducing the impact on gas emission by transportation sector and aiding in adherence to the environmental regulations [25]

2.2.7 Catalyst Reusability Test

The ability to reuse a catalyst is a key factor in evaluating the sustainability and economic viability of a heterogeneous catalyst. For industrial and environmental use, the ideal catalyst is one that consistently retains its activity, selectivity, and structural stability across multiple reaction cycles. The most practice method in catalyst

reusability is filtration and centrifugal, where it can directly separate solid catalyst from the reaction mixture to be reused. Filtration uses porous media to trap catalyst particles, whereas centrifugation relies on centrifugal force to to separate them according to their density. Both methods are simple, effective, and commonly applied in both laboratory and industrial environments.

A study conduct by Kalita et al prove that $Ni.Al_2O_3$ can be used several times without decrease in terms of efficiency for synthesis of naphthopyrans where the catalyst exhibit high activity even after recovery and used multiple times thus proving that it is one of the sustainable processes [26]. Another example of heterogenous catalyst reusability are usage of sulfonated carbon catalyst in biodiesel production from palm fatty acid distillate (PFAD). First of all, palm seed cake need to be sulfonated with pure sulphuric acid to enhance its acidity by introducing sulfonic acid. Biodiesel from PFAD then produce by reacted it with methanol and the prepared at catalyst with 2.5wt% PFAD used at 60^0C for 2 hours. After eight cycle of reusability, the biodiesel yild decrease up to 78% due to decrease of acidity from 12.08($\frac{\text{mmol}}{\text{g}}$) to 9.17$\left(\frac{\text{mmol}}{\text{g}}\right)$. Meanwhile, BET analysis show decrease in surface area from 483.07 ($\frac{\text{m}^2}{\text{g}}$) to 412.56 ($\frac{\text{m}^2}{\text{g}}$) and FTIR analysis shows decreasing in peak for intensity of sulfonic acid which clearly indicating the leaching of active sites. However, before every reused, catalyst can be re sulfonated back to regain its acidity [27].

2.2.8 Performic Acid as Oxidizing Agent

During in-situ epoxidation process, oxidising agent are needed where it act as oxygen donor and carrier. Both performic acid and peracetic acid are commonly used for this process. Performic acid are form by mixing formic acid with hydrogen peroxide while peracetic acid is a mixture of acetic acid an hydrogen peroxide. Both formic and acetic acid act as an oxygen carrier while hydrogen peroxide act as oxygen donor for epoxidation reaction. However, the choice of either formic acid or acetic acid as an oxygen carrier are depending on the researchers other parameter and condition involves for epoxidation. According to Turco et al, due to instability of peracetic acid at higher reaction condition at above 60^0C, performic acid is chosen as the oxidising agent [13].

2.2.9 Amberlite IR 120H

Amberlite IR 120H resin is a strong acidic cation exchange resin in hydrogen form which manufactured as gel- type bead form with a sulfonated polystyrene divinylbenzene (PS-DVB) matrix and have high affinity for cations like magnesium, calcium and heavy metals and beneficial as catalyst where it can turn them into hydrogen ion in a solution. It facilitate more by character of amberlite itself which are high exchange capacity, react effectively in wide PH ranging from (0-14) which make it suitable for various condition especially for water treatment. Insoluble in aqueous solution is one of the winning factor in cost optimization where it would be easily separated and reuse for several times for the same reaction.

In epoxidation, amberlite IR 120H act as a strong acid cation exchange resin and catalysed the epoxidation process by providing a solid acidic environment where it protonates hydrogen peroxide thus generating more reactive electrohlic species. Double bond of unsaturated oleic acid react with the activated peroxide produce from reaction of amberlite IR 120H and hydrogen peroxide earler, forming oxirane ring or well known as epoxide. Mechanism of oleic acid epoxidation are as below.

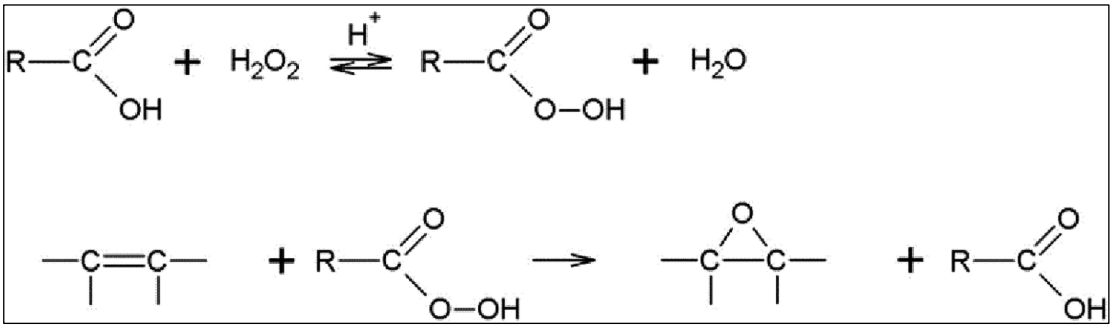


Figure 2.5 Mechanism of Oleic Acid Epoxidation

2.3 Oxirane Ring Opening

Epoxide or known as oxirane are three membered ring structure where one of the vertices is an oxygen and another two are carbon. Strain created by the three - membered ring make epoxide behave differently from other esters. Epoxide are much more reactive compared to other esters due to the fact that ring strain is relieved when there is ring opening upon nucleophilic attack as shown in Figure 2.6 below [17].

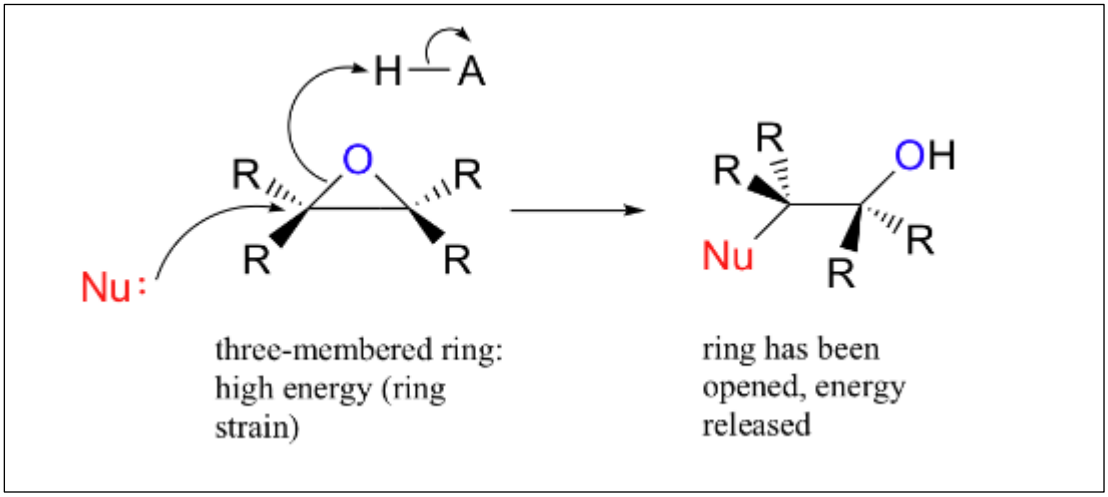


Figure 2.6 Nucleophilic Attack on Oxirane Ring [17]

Sylwia, et al. [23], in their study in synthesis of polyols from epoxide of rapeseed oil had implemented the nucleophilic attack to the epoxide by using peracid mechanism during the epoxidation process. Presence of water, mineral and organic acid in the reaction due to the intermediate of peracid formation further hydrolyse the oxirane ring of rapeseed oil into becoming polyols. However, the reaction is done with microwave as heating mechanism compared to conventional heating to reduce time and energy saving purpose. Due to reactivity of oxirane ring and by product obtain from earlier process of epoxidation, opening of oxirane ring can be develop into several new products. Figure 2.7 shows several product that can be formed prior to opening of oxirane ring [24].

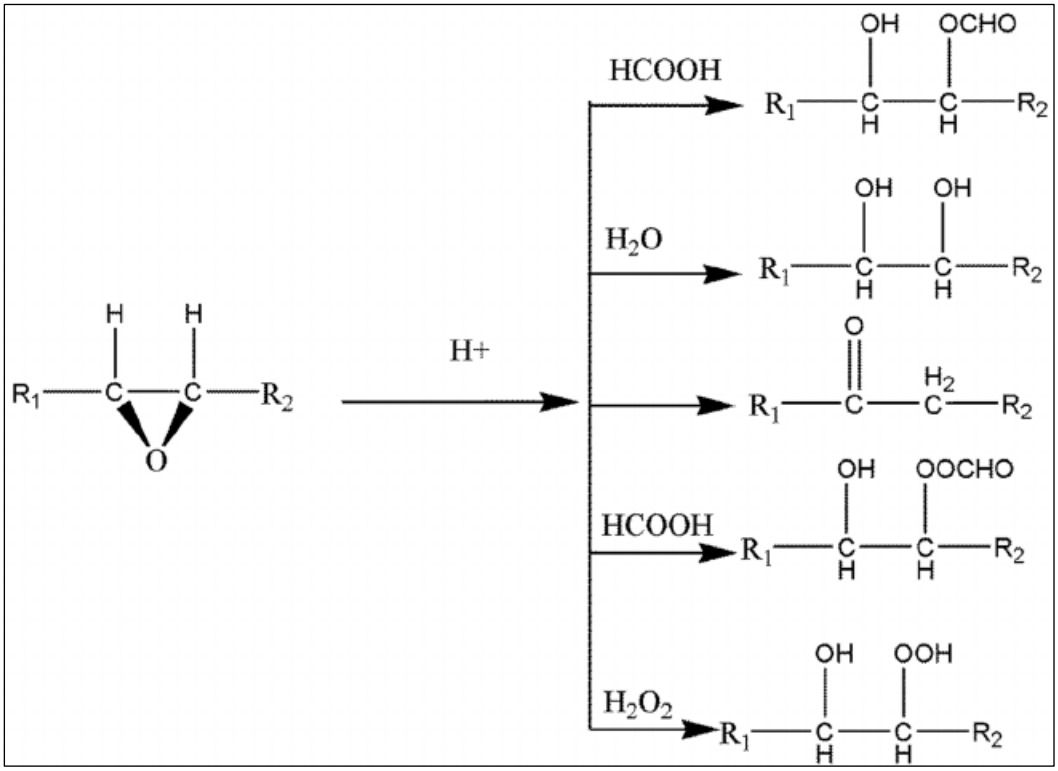


Figure 2.7 Possible Side Reaction During Oxirane Ring Opening [24]

Ring opening, also known as fusion process of epoxide that had been studied by other researchers with different area of research, in terms of reactant used for ring opening, type of epoxidized used and their own findings are tabulated in Table 2.4

Table 2.4
Finding of Other Researchers on Ring Opening

Author	Epoxide	Ring opening reactant	Finding
Sienkiewicz et al [25]	Palm oil	Bisphenol A (BPA)	Hydroxyl group of BPA to palm oil epoxide initiate the reaction of free phenolic group followed by macromolecule of modified oil, hence higher possibility to obtain new epoxy polyurethane materials.
Sylwia et al [23]	Rapeseed oil	Diethylene glycol (DEG)	Polyol with OH values between 114 to 196 mg KOH/g were obtained. Partially substitution of petroleum polyol with rapeseed polyol increase the cellular structure and tensile strength for the formation of flexible polyurethane form.

Author	Epoxide	Ring opening reactant	Finding
Salimon J et al [26]	Linoleic acid	Oleic acid with *p*-toluene sulfonic acid	Hydroxyl ester, which can use to produce biolubricant are successfully obtained, where one oxirane ring present in epoxidized linoleic acid would one each of ester and hydroxyl group would be generate as the final product.
Ren Q et al [27]	Biodiesel	2-ethyl hexanol with amberlyte as catalyst	Ring opening product have better viscosity, higher flash point, and excellent tribological properties compared to epoxidized biodiesel.

2.4 Dihydroxystearic Acid (DHSA)

The oxirane rings' instability offers possibilities for further chemical modification. One of these potential chemical modifications is the conversion of the oxirane rings to dihydroxystearic acid (DHSA) by further hydrolysis and DHSA structure as in Figure below [19].

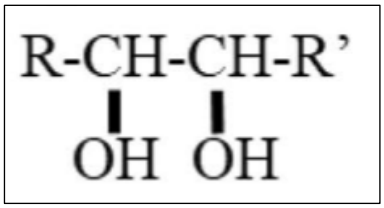


Figure 2.8 DHSA [19]

DHSA is one of the hydroxyl fatty acid that contain three functional group that made up by one carboxyl and two hydroxyl functional group. DHSA are typically produce by further hydrolysis of epoxide product from chosen vegetable oil, with water from by product of the oxidizing agent for epoxidation process or known as peracid mechanism. Figure 2.9 show structure of DHSA [28].

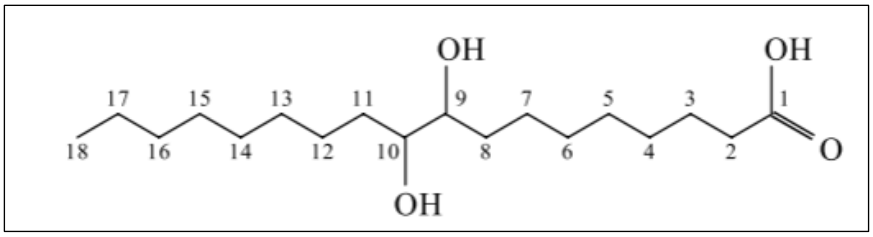


Figure 2.9 DHSA Structure [28]

According to Rafizan et al [29], epoxidation of palm kernel oil based oleic acid was carried out by reacting the oleic acid with performic acid which form by the mixture of formic acid with hydrogen peroxide. Reaction of carbon to carbon double bond with active oxygen from performic acid would result in converting the original double bond into a three-membered epoxide or oxirane ring. However, this research does not produce DHSA by in-situ hydrolysis because hydrolysis for the formation of DHSA was conducted by pre-prepared of epoxidized crude oleic acid.

Compared to study by Hadi et al [30], where the production of DHSA was done in-situ whereby the epoxidation of palm oleic acid was done by peracid acid as the oxidising agent, and epoxide oleic acid sample was taken every 5 minutes to measure the oxirane oxygen content (OOC). Epoxide form are let to further undergone hydrolysis in order to produce DHSA. Meanwhile, research done by Zahariah et al [31] is the comparison by using two different oxidising agent which are peracetic acid which form by the mixture of acetic acid and hydrogen peroxide and performic acid. Production yield of DHSA show the performic acid used give 96% with 30% cheaper cost compared to peracetic acid with production yield 72.5%. Study done by Hanisah et al [28] prove that DHSA can be produce by using oleic acid as the source for unsaturated fatty acid while using performic acid after purification method where the mixture is cooled at room temperature and precipitated that believed are DHSA are wash with ethanol to remove the residue and rinse with cold water. After dry under vacuum, the yield obtain are 85%. DHSA have distinct qualities for a variety of uses, including emulsifiers in oil phase/gel candles and water in cosmetic formulations [32]. Applications of DHSA in personal care finish product as in Table 2.5 below.

Table 2.5
5 Application of DSHA in Personal Care Product

Personal Care Products	**Application / DHSA Functionalities**
Lipsticks	Increase its mechanical properties. Lipsticks coated with DSHA possessed higher breaking point.
Liquid foundation	Gave better colour development and ease for spreading on the skin.
Mascaras	Added volume to eyelashes, hence better curling effects.
Compact powders	As a binding agent, hence increase it adhesion properties to the skin and was found to be easily distributed when applied on the skin.
Metal soaps	Sodium soaps of DHSA had better detergency effect than stearic soap at room temperature and were more biodegradable.

An in situ process describes a method or reaction that occurs directly within its original or intended environment, without extracting or separating the components from the system. Research done by Zulkifli et al proves that DHSA can be produced by in situ hydrolysis of palm based oleic acid by using both performic and peracetic acid where the result is varied when using different vegetable oil as raw material [29].

Meanwhile, Jumain et al found that DHSA can be formed with high yield as 80.7% by using this method, where the most optimum parameter temperature at 75^0C, ratio formic acid/oleic acid 1.0, hydrogen peroxide/oleic aicd 1.5 and stirring speed at 300RPM [30]. The in situ hydrolysis of epoxidized vegetable oils particularly those derived from palm oil offers an effective and environmentally friendly approach to synthesizing DHSA. To improve both the yield and purity of the final product, it is essential to fine-tune the reaction conditions and gain a clear understanding of the process kinetics. This makes DHSA a highly useful compound for a wide range of industrial uses.

2.5 Taguchi Optimization

Taguchi method are statistically method develop to improve quality of goods manufacture by eliminate the variability which cost many resources and this method are develop by Genichi Taguchi. Main purpose of taguchi method is to design the robust system, which are reliable under uncontrolled condition where it aims to adjust the design parameters to their optimum levels [33]. In the Taguchi method, the goal is to minimize the variability in the output of a process so that the quality of the product produced is consistent and predictable. Carefully selecting the process parameters that have the greatest impact on the product quality and then conducting experiments to determine the optimal values for these parameters do this.

The Taguchi method uses statistical analysis to identify the factors that contribute most to the variability in the output and then optimizes the process so that these factors are minimized. This method had been applied to many different types of products and processes, including electronics, automobiles, and consumer goods. It has been widely adopted in industry because of its ability to provide efficient and effective solutions to quality control problems, and it is considered a valuable tool for quality engineers and practitioners.

Based on Taguchi, the best opportunity to eliminate the unwanted variation is during the design of product and manufacturing process. Hence, three steps of process strategy are developed in order for quality engineering where the first step is system design, where the design is at conceptual level, involving creativity and innovation. Secondly, parameter design. Once the concept is established, the nominal values of the various dimensions and design parameters need to be set, the detail design phase of conventional engineering. This is sometimes called robustification. Lastly, tolerance design. With a completed parameter design and understanding of the effect of various parameter on performance, resources can be focused on reducing and controlling variation in the critical few dimensions.[33]

2.5.1 Orthogonal Array and ANOVA Analysis

Orthogonal array significantly reduce the number of experimental configuration to be studied which are likely to be implement in manufacturing industry since it would reduce the cost. The effect of many different parameters on the performance characteristic in a process can be defined by using orthogonal array experimental design proposed by Taguchi. Once the parameters affecting experiment that can be controlled had been determined, the levels at which these parameters should be varied must be determined, including its variables level.

Rao *et al* [34] had use L_9 orthogonal array to observe the degree of influence of the process parameters in electrochemical machining for metal recovery rate as a result. The following Table 2.6-2.7 show the process parameter involve followed by the L_9 orthogonal array.

Table 2.6
Electrochemical Machining (ECM) Process Parameters [31]

Parameter	**Code**	**Levels**		
		1	**2**	**3**
Applied voltage (V)	A	12	16	20
Feed rate (mm/min)	B	0.1	0.2	0.3
Electrolyte concentration (g/litre)	C	10	20	30

Table 2.7
Taguchi L9 OA for Metal Removal Rate (MRR) [31]

Experiment No	A	B	C	MRR(g/min)
1	1	1	1	0.0559
2	1	2	2	0.0789
3	1	3	3	0.1023
4	2	1	2	0.0765
5	2	2	3	0.0987
6	2	3	1	0.1012
7	3	1	3	0.0923
8	3	2	1	0.1005
9	3	3	2	0.1200

ANOVA is a statistically based, objective decision making tool for detecting any differences in the average performance of groups of items tested. ANOVA help in identifying the significance of all the main factor and their interaction by comparing the mean square against an estimate of the experimental errors at specific confidence levels. First, total sum of squared deviation SS_T from the total mean S/N ratio n_m can be calculated as Equation 2.3.

$$SS_T = \sum (n_i - n_m)^2 \tag{2.3}$$

Where n is the number of experiments in the orthogonal array and n_i is the mean S/N ratio for the ith experiment. The percentage contribution of P can be calculated as Equation 2.4.

$$P = \frac{SS_d}{SS_T} \tag{2.4}$$

Where SS_d is the sum of squared deviation. The ANOVA result as per Rao *et al* research are as Table 2.8.

Table 2.8
ANOVA Results for Metal Removal Rate for Electrochemical Machining (ECM) [31]

Source of Variation	Sum of squares (S)	P value (P)	Percentage (%)
A	0.0009555	0.009	34.04
B	0.0016305	0.005	58.09
C	0.0002124	0.038	7.57

From the result, percent (%) is defined as the significance rate of the process parameter on metal removal rate. The percent number shows that (A), (B), and (C), effect the MRR by 34.04%, 58.09% and 7.57% respectively [34].

2.6 Kinetic Modelling

Kinetic modeling is a branch of mathematical modeling that deals with the study of the rate of change of a system's behaviour. It is used to describe the time evolution of physical, chemical, biological, and engineering systems and can provide a quantitative understanding of the underlying processes and mechanisms. In kinetic modeling, mathematical equations are used to describe the relationships between the concentrations or amounts of different components in a system and the rate at which they change over time. The equations are derived from the fundamental laws of physics and chemistry and can include reactions, transport processes, and other phenomena. The model can then be used to simulate the behaviour of the system under various conditions and make predictions about future behaviour.

Kinetic modeling of the epoxidation of oleic acid involves the development of mathematical models to describe and predict the behaviour of the reaction as a function of time and reaction conditions. These models can provide insight into the mechanism and rate-determining steps of the reaction, as well as the effects of variables such as temperature, concentration, and catalyst type. Several kinetic models have been proposed for the epoxidation of oleic acid, including single and multiple-step models, as well as models based on various reaction mechanisms, such as free radical and ionic mechanisms. The choice of model depends on the specific reaction conditions and the information required. The most common approach for developing a kinetic model is to perform a series of experiments under controlled conditions and to measure the reaction rate as a function of time.

The data from these experiments can then be fit to a kinetic model, either through nonlinear regression or through the use of computational optimization techniques. The accuracy of the model can be validated through comparison with additional experimental data and by performing sensitivity analysis to examine the effect of varying the model parameters. Kinetic modeling of the epoxidation of oleic acid can provide valuable information for further process optimization, reaction design, and scale-up. It can also be used to develop predictive tools for monitoring and controlling the reaction, as well as to improve our understanding of the underlying reaction mechanisms.

Matrix Laboratory or known as MATLAB is a computer aided engineering software that can be used as a solvers for ordinary differential equations (*ode*s). This software is equipped with various tools for technical computing, statistical analysis, data analysis optimization and solving partial different equations. The MATLAB *ode* solver computes the solution at each time step within the predefined time interval. There are various *ode* solver available in MATLAB and the description for each type as per Table 2.9. *Ode* 45 solver is typically use to compute the ode.

Table 2.9
Type of Ode Solver and Their Description

***ode* solver**	**Description**
ode 45	A one –step solver based on the explicit fourth-order and fifth-order Runge-Kuttha method
ode 23	A solver that provides a more efficient crude tolerance based on the second-order and third-order Runge-Kuttha method.
*ode*113	A multistep solver that typically requires the solutions at several preceding time points.
*ode*15s	A solver that uses the backward differentiation formula
*ode*23s	A one step solver that has a more efficient crude tolerance than the *ode* 15s solver
*ode*23t	A solver that use the trapezoidal rule and “free” interpolant

Ode 45 is a numerical integration method used in MATLAB to solve ordinary differential equations (*odes*). It is a variable-step, variable-order method that uses a combination of explicit Runge-Kutta methods and adaptive step-size control to accurately solve *odes* with a high degree of accuracy. In the context of kinetic modeling,

ode45 can be used to solve the differential equations that describe the time-dependent behaviour of a chemical reaction system. To use *ode45* to solve a set of *odes*, you need to define the function that describes the right-hand side of the *odes*, specify the initial conditions for the system, and call the *ode45* function with the desired time range and integration options. The function will then return the numerical solution for the *odes* over the specified time range. In the context of kinetic modeling, the use of *ode45* can be an effective way to obtain solutions for complex reaction systems, as it allows to account for multiple reactions, multiple species, and other factors that can impact the behaviour of the system over time.

However, it is important to carefully validate the *odes* and the numerical solution obtained using *ode45*, as small errors in the model or in the numerical solution can lead to significant inaccuracies in the results. The *ode* 45 solver is a one-step solver, which only requires the solution immediately at the preceeding time point other than relatively easy to simulate and generate the results. Thus, the *ode* 45 solver is best used as a first option for most problems.

La ifa *et al*, [24] with their research title study on the "kinetics of epoxidation of refine, bleach and deodorize (RBD) palm olein" to studying the effect of reaction time, temperature and the type of catalyst on oxirane number and determining the reaction kinetics data. Results show bentonite catalyst optimally working at 70^0C temperature for 4 hours with oxirane level 1.02% while H_2SO_4 was optimally working at temperature 60^0C for 3 hours with oxirane level 2.31%. Meanwhile, activation energy (Ea) and frequency factor (A) for the reaction rate equation approximately as at Table 2.10 and the overall epoxidation reaction order is a pseudo first order and H_2SO_4 is more optimum as a catalyst due to the higher value of EA compared to bentonite. Reaction rate formula as Equation 2.5.

$$-r_{A=KCA} \tag{2.5}$$

2.6.1 Kinetic Modelling Epoxidation

Modeling the kinetics of vegetable oil epoxidation, especially for oleic acid is crucial for refining reaction conditions, increase yield, and promotes it for industrial scale production. This process involves unsaturated fatty acids reacting with peracids

to produce epoxides. A solid grasp of the reaction kinetics helps manage key factors like temperature, reactant levels, and catalyst performance in order to promote high yield of epoxide [25].

Kinetic models for epoxidation reactions are generally derived from experimental observations and serve to predict reaction rates under different conditions. These models typically account for variables like temperature, concentrations of reactants, and catalyst presence. Study by Cai et al [32] found that the epoxidation of soybean oil using peracetic acid follows first-order kinetics in relation to the peracid concentration. The reaction's activation energy has been estimated at approximately 43.11 kJ/mol, suggesting a moderate sensitivity of the reaction rate to temperature changes. In addition, Gustavo et al had conclude that the speed of the epoxidation reaction is affected by several factors, such as temperature, reactant levels, and the use of solvents or catalysts. Typically, raising the temperature speeds up the reaction by supplying more energy to surpass the activation energy threshold. Likewise, increasing the concentration of peracetic acid can enhance the reaction rate; however, excessively high levels may trigger unwanted side reactions or cause the peracid to break down [33].

2.6.2 Kinetic Modelling for DHSA Production

An in-depth literature survey on kinetic modeling approaches for dihydroxystearic acid (DHSA) synthesis underscores an expanding research emphasis on process intensification, particularly through the detailed mechanistic study of oleic acid epoxidation and subsequent hydrolysis pathways, utilizing feedstocks derived from renewable vegetable oil sources. The reaction rate constant, (k) define as the reaction time of the process at the reaction temperature and proportionally constant with the rate law of chemical reaction.

Arif et al had done research on determining the reaction kinetics of DHSA production from different source of vegetable oils such as palm olein, palm kernel oil and sunflower oil at optimum condition of reaction which rely on previous study for each vegetables oils. They found that the kinetic modelling of oxirane content and reaction rate regression line, R^2 = 0.935 that prove DHSA produce in this study supported by kinetic intrepretation [29]. Meanwhile, Abdul et al find the reaction kinetic through kinetic modelling because it is important used for reactor modelling. The literature indicates that kinetic parameter estimation is highly sensitive to numerical

fitting techniques, and inadequate convergence has frequently been identified as a limitation in determining reliable reaction rate constants (k).this issue had been tackled by using the reaction rate constant, k from previous study where it is applied in Arhenius plot to determine the activation energy (Ea). From (Ea) determined, it can be concluded that DHSA production is slow process due to high (Ea) need for every reaction to occur which make the reaction under kinetic limited regime and free from mass transfer coefficient and strong recommendation from researcher is fitting needs to be performed with different methods [34]. Jumain et al implement Rungge Kutha 4^{th} order method with genetic algorithm optimization to determine the process model that fit with experimental data using MATLAB software. Reaction rate for DHSA production by optimization of epoxidized palm oleic acid are obtained where there is a good agreement between simulation and experimental data which validate the kinetic model and strongly supported by high yield 80.7% and 98.5% DHSA was produced [35].

2.7 Concluding Remarks

This chapter has provided an in-depth review of neem oil, its extraction methods, physicochemical properties, and diverse applications ranging from traditional medicine to industrial use. Particular emphasis was placed on the potential of neem oil as a renewable feedstock due to its rich unsaturated fatty acid composition, making it suitable for epoxidation reactions. A comprehensive discussion of epoxidation methods including in situ processes, homogeneous and heterogeneous catalysis, and catalyst reusability highlighted both the opportunities and limitations of each approach. The instability and reactivity of the oxirane ring were shown to be central to the subsequent conversion into value-added compounds, particularly dihydroxystearic acid (DHSA), which has wide industrial relevance in cosmetics, lubricants, and polymers.

Furthermore, the review explored optimization approaches such as the Taguchi method, which provides a systematic framework to reduce experimental complexity while ensuring process robustness. Kinetic modelling was also examined as an essential tool for understanding reaction mechanisms, predicting performance, and enabling process scale-up from laboratory to industrial settings.

Overall, the literature establishes neem oil as a promising but underutilized non-edible oil for epoxidation and DHSA production. However, gaps remain in areas such as reaction kinetics, optimization strategies, and large-scale feasibility. Addressing

these gaps forms the foundation of the present study, which aims to optimise reaction parameters, introduce a hybrid hydrolysis approach, and develop reliable kinetic models for the epoxidation of neem oil and subsequent DHSA synthesis.

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Overall Research Methodology

The materials and procedure to produce DHSA from epoxidized neem oil will be briefly explained in detail in this section. The synthesis of the DHSA route consists of three parts: (1) Obtaining the design experiment needed for five parameters involved, each having four levels. (2) Obtain the relative conversion oxirane (RCO) from each design experiment and optimize it with the Taguchi method. (3) Proceed with hybrid ex-situ and in-situ hydrolysis of epoxidized neem oil with additional water.

In this study, the epoxidation process of neem oil involves 5 parameters, each having 4 levels. However, there are too many combinations of parameters and levels to be matched to obtain the highest epoxidation rate of neem oil. Hence, Taguchi's experiment (DoE) design is used to analyse the best parameter combination and their levels to obtain the highest RCO. Experiments are set up accordingly, and the oxirane oxygen content (OOC) is measured by titration with hydrobromic acid (HBr) to get RCO.

RCO obtained from a list of experiments suggested by Taguchi is used to find the most optimum parameter for the epoxidation of neem oil. An experiment is set up accordingly, and the titration is done every 5 minutes until it gets the highest RCO and proceeds until it decreases back to almost zero, where the oxirane ring starts to degenerate, or the bond breaks, where DHSA start to form. A sample from the highest RCO is taken and analysed for its physicochemical properties. The experiment is repeated with different levels of one parameter each, while other optimum parameters with their levels are maintained.

Optimum parameters with their levels are set up once again. This time, water is added when the oxirane ring degenerates for an additional OH group to promote the ring opening and higher DHSA formation rate. DHSA form later to be purified and analysed for its physicochemical properties. Kinetic modelling is further used to obtain the rate of reaction, activation energy, and enthalpy of activation of epoxidation of neem oil. The process flow diagram for the whole study is simplified in Figure 3.1

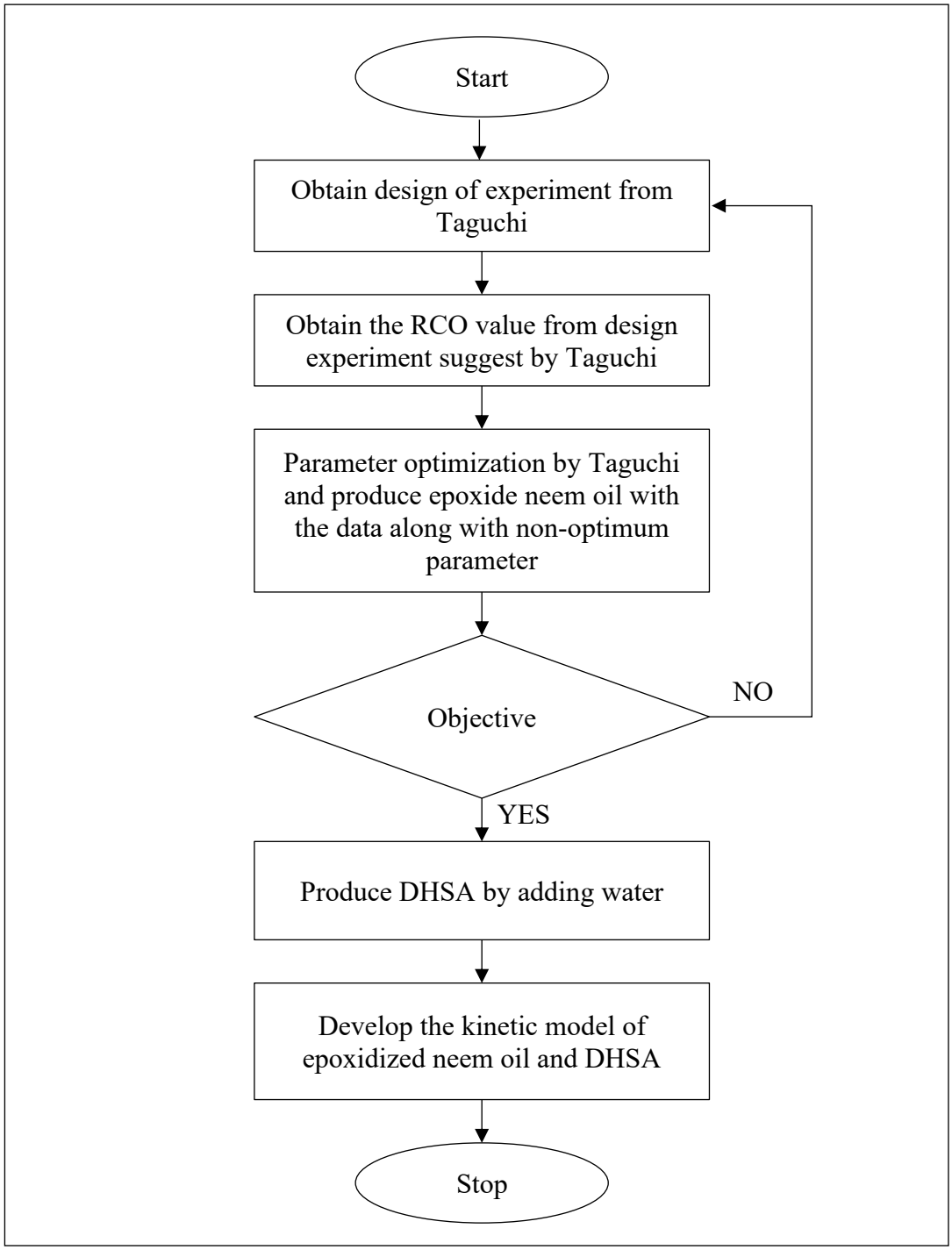


Figure 3.1 Process Flow Diagram

3.2 Materials

Neem oil, Hydrogen peroxide 50%, formic acid 85%, glacial acetic acid, and hydrobromic acid are supplied by Qrec; meanwhile, amber lite IR 120H catalysts are by Supelco as summarised in Table 3.1. Neem oil is composed primarily of fatty acids, with a significant portion being unsaturated, making it suitable for reactions like

epoxidation. The major fatty acids in neem oil include oleic acid (40%), linoleic acid (11%), and palmitic acid (45%).

Table 3.1
List of Chemicals for the Production of DHSA

Materials	**Range/Amount**	**Purity/Molarity**	**Supplier**
Neem oil	100g		
Hydrogen peroxide molar ratio	1,2,3,4,	50%	Qrec
Formic acid molar ratio	1,2,3,4	85%	Qrec
Glacial acetic acid	10ml		Qrec
Hydrobromic acid	4ml		Qrec
Amberlite IR 120H	1g		Supelco

3.3 Experimental Setup and Procedure for the Production of Epoxidized Neem Oil

100g neem oil is heated to the desired temperature. Peracid form, along with the suggested weight of the catalyst, is then mixed into the neem oil when the desired temperature is reached. Stirring speed is maintained accordingly throughout the formation of epoxide. This reaction is exothermic, and the temperature would be risen slightly earlier in the experiment. Hence, performic acid and catalyst must be added slowly to the heated neem oil. A suitable size of beaker needs to be chosen to reduce the losses of the mixture since there would be a bubbling during usage of high-level hydrogen peroxide and performic acid, and the experiment must strictly be done in the fume hood due to the high toxicity of formic acid if inhaled.

3.4 Optimization by Taguchi Method (Epoxidation)

The levels of neem oil, formic acid, and hydrogen peroxide were selected based on the degree of unsaturation in the crude oleic acid, as only unsaturated fatty acids contribute to the epoxidation reaction. Reactant ratios were therefore defined relative to the available double bonds rather than the total oil mass. The selected levels represent a gradual increase from near-stoichiometric conditions to excess formic acid and

hydrogen peroxide, allowing evaluation of their effects on epoxide formation, oxidant efficiency, and potential side reactions. These ranges were chosen to cover conditions commonly reported in the literature while remaining within practical and safe operating limits, as tabulated in Table 3.2.

Table 3.2
Mass of Reactant and Its Level

Reactant	**Unsaturation Molar Ratio**	**Mole**	**Mass (g)**	**Level**
Neem oil	1	0.354	100	
Formic acid	0.5: 1	0.177	8.147	1
	1:01	0.354	16.29	2
	1.5: 1	0.531	24.44	3
	2.0:1	0.708	32.59	4
Hydrogen peroxide	0.5: 1	0.177	6.01	1
	1:01	0.354	12.06	2
	1.5: 1	0.531	18.04	3
	2.0: 1	0.708	24.07	4

In this study, the experiment (DoE) design will be applied using the Taguchi design, where the process parameters are optimized to minimise the number of experimental runs to get optimum production of epoxidized neem oil or oxirane ring. The epoxidation parameters that would be investigated are formic acid molar ratio to neem oil (A), hydrogen peroxide molar ratio to neem oil (B), catalyst loading (C), reaction temperature (D) and stirring speed, RPM (E). These parameters were investigated using different levels. For hydrogen peroxide and formic acid, the levels are the molar ratio against neem oil variation, the amount of catalyst each experiment set up, temperature variation and the stirring speed from high to lowest, as tabulated in Table 3.3. Meanwhile, for the level of each parameter is supported by previous researcher [3].

Table 3.3
Parameter with Its Level [36]

Parameter	Level			
	1	**2**	**3**	**4**
Formic acid /neem oil molar ratio, (A)	1	2	3	4
Hydrogen peroxide/neem oil molar ratio, (B)	1	2	3	4
Catalyst loading (g), (C)	0.5	1	1.5	2
Reaction temperature (°C), (D)	40	50	60	70
Agitation speed (RPM), (E)	300	400	500	600

Each DoE suggested by Taguchi would be set up accordingly to produce an epoxide of neem oil. RCO obtained for each experiment set up were then further analysed based on the Signal to Noise ratio (S/N) and Analysis of variance to determine the most optimum and reliable level for each parameter towards epoxidation of neem oil. The design of the experiment by Taguchi is tabulated in Table 3.4.

Table 3.4
Design of Experiment by Taguchi [36]

No	Formic acid/neem oil molar ratio (A)	Hydrogen peroxide/neem oil molar ratio (B)	Catalyst (C)	Temperature (D)	Stirring speed (E)
1	1	1	1	1	1
2	1	2	2	2	2
3	1	3	3	3	3
4	1	4	4	4	4
5	2	1	2	3	4
6	2	2	1	4	3
7	2	3	4	1	2
8	2	4	3	2	1
9	3	1	3	4	2
10	3	2	4	3	1
11	3	3	1	2	4

No	Formic acid/neem oil molar ratio (A)	Hydrogen peroxide/neem oil molar ratio (B)	Catalyst (C)	Temperature (D)	Stirring speed (E)
12	3	4	2	1	3
13	4	1	4	2	3
14	4	2	3	1	4
15	4	3	2	4	1
16	4	4	1	3	2

RCO obtained from each set-up is further analysed by Taguchi to get the most optimum set-up for the epoxidation of neem oil. Later, the optimum parameter with its level is used to set up the epoxidation process. Here, the sample at the highest RCO is taken as it is supposed to be the highest concentration of epoxidized neem oil compared to the other 16 sets of set-up previously and would be sent for analysis such as Fourier Transform Infrared (FTIR), Deterioration of Bleachability Index (DOBI), iodine value, hydroxyl value, moisture content. The effect of other levels that are at lower priority as design by Taguchi method towards neem oil epoxidation for each parameter will also be studied. This can be achieved by setting up an experiment by substituting the non-optimum level for each parameter while maintaining another parameter at the optimum level.

Table 3.5
Effects of Parameter Levels with Lower Priority in the Taguchi Analysis

Experimental run	Water (g), (F)	Time to add water (min), (G)	Time to stop (Hr)
1	5	80	2
2	5	90	3
3	5	100	4
4	5	110	5
5	10	80	3
6	10	90	2
7	10	100	5
8	10	110	4

Experimental run	Water (g), (F)	Time to add water (min), (G)	Time to stop (Hr)
9	15	80	4
10	15	90	5
11	15	100	2
12	15	110	3
13	20	80	5
14	20	90	4
15	20	100	3
16	20	110	2

3.5 Analytical Technique

3.5.1 Relative Conversion Oxirane (RCO)

Every 5 minutes, 3 grams of the sample will be titrated with 10ml of acetic acid and crystal violet with HBr for oxirane content analysis according to the AOCS Tentative Method Cd 957. Samples are titrated until the highest RCO is obtained, and the epoxidation continues until RCO decreases, where the formation of DHSA happens and finally stops at 120 minutes. During titration, the colour changes from blue to yellow, the turning point of oxirane ring formation. The volume of HBr used and the sample weight is needed to obtain the OOC experimental; hence, the RCO can be obtained.

The experiment was conducted to determine the RCO by obtaining the OOC experimental. OOC was obtained by using a direct titration of HBr solution to indicate the degree of unsaturation in the palm oil to determine the OOC experimentally. The RCO, Equation 3.2, is determined based on the OOC value, which is theoretically Equation 3.3 and experimentally determined in Equation 3.4.[36]

$$\mathrm{RCO}= \frac{\mathrm{OOC}_{\mathrm{experimental}}}{\mathrm{OOC}_{\mathrm{theoretical}}} \times 100 \tag{3.1}$$

$$OOC_{theoretical}=\left\{\left(\frac{X_0}{A_i}\right)/\left[100+\left(\frac{X_0}{2A_i}\right)(A_o)\right]\right\}\times A_o \times 100 \quad (3.2)$$

$$OOC_{experimental}=1.6 \times N \times \frac{(V\text{-}B)}{W} \quad (3.3)$$

Here, X_0 is the initial iodine value, A_i is the molar mass of iodine, A_o Is the molar mass of oxygen, N is the normality of HBr, V is the volume of the HBr solution used for the blank in millilitres (mL), V is the volume of HBr solution used for titration, and W is the weight of the sample.

3.5.2 Fourier Transform Infrared (FTIR)

FTIR spectroscopy is a widely used analytical technique for analysing solid and liquid samples. The method works by shining an IR light through the sample and measuring the absorption or transmission of the light. FTIR is an important technique that provides an easy way to identify certain functional groups in an organic molecule by identifying each of the group vibration frequency characters which fall within the infrared frequency range.

FTIR is an important technique that provides an easy way to identify certain functional groups in organic molecules by identifying each group's vibration frequency within the infrared frequency range. As such, passing an infrared signal through the organic compound causes the functional group to vibrate at specific frequencies. In this study, the functional group of the samples were identified using an FTIR spectrometer (Spectrum One, Perkin Elmer, USA). The FTIR spectra were recorded within a wavenumber range of 400-4000 cm^{-1} which is the mid-infrared region corresponding to the fundamental vibration modes of the molecules

3.5.3 Hydroxyl Value Method

Hydroxyl value, also called as hydroxyl is one of the parameter that commonly used in industries such as polymer, coatings, and polyurethane industries to identify the quantity of hydroxyl groups in a chemical substances. It is define as the number of

milligrams of potassium hydroxide (KOH) equivalent to the hydroxyl content of 1 gram of the sample with unit ($\frac{\text{mg KOH}}{\text{g}}$).

Acetylation titration are the principle method of obtaining the hydroxyl value. Acetic anhydride with solvent commonly pyridine are react with OH group in a samples to produce esters and acetic acid. An unreacted acetic anhydride are further hydrolysed with water to form acetic acid. Free acetic acid produced is titrated with standard solution of potassium hydroxide (KOH). Difference in acetic acid between a blank and the test sample tells us how much acetic acid anhydride react with the hydroxyl group and allowed hydroxyl value calculateaction steps are as following equation for acetylation reaction, hydrolysis of excess acetic anhydride and titration of resulting acetoc acid.

$$\text{ROH (hydroxyl group)} + (CH_3CO)_2O) \rightarrow \text{ROCOCH}_3\text{(ester)} + CH_3COOH \quad (3.4)$$

$$(CH_3CO)_2O + H_2O \rightarrow 2CH_3COOH \quad (3.5)$$

$$CH_3COOH + KOH \rightarrow CH_3COOK + H_2O \quad (3.6)$$

For sample preparation, sample weigh is depending on the expected OH content which is usually 0.5-5g and placed in a dry flask. A known amount of acetic anhydride and pyridine are added in excess to the flask and refluxed for 1 hour to allow the acetylation reaction taking places. After reaction completed, the mixture is cooled and distilled water are added to hydrolyze unreacted acetic anhydride. Titration take places with standard KOH with 0.5 molarity (N) using phenolphthalein as an indicator until endpoint where solutions tirn to pink. Blank determination are performed with all reagents without the sample to obtain the exact amount of acetic anhydride originally present and finally OH value can be calculated.

$$\text{Hydroxyl value (OH)} = \frac{(B-S) \times N \times 56.1 \times 1000}{W} \quad (3.7)$$

Let

- B = Volume of KOH (ml) used for blank

- S = Volume of KOH (ml) used for sample
- N = Normality of KOH
- W = Weight of sample (g)
- 56.1 = weight of KOH ($\frac{g}{mol}$)

3.5.4 Deterioration of Bleachability Index (DOBI)

DOBI is important to evaluate the thermal and oxidative degradation impact on the bleachability of neem oil that would effect the bleaching process. Main procedure is divided by three steps. Firstly, thermal deterioration simulation where 100g of neem oil and 5g of deionized eater are wighed and put into a 250 ml rund bottom flask and heated until it reach temperature in range 95-100^{0}C for 5 hours and let it cooled to 80^{0}C. Next part is bleaching. 1 g of bleaching earth are dried at 105^{0}C at 1 hour. After that, mixed it with the deteriorated neem oil earlier and stirred at 80^{0}C for another 30 minutes under low vacuum to minimize further oxidation. Lastly, measure colour of the sample of raw neem oil, deteriorated neem oil and bleached neem oil by using tintometer and DOBI can further calculated using formula below.

$$\text{DOB\%} = \frac{(\text{Colour of Deteriorate oil} - \text{Colour after bleaching})}{(\text{Colour of raw neem oil} - \text{Colour after bleaching})} \text{x100} \quad (3.8)$$

3.5.5 Acid Value

Acid value is defined as quantity of potassium hydroxide in milligram required to neutralize the free fatty acid in 1 gram of sample. The procedure are as follows. Firstly, 1 gram of sample and solvent (ethanol) for 50ml are measured and poured into a 250ml Erlenmeyer flask and swirl. 2-3 drops of phenolphthalein areadded to the solution. Titration is followed by using 0.1N KOH using a burette until persistent pink colour that last for 30 seconds appear. Lastly, acid value can be calculated using formula stated.

$$\text{Acid value } \left(\frac{\text{mg KOH}}{\text{g}}\right) = \frac{\text{VxNx56.1}}{\text{W}} \quad (3.9)$$

Where,

- V = volume of KOH used (ml)
- N = normality of KOH solution
- W = weight of oil sample (g)
- 56.1 = molecular weight of KOH

3.5.6 Moisture Content

Moisture content is used to determine the percentage of moisture and volatile matter that present in neem oil and its final form, and this study is DHSA produce using oven drying method. Moisture content can be determined by measuring the loss in weight of the sample after heating it to the 105°C until a constant weight is achieved. The loss is attributed to the water and other volatiles which had been evaporated. Procedure are as follows. Moisture dish are dried first in an oven at 105°C for 30 minutes and further cooled in a dessicator for 15 minutes. Weight of dish is recorded and known as empty weight (W1). 5 g of sample are added on the moisture dish and weight is recorded (W2). Sample dish with oil sample are place in the oven at 105°C for 1 hour. Remove the sample dish properly using tongs and placed in dessicator to further remove the moisture for 30 minutes. Weight of oil + dish (W3) after drying are recorded. Moisture content can be calculated using the equation.

$$\text{Moisture content } (\%\frac{w}{w}) = \frac{(W2-W3)}{(W2-W1)} x100 \quad (3.10)$$

3.6 Catalyst Reusability

Catalyst reusability is referring to its ability to be used multiples time in a reaction without jeopardizing its catalytic activity hence reducing the yield or prolong the time taken for reaction to takes places. For this study, reusability test is conduct after each epoxidation takes places. Amberlite IR 120H are filtered, cleaning using acetone to remove the greasy from neem oil then washed thoroughly with excess water to remove the performic acid. Then, it was heat in the oven at 70^0C for 30 mins to remove

excess moisture before the catalyst is used back for the next reaction. Relative conversion of oxirane is measured for the second time usage of the catalyst. Reaction is repeated until RCO obtained are reduce until 40% from the initial.

3.7 Formation of DHSA From Epoxidized Neem Oil by Hybrid In Situ and Ex-Situ Hydrolysis

DHSA is formed due to the oxirane ring opening by the hydrolysis process by water since it is readily available in the reaction before the by-product of performic acid formation used for the epoxidation process of neem oil earlier. In this study, to the DHSA production, an additional group of OH is introduced by additional water ex-situ process, which would this study to produce Figure 3.2 below shows the graph of neem oil epoxidation and the point where water is added and its category, whether it can be considered as in-situ, ex-situ or in situ and ex-situ hydrolysis.

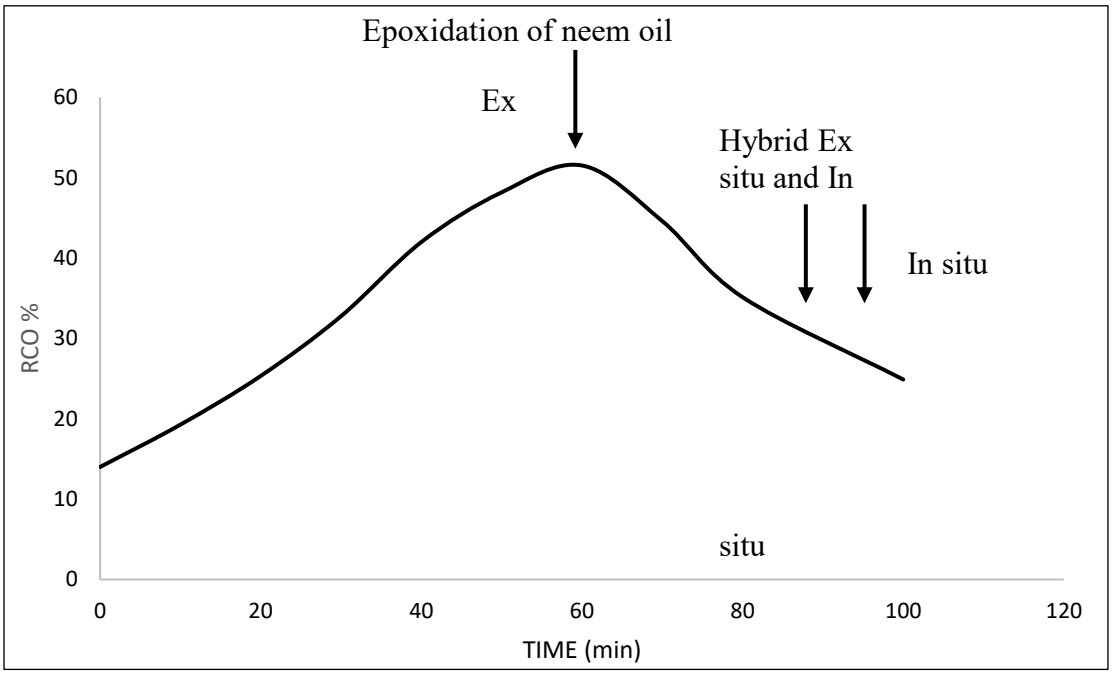


Figure 3.2 Comparison of Hydrolysis Via Ex-Situ, A Hybrid of Ex-Situ and In-Situ and In- Situ Only

This reactor is set to operate under isothermal conditions with a controlled temperature setting. This study focuses on the hybrid of ex-situ and in-situ hydrolysis, where water is added during the degradation of an epoxide ring. The process starts with the epoxidation of neem oil to create an epoxide intermediate. In the in-situ phase, water

is introduced directly into the reaction mixture, facilitating hydrolysis of the epoxide ring and forming hydroxyl groups to boost the reaction rate and DHSA yield. Following this, the ex-situ phase allows further optimization by adjusting water concentration separately, enabling precise control over hydrolysis and minimizing byproducts. By integrating the direct reactivity of in-situ hydrolysis with the precision of ex-situ adjustments, this approach aims to achieve efficient and scalable DHSA production.

3.7.1 Influence of Parameter Towards OH Value DHSA

OH value for final product, which is DHSA is the quantitative analysis to identify whether hydroxylation reaction process where it can prove successful incorporation of hydroxyl group into the fatty acid structures of fatty acid structure. Continuous with the in situ epoxidation process at the optimum condition, hydrolysis process are continue by ex-situ method, where water are added to promote DHSA formation. However, there are a few parameter involved for this process, which is effect of reaction time and amount of water added to the reaction. Reaction time and water volume were chosen because both influence the ring opening (hydrolysis) of oxirane groups which determine the OH values of DHSA produced. A range of 80-110 minutes are as what found based on previous study where hydrolysis of epoxidized vegetable oils generally reaches significant oxirane conversion within 60-120 mins while shorter duration often result in incomplete ring opening and excessive may promote other side reaction[4]. Meanwhile, for volume of water, lower volume would limit hydroxyl group formation while excess of it eventually dilute the system and reduce efficiency [5]. A set of experiment were set up in order to determine the pattern for both parameter as tabulated in Table 3.6.

Table 3.6
Parameter Level Affected OH Value DHSA

Reaction Time (Min)	Volume of Water (G)
80	5
90	10
100	15
110	20

All other optimum parameters for epoxidation that had been determined earlier are kept constant throughout all the experiment set up. All DHSA formed at end of each run are measured for its hydroxyl value and the pattern are to be discussed.

3.8 Optimization of DHSA via Taguchi Method

This reactor is set to operate under isothermal conditions with a controlled temperature setting. This study focuses on the hybrid of ex-situ and in-situ hydrolysis, where water is added during the degradation of an epoxide ring. The process starts with the epoxidation of neem oil to create an epoxide intermediate. In the in-situ phase, water is introduced directly into the reaction mixture, facilitating hydrolysis of the epoxide ring and forming hydroxyl groups to boost the reaction rate and DHSA yield. Following this, the ex-situ phase allows further optimization by adjusting water concentration separately, enabling precise control over hydrolysis and minimizing byproducts. By integrating the direct reactivity of in-situ hydrolysis with the precision of ex-situ adjustments, this approach aims to achieve efficient and scalable DHSA production. There are three parameters, and each has four levels, as tabulated in Table 3.6.

Table 3.7
Parameter on a Hybrid of Ex-Situ and In-Situ Hydrolysis of Epoxidized Neem Oil [37]

Parameter	**Level**			
	1	**2**	**3**	**4**
Water/neem oil molar ratio, (A)	1	2	3	4
Time for water additional (min), (B)	80	90	100	110
Time to stop reaction (min), (C)	120	180	240	300

Samples were analyzed using the hydroxyl value test to monitor reaction progress and assess DHSA yield, providing a quantitative measure of hydroxyl group formation as the epoxide ring opens during hydrolysis. This test enables precise tracking of the conversion to DHSA, allowing for adjustments in reaction conditions to optimize yield and ensure process consistency. The diversity of factors was examined using the orthogonal array of control parameters (Table 3.7). Results from the L16 experimental runs were analyzed using the signal-to-noise (S/N) ratio and ANOVA. The Taguchi

method predicted optimal reaction conditions, which were then validated through a confirmation reaction [38].

Table 3.8
L16 Experiment Design for Hybrid In Situ and Situ Hydrolysis of Epoxidized Neem Oil

No	Water/Neem Oil Molar ratio (A)	Time For Water Addition (B)	Time to Stop Reaction (C)
1	1	1	1
2	1	2	2
3	1	3	3
4	1	4	4
5	2	1	2
6	2	2	1
7	2	3	4
8	2	4	3
9	3	1	3
10	3	2	4
11	3	3	1
12	3	4	2
13	4	1	4
14	4	2	3
15	4	3	2
16	4	4	1

3.9 Numerical Modelling of Epoxidation of Neem Oil

The epoxidation of neem oil can be modelled kinetically by determining the rate equation for the reaction. The kinetic studies were conducted using MATLAB software (The MathWorks, Inc., Natick, MA, USA). The version used for the analysis was MATLAB R2021b (9.11.0). The development model in the kinetic study of the epoxidation process is based on the assumptions that: 1) The epoxidation process takes

in a single phase to avoid distribution constant for the qualification of different species in aqueous and oil phases; 2) Each phase's volume remains constant throughout the process. In situ, epoxidation is characterized by two main reactions: performic acid and epoxide formation, as illustrated in Equations 3.12.

$$FA + H_2O_2 \underset{k12}{\overset{k1}{\rightleftharpoons}} PFA + H_2O \tag{3.11}$$

In this study, the symbols are defined as follows: formic acid (FA), hydrogen peroxide (H_2O_2), performic acid (PFA), water (H_2O), neem oil (NO), and EPOXY, which represents epoxidised vegetable oil derived from oleic acid. The kinetic constants K_{11}, K_{12}, and K_{21} correspond to the reaction rates associated with Equations 3.6 and 3.7 [39]. Based on the epoxidation mechanism, the system of differential equations governing the concentration changes of each species was subsequently formulated and expressed as Equations 3.13 to 3.18.

$$\frac{d[FA]}{dt} = -k_{11}[FA][H_2O_2]+k_{12}[PFA][H_2O]+k_{21}[PFA][OA]-k_{22}[EPOXY][FA] \tag{3.12}$$

$$\frac{d[H_2O_2]}{dt} = -k_{11}[FA][H_2O_2]+k_{12}[PFA][H_2O] \tag{3.13}$$

$$\frac{d[PFA]}{dt} = k_{11}[FA][H_2O_2]-k_{12}[PFA][H_2O]-k_{21}[PFA][OA] \tag{3.14}$$

$$\frac{d[H_2O]}{dt} = -k_{12}[PFA][H_2O] \tag{3.15}$$

$$\frac{d[OA]}{dt} = -k_{21}[PFA][OA] \tag{3.16}$$

$$\frac{d[EPOXY]}{dt} = k_{21}[EPOXY][FA] \tag{3.17}$$

Additionally, the rate equations were integrated in MATLAB employing the fourth-order Runge–Kutta algorithm. Parameter accuracy was confirmed by minimising

deviations between experimental observations and simulated outputs, demonstrating the robustness of the kinetic model.

$$e=\sum_{i=1}^{n}\frac{\left|[EPOXY]^{sim}{}_{i}-[EPOXY]^{exp}{}_{i}\right|}{n} \tag{3.18}$$

Where $[EPOXY]^{exp}{}_{I}$ and $[EPOXY]^{sim}{}_{I}$ are the experimental and estimated concentration of epoxy in respected data points, i and n are the number of data points in the experiment and simulation. A closer error, e value to zero, indicates an excellent correspondence to experimental data.

3.10 Kinetic Modelling DHSA from Neem Oil

A kinetic study based on finding a rate constant for each reaction involved in epoxidation and hydrolysis of neem oil is shown in Equation 3.20 - 3.22 where FA, HP, PFA, NO, and ENO are formic acid, hydrogen peroxide, performic acid, neem oil and epoxidized neem oil consecutively. This model assumes all reactants, intermediates, and products are in a single liquid phase, eliminating phase boundary effects and mass transfer limitations. It also assumes constant temperature, pressure, and ideal mixing, ensuring uniform concentration and stable reaction rates, which simplifies the analysis and allows the Runge–Kutta method to focus solely on reaction kinetics in a homogeneous system. However, the model's constraints include accurate reaction kinetics, controlled time steps for Runge–Kutta, and energy limits in simulated annealing to prevent premature convergence, with convergence criteria ensuring reliable results.

$$FA+ HP \underset{k12}{\overset{k11}{\rightleftharpoons}} PFA+ H_2O \tag{3.19}$$

$$PFA+NO \xrightarrow{k_2} ENO+FA \tag{3.20}$$

$$ENO+H_2O \xrightarrow{k_3} DHSA \tag{3.21}$$

A novel genetic algorithm was developed and applied to solve ordinary differential equations. The data for FA, HP, and NO were obtained experimentally. ENO and other intermediates like PFA reach constant levels, indicating that the reaction rates of the reversible steps are balanced. Since the system reaches a steady state, it implies that the final step effectively consumes ENO and promotes its irreversible transformation into DHSA. In reaction kinetics modeling, assuming irreversibility for the final step can simplify the system of equations, making the model easier to solve and interpret. This self-learning system is intrinsically parallel and can handle linear and nonlinear equations [40]. Genetic algorithms are a key element in artificial intelligence and machine learning, playing a significant role in optimization and kinetic study [41]. The rate and simultaneous differential equations are provided in Equations 3.23-3.28.

$$\frac{d[FA]}{dt} = -k_{11}[FA][HP]+k_{12}[PFA][H_2O]+k_2[PFA][NO] \tag{3.22}$$

$$\frac{d[HP]}{dt} = -k_{11}[FA][HP]+k_{12}[PFA][H_2O]-k_3[ENO][HP] \tag{3.23}$$

$$\frac{d[PFA]}{dt} = k_{11}[FA][HP]-k_{12}[PFA][H_2O]-k_{21}[PFA][NO] \tag{3.24}$$

$$\frac{d[H_2O]}{dt} = k_{11}[FA][HP]-k_{12}[PFA][H_2O] \tag{3.25}$$

$$\frac{d[NO]}{dt} = -k_{21}[PFA][OA] \tag{3.26}$$

$$\frac{d[ENO]}{dt} = k_{21}[PFA][NO]-k_3[ENO][H_2O] \tag{3.27}$$

$$\frac{d[DHSA]}{dt} = k_3[ENO][H_2O] \tag{3.28}$$

Kinetic modeling involves (1) numerically solving rate equations using MATLAB's ode45 solver with the fourth-order Runge–Kutta method and (2) minimizing the error e between simulation and experimental values. The error function e is given by Equation 3.30.

$$= \sum_{i=1}^{n} \frac{\left| -ENO_i^{sim} - ENO_i^{exp} \right|}{n} \quad (3.29)$$

Where ENO_i^{sim} and ENO_i^{exp} denote the estimated and experimental epoxide concentrations, i is the i^{th} data point, and n is the total number of data points in the simulations and experiments.

3.11 Concluding Remarks

This chapter has outlined the methodology adopted for the production of DHSA from neem oil via epoxidation and subsequent hydrolysis. A systematic experimental approach was designed, beginning with parameter optimization for epoxidation using the Taguchi method to identify the most influential factors affecting oxirane formation. The optimized parameters were then applied to a hybrid in situ and ex situ hydrolysis strategy to enhance hydroxyl group incorporation and improve DHSA yield.

A range of analytical techniques including FTIR, hydroxyl value determination, acid value, DOBI, and moisture content were incorporated to characterise the intermediates and final products, while BET and SEM analyses ensured proper evaluation of the catalyst's surface and structural properties. Catalyst reusability tests further reinforced the sustainability aspect of the study by validating Amberlite IR 120H as a cost-effective and recyclable heterogeneous catalyst.

Finally, kinetic modelling using MATLAB was integrated to simulate reaction pathways, estimate rate constants, and validate experimental results. By combining experimental design, process optimisation, analytical characterisation, and kinetic modelling, this methodology establishes a comprehensive framework for producing DHSA efficiently and sustainably from neem oil, while ensuring scalability and industrial relevance.

CHAPTER 4
RESULT AND DISCUSSION

4.1 Introduction

In this chapter, the optimization of epoxidized neem oil was discussed in detail, and validation of the result from the Taguchi method was examined by studying the effect of each reaction condition. The presence of an oxirane ring, which indicates that epoxidation is happening, is also to be discussed, other than epoxidized neem oil characterization. OFAT was first used as a simple and direct method to screen the effects of each parameter and determine feasible ranges. Once the significant factors were identified, the Taguchi method was applied to optimise the parameters simultaneously and obtain the best reaction conditions with fewer experiments. The process continues by producing DHSA from epoxidized neem oil, where the optimum process parameter is determined by (OFAT). Then, physical characterization was also examined. Lastly, kinetic modelling for the epoxidation of neem oil was developed using homogenous theory with a detailed discussion.

4.2 Optimization of the Epoxidation Process Parameter

Taguchi method was used to determine the optimum values of the epoxidation process. In general, a higher S/N ratio indicates better performances, thus providing info on the influence of each factor contributing to the optimum response. Therefore, optimum levels for all process parameters are the level with the highest S/N ratio [42]. The S/N ratio values for each set-up of neem oil epoxidation and relative conversion oxirane % (RCO) are presented in Table 4.1.

The Signal to Noise ratios were plotted for each of the three levels of parameters stated, as shown in Figure 4.1. Based on the plot, it can be concluded that RCO was maximum at the following (A) formic acid/neem oil molar ratio: 1:1 (level 1), (B) hydrogen peroxide/neem oil molar ratio: 4:1 (level 4), (C) catalyst loading: 1g (level 2), (D) reaction temperature: 70^0C (level 4) and (E) agitation speed: 300 rpm (level 1). By employing design of experiment (DoE), Uppar et al. [43] able to reduce the number of trial experiments in order to produce bio lubricant base by non edible vegetable oils

(jathropa). In his study, (DoE) reveals that the optimized yield for jatropha biolubricant with H_2SO_4 and HCL catalyst are achieved with molar ratio of 0.5:1:1.5 of organic acid, jathropa oil, and hydrogen peroxide at reaction temperature ranging from 65 to 70°C helped in achieveing maximum yield.

Meanwhile, optimization on epoxidation of cotton seed oil via taguchi (DoE) had been studied by Dodo R et al. [44] had found that molar ratio of 1.5:1 of hydrogen peroxide to cotton seed oil, molar ratio of 0.75:1 of acetic acid to cotton seed oil with 3 wt% of catalyst achieved 72.20% relative conversion oxirane. This finding align with our study where parameter (1) formic acid/neem oil molar ratio is at the lowest level and parameter (2) hydrogen peroxide/neem oil molar ratio at the highest level.

Table 4.1
S/N ratio Analysis Based on the Larger the Better-Quality Characteristic

	Process Parameters and Their Levels						
Run	**Formic acid/neem oil molar ratio**	**Hydrogen peroxide/neem oil molar ratio**	**Catalyst loading (g)**	**Temperature (^{0}C)**	**Stirring speed (rpm)**	**RCO**	**S/N ratio**
1	1	1	1	1	1	45.93	33.24
2	1	2	2	2	2	41.74	32.41
3	1	3	3	3	3	22.41	27.00
4	1	4	4	4	4	27.88	28.90
5	2	1	2	3	4	24.61	27.82
6	2	2	1	4	3	22.39	27.00
7	2	3	4	1	2	19.44	25.77
8	2	4	3	2	1	32.08	30.12
9	3	1	3	4	2	32.83	30.33
10	3	2	4	3	1	22.08	26.88
11	3	3	1	2	4	24.48	27.78
12	3	4	2	1	3	36.51	31.25
13	4	1	4	2	3	19.63	25.86
14	4	2	3	1	4	18.76	25.46
15	4	3	2	4	1	32.90	30.34
16	4	4	1	3	2	24.59	27.82

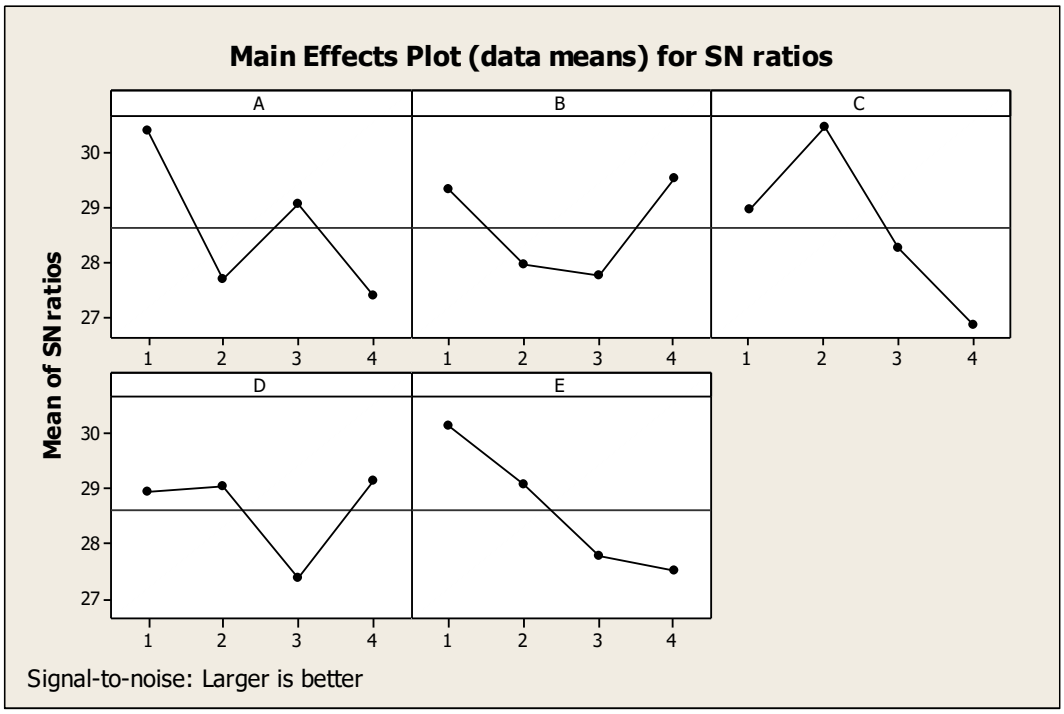


Figure 4.1 Average S/N Ratios for All Process Parameters Affecting the Epoxide Yield

*A: Formic acid/neem oil molar ratio, B: Hydrogen peroxide/neem oil molar ratio, C: catalyst loading, D: Reaction temperature, E: Stirring speed

ANOVA determined each factor's contribution to the mean response, predicting optimal process parameters. The F-value and p-value assessed the significance of each parameter on neem oil epoxide yield [45]. The contribution percentage was determined to obtain each process parameter's contribution towards the neem oil's epoxidation reaction. The ANOVA results obtained from epoxidation of neem oil are tabulated in Table 4.2. It can be seen that catalyst loading (C) was the most significant factor that affected the epoxidized neem oil (30.66%), followed by formic acid/neem oil molar ratio (A) at (29.77%) due to its role as an oxygen carrier compared to hydrogen peroxide, which acts as an oxygen donor and the third one is stirring speed (E) at (20.95%). Meanwhile for followed hydrogen peroxide/neem oil molar ratio (B) at (8.76%) and reaction temperature (D) at (9.90%) has less impact in epoxidation process since both of them had lower percentage contribution.

The ANOVA results also revealed that reaction temperature (D) had the most negligible significant effect on this study. This is because the epoxidation process is exothermic, so we cannot fix the temperature precisely at their level. Other than that, there is only a 10^0C difference for each level in reaction temperature that can be varied in this study because a reaction at more than 80^0C would lead to an explosion due to the

exothermic reaction. The optimum level for each parameter must be validated to verify the optimum process parameter with its level by ANOVA and S/N ratio analysis.

As mentioned by Ramabulana K et al. [46], larger *F-value* and smaller *P-value* p (< 0.5) show that the more significant the parameter is for the reaction. Also supported by Wahyudin et al. [47] where their studies on optimization of the dyeing process shows the largest *F-value* would lead to lowest *P-value* that indicate the most significance parameter in affecting dyeing process is dye concentration. In this study, catalyst loading (C) are the most significance parameter because heterogenous catalyst plays an impotant role in the epoxidation process, where it provides the pores where performic acid can seeping through, thus reduce the possibility of oxirane degradation [48]. Heterogenous catalyst type also chosen to reduce the by product formation due to the instability of performic acid formed earlier [49].

Mungroo R et al. [50] also states that higher oxygen carrier, which in their study was acetic acid does not increase the epoxidation rate of canola oil, where it would just increase the reactivity of reaction and epoxidation rate are lower since excess of oxygen carrier would lead to degradation of epoxide produced. This findings are going well with our second most significance parameter in this study, which are formic acid/neem oil molar ratio (A) would react if there are excess of it in the system. For the third significant parameter in this study, stirring speed (RPM) for the reaction. It does have significance impact on epoxidation because we need to maintain the optimum temperature throughout the process. Higher stirring speed would lead to dissipation of heat towards the next medium which is water bath, does would fluctuate the epoxidation rate of neem oil due to heat losses would reduce the effective collision between the reactant and halt the reaction [51].

Table 4.2
ANOVA Results for Epoxidation of Neem Oil

Factor	DOF (f) (Degree of Freedom)	The sum of Squares (S)	Mean Square(M)	F-Value	P-Value	Percentage Contribution (%)
A	3	282.7	94.2	1.56	0.251	29.77
B	3	103.9	34.6	0.46	0.716	8.76
C	3	289.1	96.4	1.61	0.240	30.66
D	3	115.3	38.4	0.52	0.679	9.90
E	3	217.8	72.6	1.10	0.386	20.95

4.3 In Situ Epoxidation of Neem Seed Oil

Epoxidation of neem oil is set up according to each optimum process parameter level decided earlier by S/N ratio, where (A) formic acid/neem oil molar ratio: 1:1 (level 1), (B) hydrogen peroxide/neem oil molar ratio: 4:1 (level 4), (C) catalyst loading: 1g (level 2), (D) reaction temperature: 70^0C (level 4) and (E) agitation speed: 300 rpm (level 1). The maximum RCO obtained by this set up is 51.50%

A higher level for parameter (B) produces higher RCO due to hydrogen peroxide is a good oxidizing agent and has a weaker pie bond where it would be easier to react with formic acid during the performic acid formation and epoxidation of the neem oil process. However, a higher concentration of hydrogen peroxide would lead to faster degradation of the oxirane ring, as we can see in Figure 4.1 where RCO% starts to decrease after 70 minutes [52]. In addition, eventhough higher molar ratio of hydrogen peroxide would lead to degradation of oxirane ring, it is crucial in order to increase the epoxidation rate. So, the right amount are needed in order to maximize it[53]. According to Milchert et al. [54], an increase in temperature reactions above 70^0C is recognised as too high and would lead to resin degeneration hence causing a significant decrease in catalyst properties to enhance the effective collision which would lead to a shorter time taken for the reaction to happen. Figure 4.2 shows the relative conversion oxirane of neem oil.

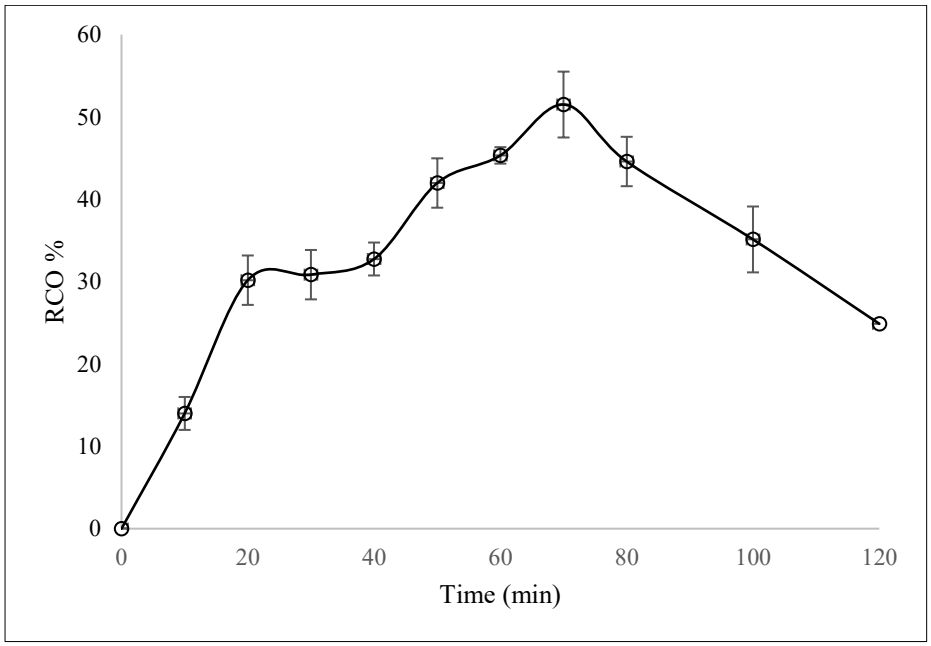


Figure 4.2 RCO% Conversion of Neem Oil

Epoxidized neem oil or known as oxirane ring are reactive compared to other ethers due to the fact that ring strain is relieved when there are ring opening upon nucleophilic attack as shown in Figure 4.3 below. Its three ring membered structure, which one vertices is an oxygen and another two carbon make it more reactive. This by product that formed can be further used in industries as precursor for new material as polyol [55], hydroxyl ester [56], biodesel [57].

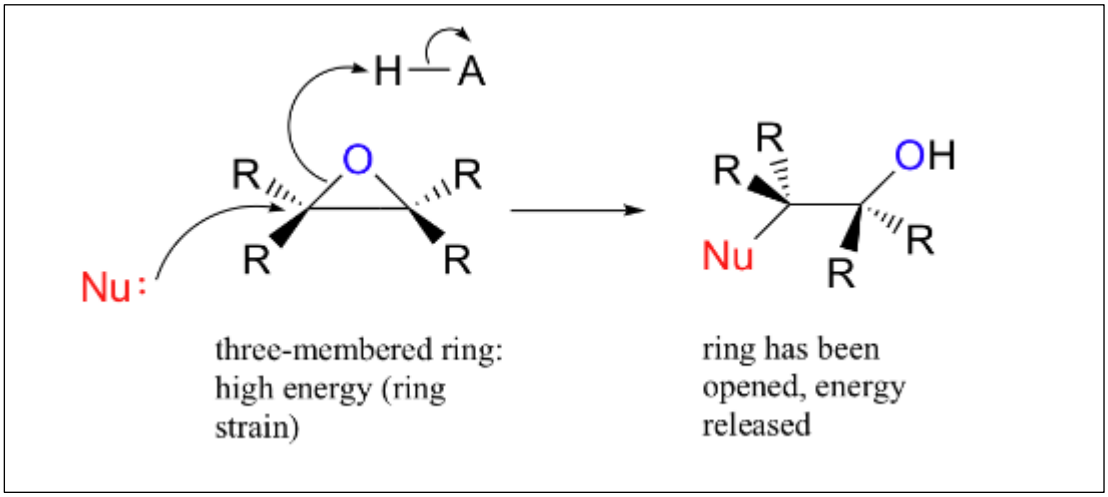


Figure 4.3 Nucleophilic Attack on Oxirane Ring [58]

4.4 Effect of Process Parameter on the Epoxidation Rate at Optimum Condition

The effect on the epoxidation rate of neem oil at optimum parameters with its level is validated by comparing the epoxidation rate at optimum conditions. It varies each process parameter with its level.

4.4.1 Effect of Formic Acid/Neem Oil Molar Ratio on Epoxidation

Formic acid in performic acid, which acts as an oxidizing agent, is also a catalyst for the epoxidation process. It is a relatively mild acid that can effectively promote the reaction while minimizing the probability of side-product formation. Specifically, formic acid acts as a proton donor that initiates the reaction between an oxidizing agent and an unsaturated double bond in the neem oil. Sobhan et al. [59] found higher formic acid conditions at a 10:10 ratio to soybean oil yield with high selectivity at 93.68% and achieved 88.80% conversion. In this study, the formic acid molar ratio to neem oil level

varies to 1:1, 2:1, 3:1, and 4:1 while maintaining the same level for other parameters. This research shows that the optimum level for epoxidation of neem oil is at a 1:1 ratio of formic acid/neem oil with 51.50% relative conversion oxirane. This study leads us to a different result from Sobhan et al. [59].

It is mainly due to the implementation of amberlite IR 120H as a catalyst for epoxidation, where its functionality is to bind with performic acid to reduce the reactivity of formic acid, whereby it can slow the oxirane ring degradation while optimizing the epoxidation of neem oil as per a study done by Rahman et al. [48]. This can be shown in Figure 4.4, where the higher molar ratio of formic acid/neem oil reaches optimum epoxidation at a faster rate, but due to the reactivity of formic acid, degradation of the oxirane ring occurs at a fast pace, thus reducing the optimum rate of epoxidation.

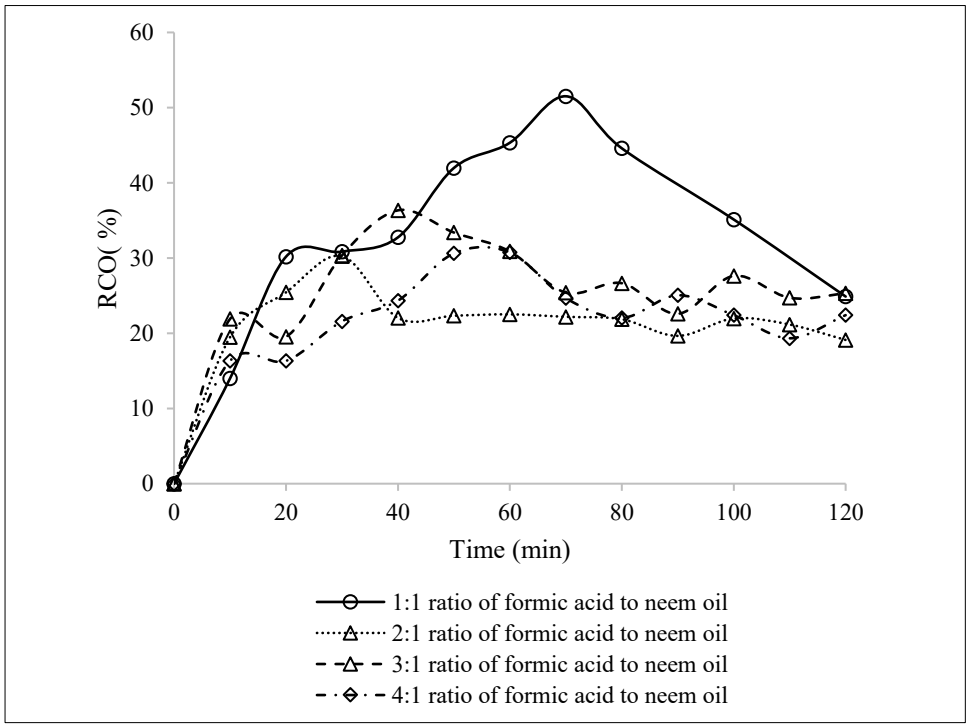


Figure 4.4 Epoxidation of Neem Oil by Varying Molar Ratio of Formic Acid to Neem Oil

4.4.2 Effect of Hydrogen Peroxide/Neem Oil Molar Ratio on Epoxidation

Hydrogen peroxide in performic acid form, which acts as an oxidizing agent, also acts as an oxygen donor in the epoxidation of neem oil to break the double bond of its unsaturated fat. According to Derawi et al. [60] higher molar ratio of hydrogen peroxide to oil chosen, which is 60%, would result in a higher epoxidation rate.

However, the stability of the oxirane ring would be lower since it would degrade with time, while a lower concentration of hydrogen peroxide makes the oxirane ring more stable. Meanwhile, Campanella et al. [61] conclude that the molarity of hydrogen peroxide does not significantly impact the epoxidation rate at a lower temperature of 40^0C but would have a considerable impact when the reaction temperature increases to 60^0C.

In this study, the hydrogen peroxide molar ratio to neem oil level varies to 1:1, 2:1, 3:1, and 4:1 while maintaining the same level for other parameters. This research shows that the optimum level for epoxidation of neem oil is at a 4:1 ratio of hydrogen peroxide/neem oil with 51.50% relative conversion oxirane. This lead to the same result obtained as other researchers mentioned since reaction temperatures are maintained at 60^0C. A higher molar ratio of hydrogen peroxide to neem oil promotes a higher epoxidation rate since the system has more free oxygen supply. Figure 4.5 shows the epoxidation rate of neem oil by varying the molar ratio of hydrogen peroxide/neem oil.

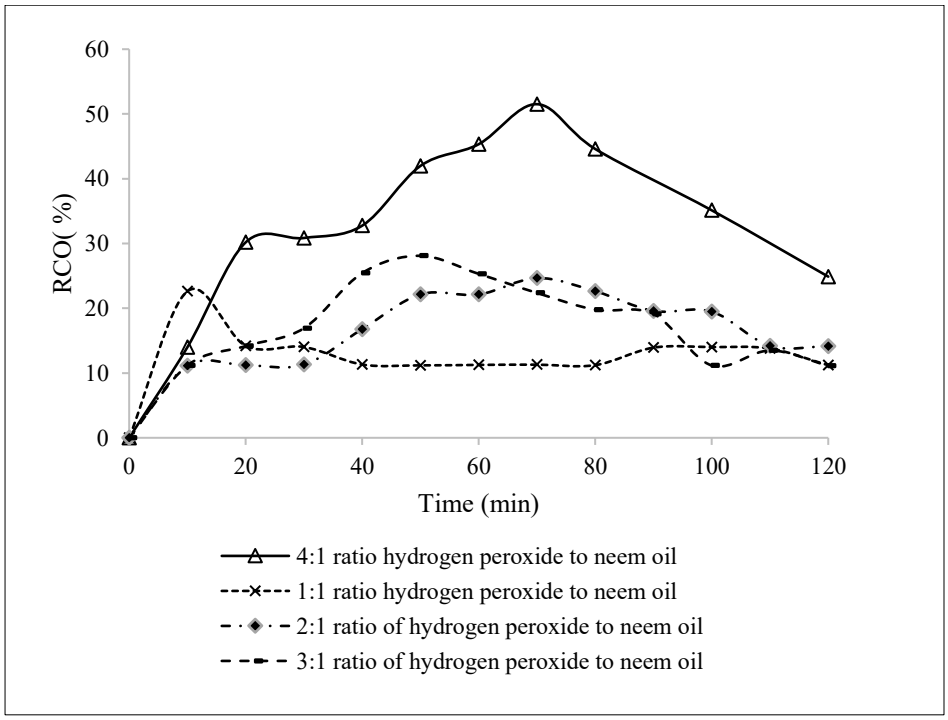


Figure 4.5 Epoxidation of Neem Oil by Varying Molar Ratio of Hydrogen Peroxide to Neem Oil

4.4.3 Effect of Catalyst Loading on Epoxidation

A heterogeneous catalyst such as Amberlite IR 120H was chosen in this study to increase the time taken for the epoxidation reaction to take place. Furthermore, heterogeneous catalysts are more favourable for epoxidation nowadays due to homogenous catalysts such as sulphuric acid and hydrochloric acid that may corrode the whole system in the long run. Other than that, heterogeneous catalysts have the potential to be reusable, other than lowering the cost of their separation [62].

In this study, the catalyst loading varies to 0.5,1.0,1.5, and 2 g of catalyst while maintaining the same level for other parameters. Results show that 1g of Amberlite IR120H used resulted in the highest RCO contain compared to other loadings, as shown in Figure 4.6. 1g of catalyst loading is equivalent to 1 wt% of neem oil used for this study as research done by Buchori et al. [63] on the effect of catalyst loading on biodiesel production shows that 1% wt of catalyst is sufficient to produce biodiesel at 92.96%. In contrast, higher loading of catalyst at 3,5 and 7wt% would inhibit the transesterification process due to slurry formation, reducing biodiesel production. Other than that, higher catalyst loading would lead to catalyst particle agglomeration blocking sites, leading to side reactions and the formation of the by product, reducing the epoxidation rate [48].

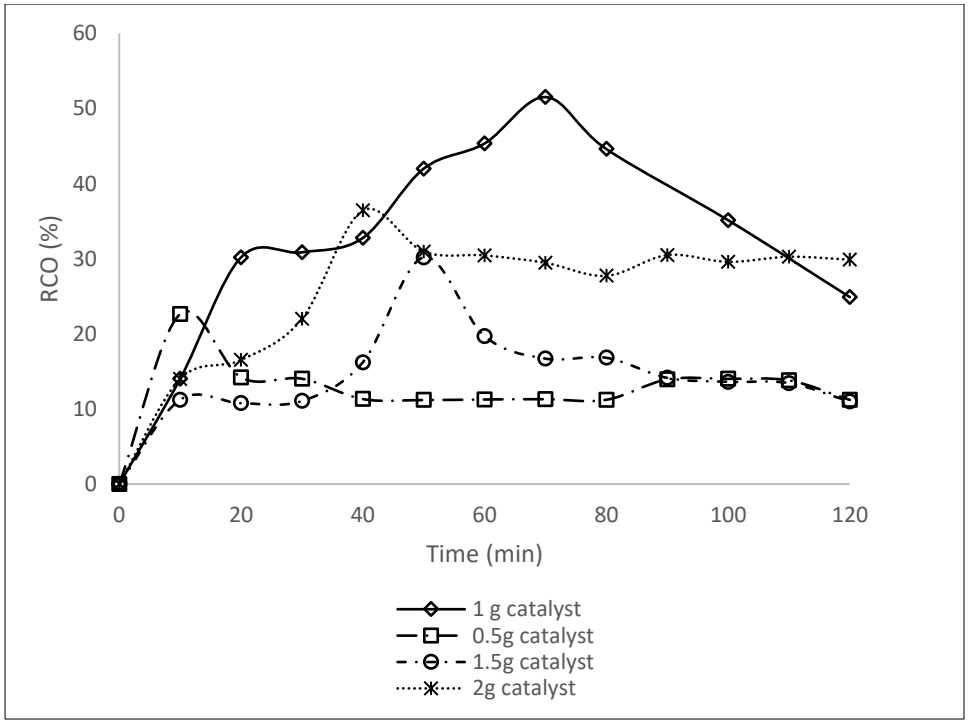


Figure 4.6 Epoxidation of Neem Oil by Varying Catalyst Loading

4.4.4 Effect of Reaction Temperature on Epoxidation

Reaction temperature also plays an essential role during the epoxidation process. This study studies the effect of various temperatures on the epoxidation rate of neem oil. Reaction temperatures are maintained and repeated at 40,50,60 and 70 °C throughout the study while maintaining other parameters. Higher operating temperatures are not favourable since there are intense bubbling and solution would splash out of beaker at high temperature. This is due to the formation of performic acid itself being exothermic [50].

It was found that the relative conversion of oxirane or rate of epoxidation was highest during the reaction temperature maintained at 70 °C. It is due to the higher temperature that provides kinetic energy that promotes more effective collisions between molecules. It increases the reactivity of the double bond in neem oil to react with oxygen from performic acid, thus producing a higher oxirane ring [64]. However, the peak epoxidation rate only occurs in a few minutes due to the degradation of the oxirane ring and the formation of by-products where the oxirane ring reacts with formic acid that is reverse from performic acid, along with water that is available in the system as a by-product from the formation of performic acid [65]. The relative conversion of oxirane at different reaction temperatures is shown in Figure 4.7.

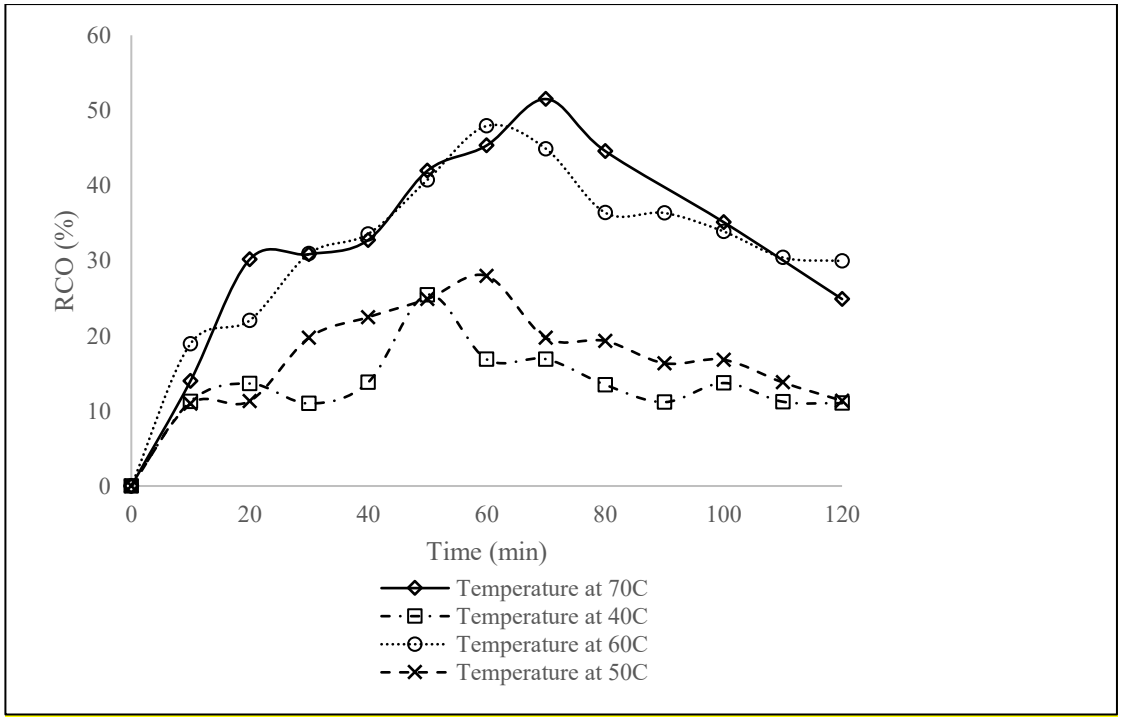


Figure 4.7 Epoxidation of Neem Oil by Varying the Reaction Temperature

4.4.5 Effect of Stirring Speed on Epoxidation

Various stirring speeds of the reaction mixture during this study affect the epoxidation rate by the heat transfer from the system to the water bath [66]. In this study, stirring speeds are manipulated at 300,400,500 and 600 rpm while other parameters are maintained. 300 rpm was the best among others because it produces the highest relative conversion oxirane due to less heat transfer from the system to the water bath. Hence, the exothermic reaction from the additional performic acid did not lose its energy and promoted the reaction.

Meanwhile, the highest stirring rate would disperse more heat transfer to the water bath system [51], thus not providing the optimum temperature for epoxidation reaction, which is 70^0C for this study to promote epoxidation reaction, other than reducing the kinetic energy for all the particles, does reduce the effective collisions. The effect of varying stirring speed on the relative conversion of oxirane is shown in Figure 4.8.

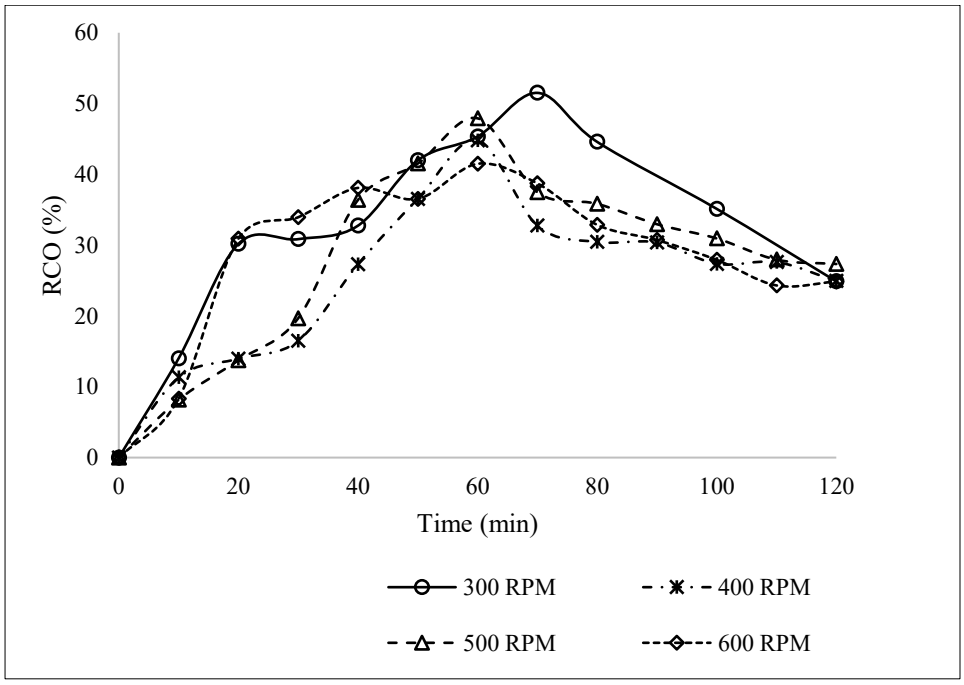


Figure 4.8 Epoxidation of Neem Oil by Varying Stirring Speed

4.5 Characterization of Raw Neem Oil Compared to Its Epoxide

Characterization of both raw neem oil and its epoxide are important for several aspects. One of it is to have better understanding on the chemical changes. Main purpose

of epoxidation is to convert the unsaturated carbon-carbon double bond. Next, identify the functional group changes. As epoxidation of neem oil could be confirmed with increase in hydroxyl group, characterization by FTIR are highly recommended by previous researchers. Table 4.3 shows the results of characterization for both raw neem oil and its epoxide by DOBI, Iodine Value and Hydroxyl value.

Table 4.3
Characterization of Raw Neem Oil and Epoxidized Neem Oil

Characterization	**Raw Neem Oil**	**Epoxidized Neem Oil**
DOBI	0.15	0.05
Iodine value ($\frac{gI_2}{g}$)	80.7	51.1
OH value	7.2	96.7

4.5.1 Fourier Transform Infrared (FTIR) Spectroscopy of Neem Oil and Its Epoxide

Fourier Transform Infrared (FTIR) spectroscopy examined epoxidized and raw neem oil properties. Both samples underwent analysis to confirm the presence of a C-O-C ring, indicative of an oxirane ring that serves as evidence for the existence of epoxidized neem oil post-epoxidation process, as shown in Figure 4.9. A comparison of the FTIR analyses for both samples, illustrated in Figure 4.10, reveals C-O-C oxirane rings at a wavenumber of 1240 cm^{-1}. The absence of a peak at 1706 cm^{-1} and 2850 $cm^{-1,}$ which indicates that oxirane ring opening due to stretching vibration of carbonyl ester and carboxylic acid [67] proves that oxirane ring or epoxidized neem oil has not degraded yet and epoxidation is at its peak.

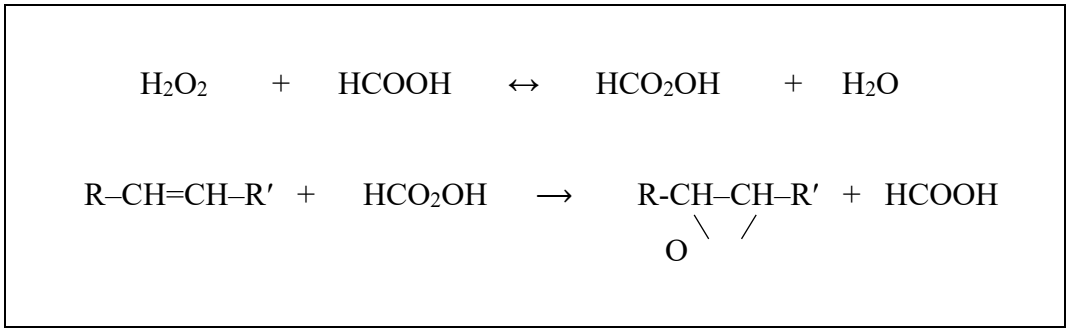


Figure 4.9 Schematic of Epoxidation of Neem Oil

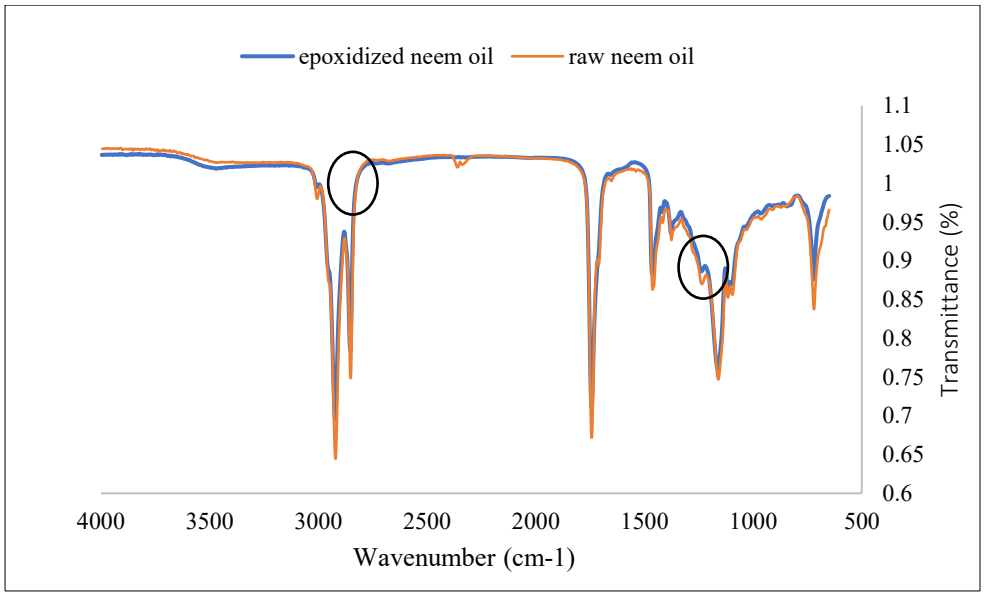


Figure 4.10 FTIR Spectrum for Raw Neem Oil and Epoxidized Neem Oil

4.5.2 Deterioration of Bleachability Index (DOBI)

(DOBI) is one of the characetrization that measure the oxidative stability and bleachabilty of vegetables oils. It is defined as ratio of UV absorbance at a range from 446 nm which absorbs the natural colurs which is carotenoid and 269 nm for the secondary oxidation products. High DOBI value means oil has high quantity of carotenoid and few oxidation products [68]. For this study, crude neem oil DOBI level is 0.15 clearly indicates that these oil have lower quantitative pigment intact which make it hard to bleach. This are proven because there are almost no colour changes for epoxidized neem oil.

Decrease in DOBI value for epoxidized neem oil shows that it has high possibilities to react to many polar oxidation products such as polymerization. This co currently with this study where epoxidized neem oil reacts as intermediate products only before further hydrolysis process which involve heating and additional of proton donor. In addition, the lower value of epoxidized neem oil DOBI shows that it is heaviliy oxidized where in context of edible oil is not consumable, which align with this study to produce DHSA as one of the raw maerial for cosmetic products[69].

4.5.3 Iodine Value

Iodine value is crucial in the characterization of oils and fats because it can show the quantity level of unsaturation bond, particularly number of carbon-carbon double bond. Iodine value measurement shows amount of iodine in grams that react with 100 grams of an oil sample. As per the analysis result, raw neem oil have higher iodine value, which is 80.7 while epoxidized neem oil at 51.1 indicates that epoxidation reaction is happening, where the number of carbon-carbon double bond decrease because the epoxidation reaction typically the double bond react with an oxygen donor from performic acid which converting them into oxirane ring or known as epoxide[70]. This reaction proven by reducing number of iodine value for epoxidized neem oil.

Reduction of iodine value between raw neem oil and epoxidized neem oil at 36% confirm a successful epoxidation reaction and a few advantages such as oxidative stability, where epoxidized oil with fewer double bonds are less prone to degradation, make it more suitable for industrial application such as plasticizers and lubricants[71]. In terms of functionality, epoxide group provides reactive sites that suitable for polymer synthesis which can enhancing the industrial usage of neem oi instead of its conventional usage.

4.5.4 Hydroxyl Value

Hydroxyl value with abbreviation OH value indicates the amount of hydroxyl groups present in oil or fat. With unit ($\frac{\text{mg.KOH}}{\text{g}}$), it shows the quantity in milligrams of potassium hydroxide (KOH) needed to balanced out the acetic acid bound by the hydroxyl group in 1 gram of sample. This number is critical in order to determine the reactivity and applicability of its oils in chemical modifications. Raw neem oil OH value is 7.2 ($\frac{\text{mg.KOH}}{\text{g}}$) while its epoxide is 96.7 ($\frac{\text{mg.KOH}}{\text{g}}$). these increase prove that significant chemical transformation during epoxidation process are taking places that lead to oxirane ring formation [69], [71]. Increase in OH value is a result of reduction carbo-carbon double bond where it converts to epoxide ring. Significance of quantitatively increasing of hydroxyl value make the epoxidized oil more valuable for industrial applicaton such as polyurethane production as bio based polyol, alkyd resins where it

enhance drying and filming properties, also for lubricants and plasticizers where it can improve their polarity and thermal stability [71].

4.6 Influence of Process Parameters on DHSA Formation.

4.6.1 Effect of Reaction Time

Reaction time significantly influences the hydroxyl (OH) value of dihydroxystearic acid (DHSA), as evidenced by the trend observed in the experimental data. The OH value increases progressively from 80 to 100 min, suggesting that the hydroxylation reaction proceeds efficiently within this time frame. The increase indicates successful incorporation of OH groups into the fatty acid structure, with the maximum OH value achieved at 100 min, identifying this duration as optimal for producing highly functionalised DHSA.

The significant increase in OH value between 90 and 100 min can be attributed to favourable reaction conditions, such as sufficient reactant concentration and optimal contact time, which enhance the rate of OH group formation. At 100 min, the reaction appears to reach peak efficiency, yielding DHSA with the highest OH content. This is particularly important for downstream applications, such as polyol production, where a high OH value is desirable for improving reactivity and performance in polymer formulations.

Beyond 100 min, a decline in OH value was observed, as demonstrated at 110 min. This reduction is likely due to secondary reactions, including overoxidation or thermal degradation, which may result in the loss of OH groups or transformation into undesired by-products. These findings highlight the importance of precise reaction time control to ensure product quality and functional integrity. Thus, a reaction time of 100 min is recommended to maximise OH functionality in DHSA while minimising degradation.

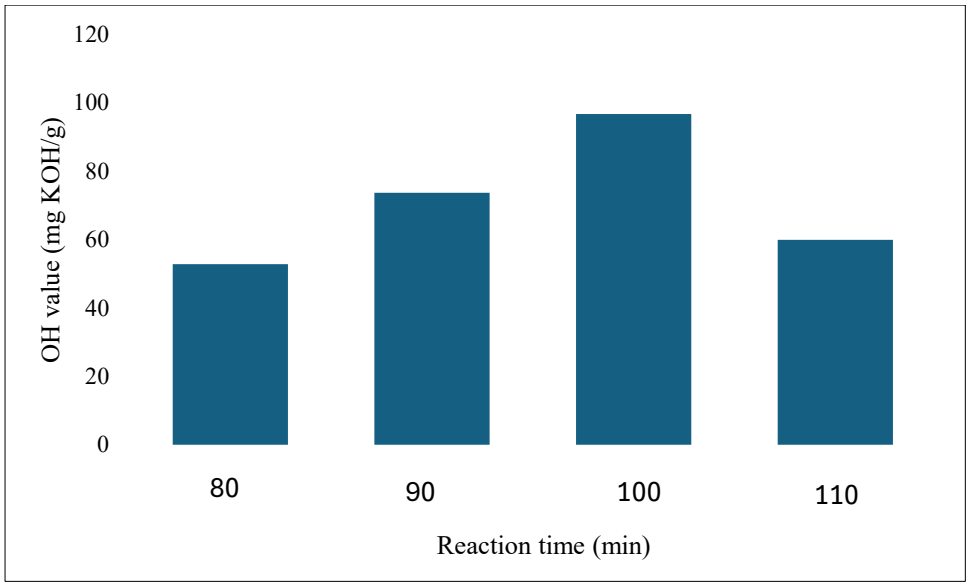


Figure 4.11 Effect of Reaction Time to OH Value

4.6.2 Effect of the Amount of Water

The OH value of DHSA demonstrates a direct relationship with the amount of water present in the reaction. At a lower water content of 5 g, the OH value is relatively low, suggesting that limited water availability may restrict the hydroxylation process. As the water amount increases to 10 g and 15 g, a progressive rise in the OH value was observed, indicating improved OH group formation. This trend highlights the critical role of water in facilitating the reaction, likely by increasing the availability of reactive species required for hydroxylation.

A substantial increase in the OH value was noted when 20 g of water was added, reaching the highest level recorded in this study. This suggests that a higher water concentration promotes more efficient hydroxylation, potentially by optimising reagent solubility, improving mass transfer, or driving hydrolysis reactions that contribute to the formation of the OH functional group. These findings underscore the importance of water in influencing the extent of hydroxylation, which is a key parameter in applications requiring high OH values, such as polymer synthesis and bio-based polyols.

Despite the observed benefits of increased water content, excessive amounts can introduce challenges such as phase separation, dilution effects, or the initiation of undesired side reactions. Further investigation is necessary to determine whether the OH value continues to increase beyond 20 g or if an optimal threshold exists.

Understanding the precise influence of water quantity is essential for optimising reaction conditions and ensuring maximum OH functionality while maintaining process efficiency.

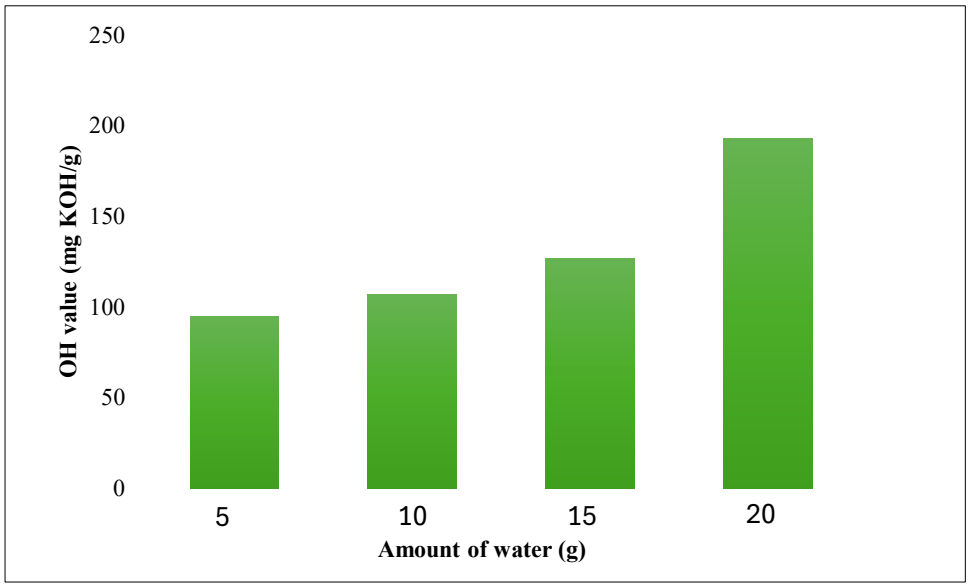


Figure 4.12 Effect on Amount of Water to OH Value

4.7 Optimization of DHSA Production by the Taguchi Method

The Taguchi method applies statistical principles to determine the factors that most strongly influence variability in process outcomes, and subsequently optimises conditions to minimise their effects [72]. It has been widely utilised across different sectors, including automotive, electronics, and consumer product manufacturing. By employing orthogonal arrays, the method substantially decreases the number of experimental trials required, thereby lowering both time and cost in industrial applications. Using Taguchi's orthogonal array design, the impact of multiple parameters on process performance can be systematically assessed [73]. Typically, a higher signal-to-noise (S/N) ratio reflects improved process stability and efficiency, offering valuable insights into the contribution of each factor towards achieving an optimum response. Consequently, the ideal parameter levels are those associated with the highest S/N ratios [74]. The hydroxyl (OH) values obtained for each neem oil epoxidation configuration are summarised in Table 4.4.

Table 4.4
Hydroxyl Value Analysis Based on the Larger-the-Better Quality Characteristic

No	Water/Neem Oil Molar Ratio (F)	Time for Water Additional (G)	Time to Stop Reaction (H)	OH Value mg KOH/g
1	1	1	1	75.4
2	1	2	2	101.2
3	1	3	3	70.1
4	1	4	4	75.9
5	2	1	2	77.7
6	2	2	1	129.4
7	2	3	4	88.8
8	2	4	3	80.4
9	3	1	3	86.7
10	3	2	4	70.4
11	3	3	1	72.6
12	3	4	2	73.3
13	4	1	4	58.1
14	4	2	3	73
15	4	3	2	45.4
16	4	4	1	126.9

The S/N ratios were plotted for each of the three parameter levels, as shown in Figure 4.13. Based on the plot, it can be concluded that the OH value was maximised at the following conditions: (1) water/neem oil molar ratio of 2:1, (2) water addition time of 90 min, and (3) reaction stop time of 120 min. The S/N ratio produces a higher epoxidation rate of neem oil to produce DHSA, with the highest OH value of 129.4 mg KOH/g. The OH value obtained using the in situ method alone was 90.8 mg KOH/g, while the maximum OH value achieved with the hybrid in situ and ex situ approach reached 129.4 mg KOH/g.

This substantial increase in OH value confirms that DHSA was successfully produced by combining in situ and ex situ hydrolysis. The higher OH value in the hybrid approach demonstrates more effective hydroxylation, validating the advantage of using

both methods to maximise DHSA yield. The S/N ratio indicates that these parameters enhance the epoxidation rate of neem oil, leading to increased production of DHSA. A high OH value indicates a higher concentration of OH groups in the product, which can enhance its functionality in various applications [75]. In epoxidation and hydrolysis, a high OH value signifies the effective conversion of the epoxide groups to DHSA, making the product more valuable and suitable for industrial uses.

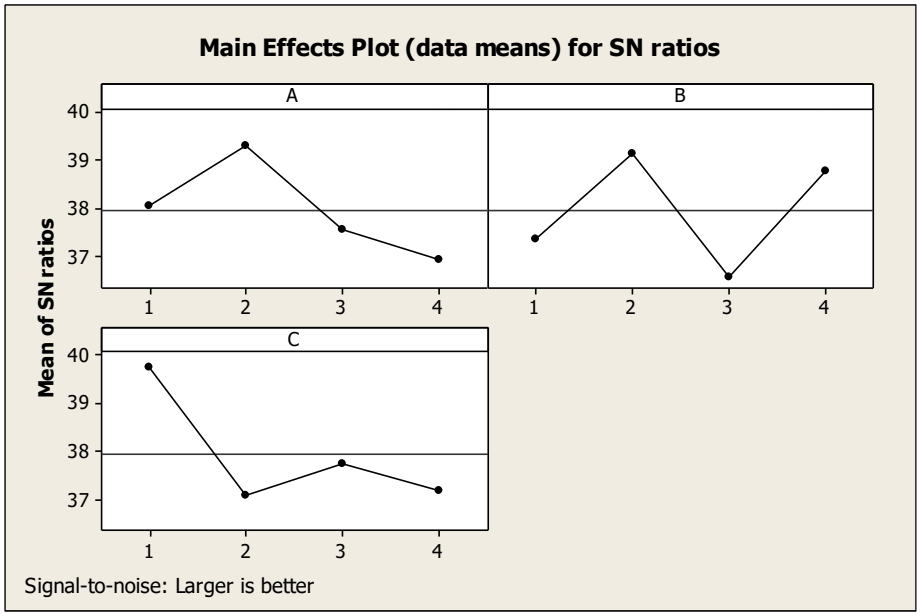


Figure 4.13 Average S/N Ratios for All Process Parameters Affecting the DHSA Yield

Analysis of variance (ANOVA) is a statistical technique used to objectively detect differences in the mean performance of groups under investigation. By comparing mean square values with estimates of experimental error at specified confidence levels, ANOVA identifies the significance of both main factors and their potential interactions [76]. This approach allows the relative contribution of each process parameter to the response to be classified, with the total variability partitioned into sums of squares representing deviations from the overall mean [77]. The ANOVA results for the epoxidation of neem oil are summarised in Table 4.5, showing the relative influence of each parameter on dihydroxystearic acid (DHSA) yield.

Among the tested parameters, reaction stop time emerged as the most significant factor, accounting for 48.3% of the overall variability in DHSA production. This high contribution reflects its critical role in determining the duration of the process, directly

influencing the extent of epoxidation and subsequent hydrolysis. An optimised stop time ensures maximum conversion of oxirane groups into DHSA, while avoiding excessive reaction periods that may lead to side reactions or product degradation.

The second most influential parameter was water addition time, contributing 34.44% to the overall effect. This highlights the importance of controlling the timing of water introduction to ensure efficient ring opening without unnecessary dilution or inhibition of the reaction. In contrast, the water/neem oil molar ratio accounted for 17.3% of the variability. Although its effect is less dominant, the ratio remains relevant as it dictates the relative availability of water molecules for hydrolysis. However, its influence is secondary to the timing-related factors, which more directly govern the kinetics and efficiency of the conversion process.

Overall, the combined analysis confirmed that the optimum operating conditions at water/neem oil molar ratio of 2:1, a water addition time of 90 minutes, and a reaction stop time of 120 minutes produced the highest hydroxyl value of 129.4 mg KOH/g. This outcome demonstrates not only the importance of carefully balancing reaction parameters but also the effectiveness of Taguchi optimisation validated through ANOVA in maximising DHSA yield.

Table 4.5
ANOVA Results for DHSA Formation [78]

Factor	DOF (f) (Degree of Freedom)	The sum of Squares (S)	Mean Square (M)	*F*-Value	*P*-Value	Percentage Contribution (%)
A	3	895	298	0.57	0.648	17.3
B	3	1609	536	1.14	0.371	34.44
C	3	2066	689	1.60	0.241	48.34

4.8 Characterization, FTIR Analysis, and Moisture Content of DHSA

4.8.1 FTIR Analysis of Epoxidized Neem Oil and DHSA

The FTIR spectra compared are those of epoxidized neem oil and DHSA produced after a hybrid approach of in situ and ex situ hydrolysis. The focus is on how their functional groups differ. Several key points stand out, including the loss of C=C

double bonds, the presence of OH groups in DHSA, and the carbonyl (C=O) and C–O stretching bands, which will be discussed further.

Loss of C=C double bonds and formation of oxirane (epoxy) rings. Pure neem oil and DHSA, both derived from unsaturated fatty acids, typically exhibit a C–H stretch for non-conjugated alkenes around 3010 cm^{-1}. In epoxidized oils, this band disappears because the carbon-carbon double bonds have been consumed during epoxidation. Simultaneously, a new absorption band appears around 820–830 cm^{-1}, which is a characteristic of epoxy ring vibrations that is absent in the unmodified oil. This band arises from the three-membered oxirane ring (C–O–C) and provides the most direct evidence of epoxide formation. In the spectrum, the C–O epoxide marker near approximately 900–1000 cm^{-1} corresponds to this new oxirane vibration [79].

Presence of hydroxyl groups in DHSA. Pure DHSA is produced by dihydroxylating an unsaturated fatty acid, which contains both a carboxylic acid and two secondary alcohol (–OH) groups on the 9- and 10-carbon atoms. The FTIR spectra of such compounds show a very broad O–H stretching absorption centred around 3300–3500 cm^{-1}. This band is also known as O–H by-product, which is prominent in the DHSA spectrum but much less intense in the epoxidized oil because epoxidation consumes double bonds rather than introducing hydroxyls. Any O–H band in the epoxidized oil would originate from minor ring-opening side reactions or residual moisture rather than from the main functional groups.

Carbonyl (C=O) and C–O stretching. Both samples contain ester/carboxylic acid functionalities from the triglyceride backbone. The ester carbonyl stretch usually occurs around 1745–1750 cm^{-1}, while carboxylic acid carbonyls appear slightly lower. Hydrolysis, which can accompany epoxidation, reduces the carbonyl stretching frequency and produces additional C–O bands near 940 and 1300 cm^{-1}. In epoxidized oil, these peaks may shift because the neighbouring epoxide ring alters the electronic environment of the carbonyl. In contrast, DHSA retains the typical fatty acid carbonyl band and shows strong C–O stretching bands for alcohols in the 1000–1200 cm^{-1} region.

In summary, the main spectral differences between pure DHSA and epoxidized neem oil are the presence of broad O–H stretching bands and alcohol C–O bands in DHSA, versus the disappearance of the C=C stretching band and the appearance of an oxirane ring band around 820–830 cm^{-1} in the epoxidized oil. These differences correspond directly to the chemical changes that occur during epoxidation. Dihydroxystearic acid is more polar because of its two free OH groups and acid

function, whereas epoxidized neem oil has non-polar oxirane rings and fewer hydroxyls. This difference is reflected in the relative intensities of the O–H band and the epoxide ring band. The following figure shows the FTIR spectra for both epoxidized neem oil and DHSA.

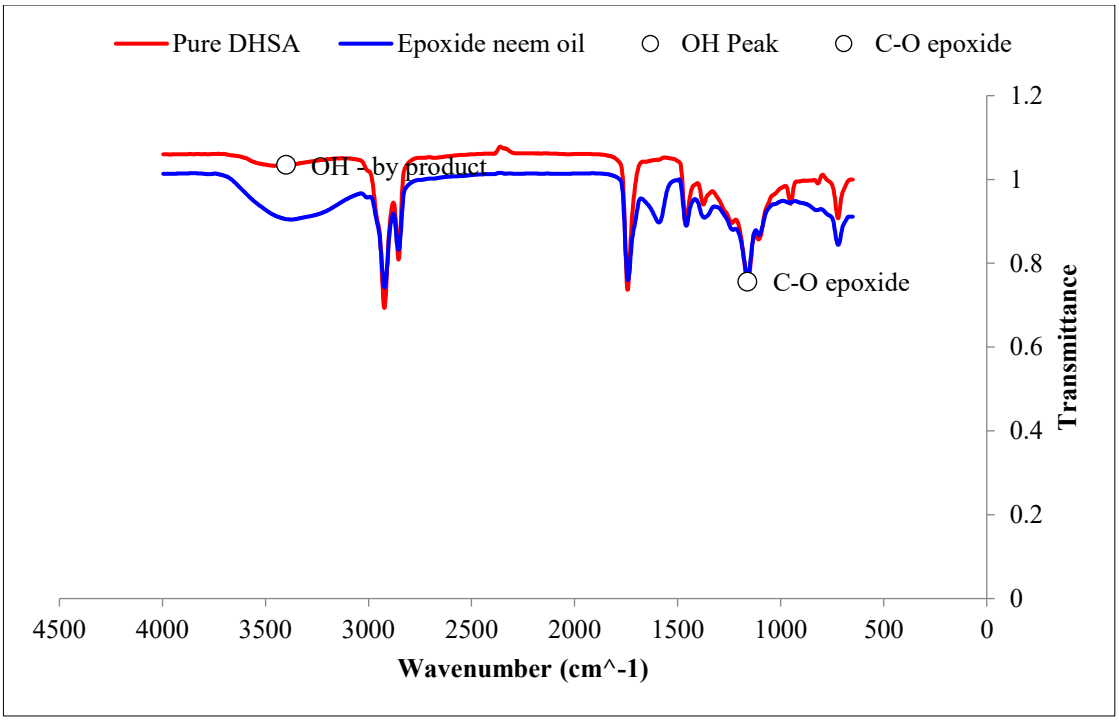


Figure 4.14 FTIR Spectrum of Epoxidized Neem Oil and DHSA

4.9 Moisture Content of DHSA

Moisture is a critical quality parameter for oils and oil-derived materials because it affects shelf stability, susceptibility to hydrolysis, and microbial growth. Neem oil contains about 6% water, while DHSA contains around 8% water. The higher moisture level in DHSA can be rationalised by its chemistry, in which DHSA has two secondary OH groups and a carboxylic acid, making it more hydrophilic and more prone to absorb atmospheric moisture than the relatively hydrophobic triglyceride molecules in neem oil. This hygroscopic nature can lead to a higher equilibrium moisture content even under the same storage conditions, where it can even crystallise with a molecule of water, demonstrating its ability to form strong polar interactions. This inherent hydrophilicity means that DHSA will readily adsorb and retain moisture from its surroundings, leading to a higher equilibrium water content.

Epoxidized neem oil, on the other hand, is still largely composed of triglycerides. During epoxidation, the double bonds in the fatty acid chains are converted to oxirane rings, but no new OH groups are introduced. As a result, the resulting molecule is relatively non-polar and lacks the hydrogen-bonding sites that would attract water. Oils of this type tend to repel water rather than absorb it, stabilising at a lower moisture level. Excess water in oils promotes hydrolytic breakdown and rancidity; therefore, the lower moisture content in epoxidized neem oil is actually beneficial for its storage stability [80].

The difference of 2% points might be small, but its implications are significant. Studies on edible oils show that as moisture content rises, water activity increases, and the oil becomes more susceptible to hydrolytic degradation. Elevated water levels promote hydrolysis of triglycerides, which results in an increase in free fatty acid content during storage. High moisture content also encourages rancidity and supports the growth of moulds, such as *Aspergillus niger* and *Mucor* species. These effects shorten the shelf life and reduce product quality. From a processing perspective, excess water in oils can interfere with chemical modifications. For example, water readily opens epoxide rings; hence, controlling moisture is crucial when producing or storing epoxidized products [81].

In practical terms, keeping neem oil at a moisture level around 6% helps maintain stability, but the slightly higher 8% moisture in DHSA suggests the need for more careful drying and storage. Ensuring low moisture content not only helps preserve quality but also prevents unwanted side reactions and microbial contamination.

4.10 Amberlite IR 120H as Catalyst

Amberlite IR-120 H is a strong-acid ion-exchange resin produced by sulfonating a cross-linked polystyrene-divinylbenzene framework. In the hydrogen form, the resins exist as tiny, water-insoluble beads bearing sulphonic acid groups. As the acid functionality is fixed on a solid support, the material acts as a heterogeneous catalyst. It provides Brønsted acidity without dissolving into the reaction medium, can be separated by filtration, and can often be used repeatedly. These qualities make Amberlite resins attractive replacements for liquid mineral acids in many acid-driven transformations [82], [83].

Unlike strong mineral acids such as sulphuric acid, Amberlite IR-120 H does not corrode equipment and eliminates the need for neutralisation steps. Its high internal surface area provides numerous acid sites, and its polymeric matrix is compatible with organic substrates. These attributes have made the resin useful in epoxidising various vegetable oils and oleic acid derivatives, as well as in esterification and dehydration. In the context of neem oil epoxidation, using Amberlite IR-120 H yields efficient oxirane formation with fewer by-products, positioning it as a promising catalyst for greener oleochemical processes [48].

Meanwhile, when unsaturated fats are epoxidized via in situ peracid formation, Amberlite IR-120 H plays two roles. First, its acidic sites catalyse the reaction between hydrogen peroxide and a carboxylic acid to generate a peroxyacid. Second, the peroxyacid diffuses into the porous network of the resin; consequently, the polymer beads effectively become micro-reactors. A recent investigation of oleic acid epoxidation describes Amberlite IR-120 as an “insoluble gel” whose pores are filled by peracids, and the study notes that triglyceride molecules enter this gel structure and are epoxidized inside the resin, which helps limit oxirane ring opening and thus improves selectivity [83].

4.10.1 Catalyst Recycling

Catalyst recycling refers to the recovery and repeated use of catalysts after chemical reactions, which improves both the sustainability and cost-effectiveness of industrial processes [84]. By reducing the requirement for fresh catalyst material and minimising waste generation, recycling is particularly advantageous when expensive or scarce resources are employed. In this study, the reusability of Amberlite IR-120 H was assessed by measuring the relative conversion to oxirane (RCO) during successive reaction cycles. Reusing catalysts not only conserves valuable resources but also reduces operational costs and environmental impacts by limiting the continuous procurement of new materials. Amberlite, a heterogeneous catalyst, has demonstrated effectiveness in aqueous hydrogen peroxide systems, where it enhances epoxidation efficiency while lowering the acidity of the final epoxide product. The reaction mechanism generally involves three steps: (1) the reversible generation of peroxy acid from hydrogen peroxide in the presence of a strong acid catalyst, (2) the transfer of

organic acid and peracid into the organic phase based on their partition coefficients, and (3) epoxidation within the organic phase to produce the desired epoxide.

Following each epoxidation cycle, the Amberlite catalyst was separated by filtration, washed sequentially with acetone and water to eliminate residual solution, and subsequently dried and weighed to determine any material loss. This procedure was repeated prior to each reuse. The RCO was employed as a performance indicator, providing a measure of the catalyst's ability to facilitate the formation of oxirane groups. Monitoring the RCO over successive cycles enabled an evaluation of catalyst stability and efficiency during repeated use.

The recycling results revealed a gradual decline in catalytic activity with successive runs. A reduction in RCO of approximately 5% was observed in the second use compared to the initial run, followed by decreases of 17% and 34% after the third and fourth cycles, respectively. This trend, illustrated in Figure 4.15, indicates progressive catalyst deactivation, which may be attributed to surface fouling, pore blockage, or partial loss of active sites during handling and washing. Nevertheless, the results demonstrate that Amberlite IR-120 H retains reasonable activity over multiple cycles, making it suitable for applications where sustainability and cost reduction are priorities. The findings also highlight the wider relevance of recycling in processes involving rare or costly catalysts, such as platinum-based systems, where effective reuse is essential for economic and environmental viability.

In terms of deactivation mechanisms, several possibilities may explain the observed decline in Amberlite activity. Firstly, leaching of functional sulfonic acid groups during repeated washing and reaction cycles may reduce the overall number of active catalytic sites. Secondly, adsorption of organic by-products or residual peroxy acids onto the resin surface can lead to fouling, thereby limiting accessibility of reactants to active sites. Additionally, structural degradation of the polymer matrix under prolonged exposure to acidic and oxidative conditions may contribute to the progressive loss of catalytic efficiency. Mass transfer limitations may also become more pronounced if pore blockage occurs due to accumulation of heavy intermediates or degradation products. Taken together, these factors underline the importance of optimising catalyst handling and regeneration procedures to prolong effective reusability.

Looking ahead, several strategies could help maintain or even enhance Amberlite's reusability in epoxidation processes. Mild regeneration treatments, such as

acid washing or solvent rinsing, could remove adsorbed by-products and restore active sites. Optimising reaction conditions to minimise oxidative stress on the polymer backbone would help reduce structural degradation. In industrial settings, blending recycled Amberlite with small amounts of fresh catalyst may compensate for gradual losses in activity while keeping costs low. Additionally, exploring support modifications or coating techniques could increase the catalyst's resilience against leaching and fouling. By implementing such measures, the lifetime and efficiency of Amberlite IR-120 H could be extended, thereby improving the economic feasibility and sustainability of large-scale epoxidation processes.

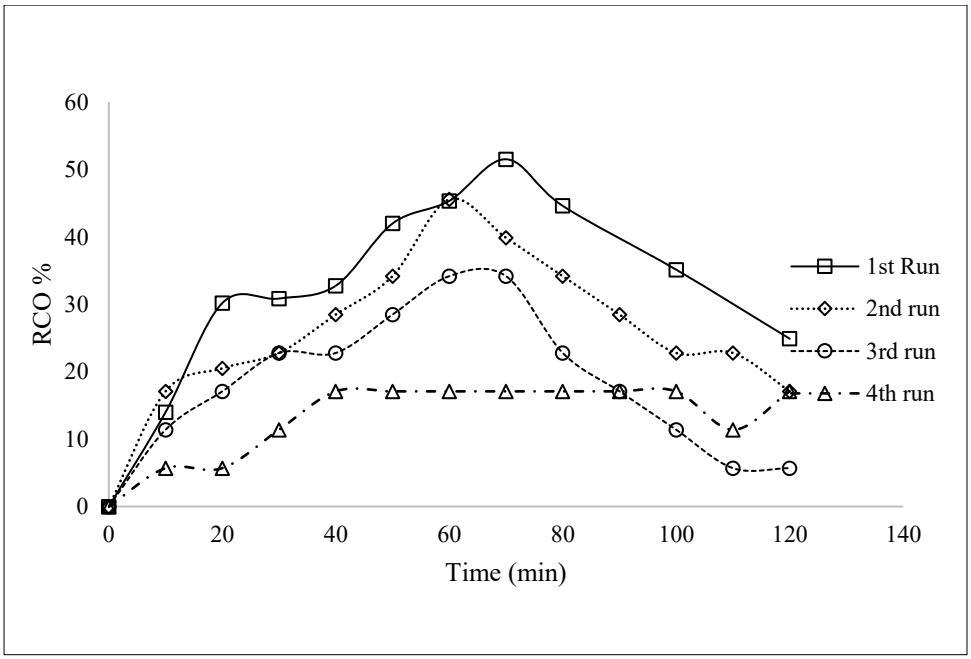


Figure 4.15 RCO% Profile for Repetitive Epoxidation

4.10.2 Catalyst Characterisation

In this investigation, Amberlite IR-120 H, a heterogeneous catalyst, was employed to promote the epoxidation of neem oil. The catalyst was utilised in its original form, without any pretreatment or chemical modification. As such, extensive characterisation was not undertaken, since the central focus of this research was on the epoxidation process itself and the optimisation of reaction parameters determined through the Taguchi method. Nevertheless, Brunauer–Emmett–Teller (BET) analysis and scanning electron microscopy (SEM) were performed to confirm the fundamental characteristics of the catalyst. BET measurements revealed a surface area of 3.27 m^2/g

and a pore volume of 1.084 mm^3/g, indicating the presence of sufficient active sites to enhance the epoxidation rate of neem oil [85]. In addition, SEM imaging (Figure 4.16) demonstrated the spherical morphology of the resin, consistent with earlier reports [86], further supporting its suitability as an effective heterogeneous catalyst.

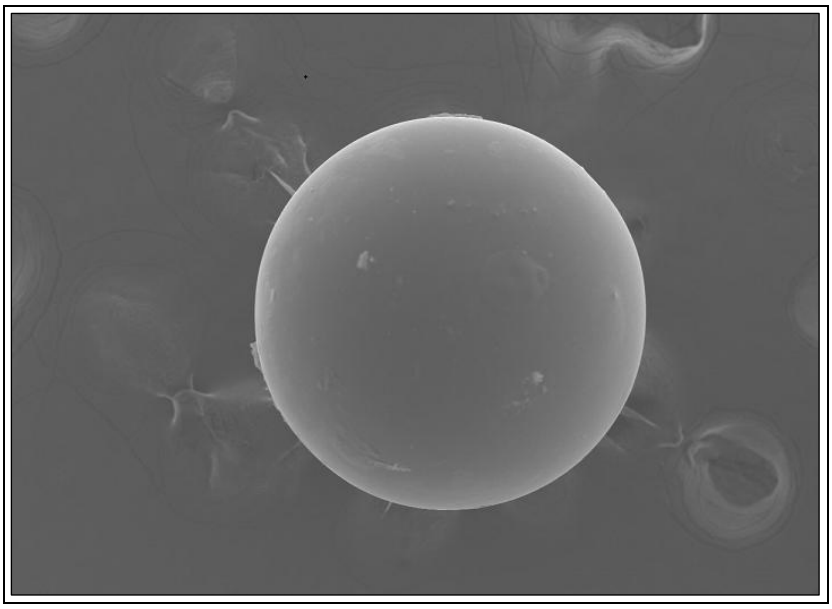


Figure 4.16 SEM Image of Spherical Shape of the Catalyst

Table 4.6
Characterization for Fresh and Recycle Amberlite

	Surface Area (m^2/g)	**Pore Volume (mm^3/g)**
Fresh Amberlite	3.27	1.084
Recycle Amberlite	0.056	0.042

The structural properties of Amberlite undergo significant changes after its use in the epoxidation reaction. Initially, fresh Amberlite exhibits a surface area of 3.27 m^2/g and a pore volume of 1.084 mm^3/g, indicating a well-developed porous network that facilitates efficient catalytic activity. The high surface area provides abundant active sites for reactant adsorption, while the substantial pore volume enhances mass transfer, both of which contribute to optimal catalytic performance in the epoxidation process. These characteristics are crucial for ensuring effective conversion and selectivity during the reaction.

However, after the epoxidation process, the recycled Amberlite undergoes a significant decline in structural properties, with its surface area dropping to 0.056 m^2/g and pore volume decreasing to 0.042 mm^3/g. This substantial reduction suggests pore blockage, adsorption of by-products, or possible structural degradation, all of which significantly impact its catalytic efficiency. The loss of active sites and diminished porosity hinder the catalyst's ability to facilitate further epoxidation, ultimately reducing reaction performance. These findings underscore the importance of implementing effective regeneration strategies to restore the structural integrity of Amberlite and maintain its catalytic functionality in repeated reaction cycles.

4.11 Kinetic Modelling

4.11.1 Numerical Kinetic Modelling of Neem Oil Epoxidation

As presented in Table 4.7, the kinetic constants obtained from the proposed models were compared with those derived experimentally. A clear understanding of the reaction kinetics is essential, as it enables the prediction of conversion behaviour under defined operating conditions. Fundamental considerations in kinetic studies include the overall reaction rate, product selectivity, and the extent to which these depend on process variables. In this case, performic acid forms rapidly from the interaction of hydrogen peroxide and formic acid, a strongly exothermic reaction that can proceed even in the absence of a catalyst. Under suitable conditions, the generation of performic acid occurs swiftly, releasing heat and thereby necessitating careful control to limit losses during the subsequent addition of neem oil for epoxide production.

The rate of epoxidation was observed to increase with higher concentrations of peracid and elevated reaction temperatures, while also showing a positive correlation with substrate concentration up to a limiting point. The optimised rate constants for neem oil epoxidation are summarised in Table 4.7. The values obtained were $k_{11} = 0.32$ mol/L·min, $k_{12} = 12.44$ mol/L·min, and $k_2 = 0.032$ mol/L·min, with a correlation coefficient (R^2) of 0.84, indicating a good fit between the model and experimental data.

Table 4.7
Rate Constant for Epoxidation of Neem Oil at Optimized Process Parameters

Reaction Rate Constant	**Value (mol/L·min)**
k_{11}	0.32
k_{12}	12.44
k_2	0.032
Correlation coefficient, $R2$ = 0.84	

Figure 4.17 shows epoxide formation starting at 20 minutes in the simulation, while experiments indicate maximum oxirane levels are reached at 40 minutes. This disparity arises from simulation assumptions, such as ignoring heat transfer and loss. In experiments, heat loss occurs post-epoxidation, and the exothermic addition of performic acid increases system heat [87]. Next, the performic acid reaction are irreversible during the simulation. However, the performic acid tend to reverse to its form during the heat loss after epoxidation thus explaining the reduction of oxirane oxygen content by experiment [88]. Both of this factor explain the disparity between simulation and experiment.

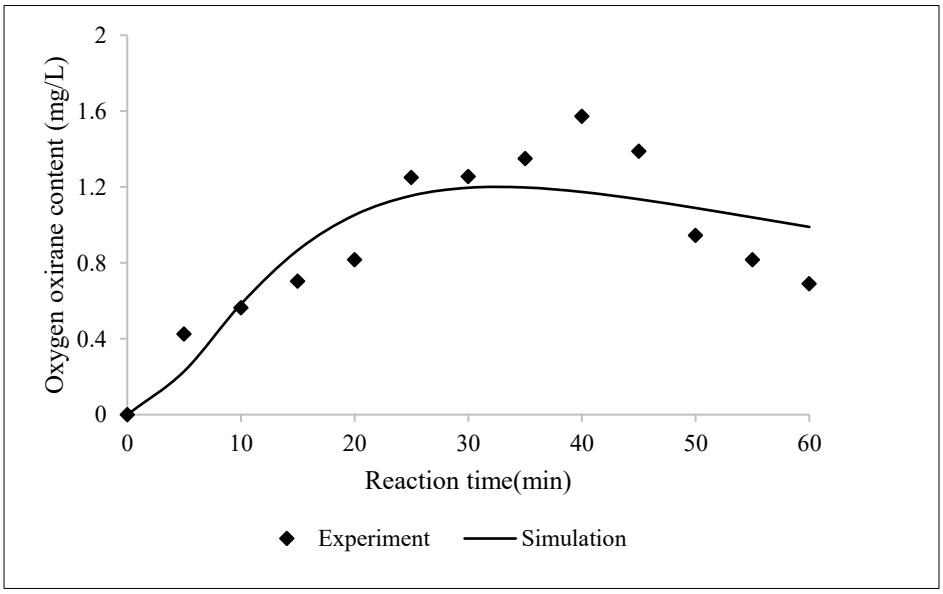


Figure 4.17 Oxygen Oxirane Content for Simulation and Experiment

4.11.2 Numerical Kinetics Model DHSA Production

Kinetic modelling plays a crucial role in optimising the epoxidation process, as it enables both the prediction of reaction rates and the estimation of residence times required to achieve target conversion levels. In this study, MATLAB was employed to simulate the kinetics of hybrid oil epoxidation. A genetic algorithm was used to fit the experimental data, while the Runge–Kutta fourth-order method (ode45) was applied to solve the system of differential equations. The integration of these advanced numerical approaches provides a reliable framework for describing and predicting the kinetic behaviour of epoxidation reactions, offering a practical tool for refining process design and optimisation.

The results demonstrated that the rate constant for performic acid formation (k_{12}) was lower than that of the reverse reaction (k_{11}), highlighting the tendency for performic acid to decompose more readily than it is formed. This observation is significant, as performic acid functions as a key intermediate in the epoxidation pathway, directly influencing the rate of epoxide production (see Table 4.8). Consequently, efficient control over performic acid stability is critical to maximising oxirane formation.

When compared with values reported in earlier studies, the kinetic parameters obtained for hybrid oil epoxidation fall within the established ranges, although oxirane ring degradation was observed to occur at an earlier stage. This consistency with literature results validates the robustness of the MATLAB-based model, while the minor deviations highlight the sensitivity of oxirane stability to process conditions. Overall, the model developed here provides a reliable predictive tool for understanding the behaviour of hybrid oils during epoxidation and can serve as a foundation for future process optimisation and scale-up studies [89].

Table 4.8
Rate Constant for Epoxidation of Neem Oil and Ring Opening by Hybrid Hydrolysis

Reaction Rate Constant	**Value (mol/L·min)**
k_{11}	3.60
k_{12}	0.94
k_{2}	0.10
k_{3}	0.02
Correlation coefficient, r = 0.90	

Figure 4.18 compares experimental and simulated EPOA concentrations at the optimal temperature of 100°C, showing close alignment with a ~0.20 difference. However, deviations occur between 30 and 40 minutes because the simulation assumes ideal conditions without accounting for heat loss or mass transfer limitations. In contrast, the experimental setup may experience heat loss, temperature gradients, and system inefficiencies that the model does not capture. Since the simulations rely solely on chemical reaction equations under ideal conditions, practical limitations in the experiment, such as non-uniform heating and imperfect insulation, contribute to these differences. The deviations observed in the initial stage of the reaction are attributed to non-steady-state conditions, including mass transfer limitations, temperature stabilisation, and early-stage sampling variability. As the reaction progresses, the experimental and simulated values converge, indicating improved model accuracy at steady-state conditions.

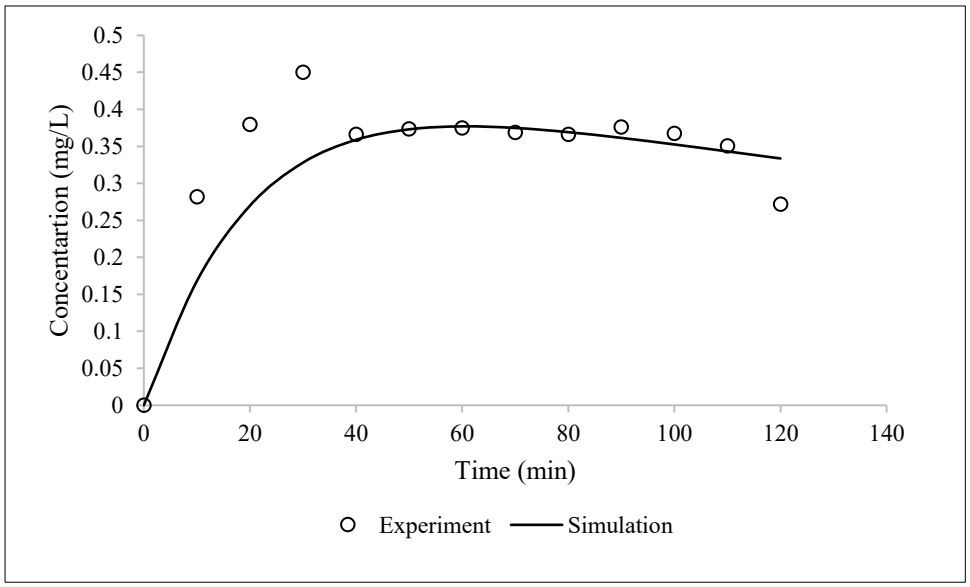


Figure 4.18 Comparison of the Oxirane Content Predicted by Genetic Algorithm from Experiment

4.12 Concluding Remarks

The results of this study clearly demonstrate the feasibility of neem oil as a promising renewable feedstock for epoxidation. Through the application of the Taguchi method, the most influential parameters, namely catalyst loading, formic acid/neem oil ratio, and stirring speed were systematically identified and optimized to enhance

oxirane oxygen content. Supporting analyses, including iodine value reduction, deterioration of bleachability index (DOBI), and FTIR confirmation of oxirane ring formation, validated the success of the epoxidation process under the chosen conditions. These findings provide a strong foundation for further valorisation of neem oil beyond its conventional applications as a pesticide or medicinal source.

The subsequent hydrolysis of epoxidized neem oil to dihydroxystearic acid (DHSA) revealed that a hybrid in situ–ex situ approach produced superior results compared to conventional single-step hydrolysis. Higher hydroxyl values, improved functional group incorporation, and increased stability of the product confirmed the effectiveness of the method in enhancing oxirane ring opening. This approach not only reduced reaction time but also eliminated the need for additional transesterification steps, making the process more cost-effective and industrially viable. The success of DHSA production from neem oil highlights its potential as a high-value bio-based material suitable for use in cosmetics, lubricants, and polymer synthesis.

Finally, catalyst reusability tests and kinetic modelling provided additional evidence of process sustainability and scalability. Amberlite IR-120H was proven to retain its catalytic performance across multiple cycles, reinforcing its role as a durable heterogeneous catalyst. Meanwhile, the developed kinetic model accurately described both epoxidation and hydrolysis behaviours, offering predictive insights into reaction mechanisms and enabling reliable process optimisation. Together, these results establish a comprehensive framework for sustainable DHSA production from neem oil and open pathways for industrial adoption of bio-based alternatives to petroleum-derived chemicals.

CHAPTER 5
CONCLUSION

5.1 Overall Conclusion

In recent years, epoxidised neem oil has gained significant importance as an industrial product due to the renewable, sustainable, and environmentally friendly nature of vegetable oils. This study specifically focused on the epoxidation of neem oil, a primary source of fatty acids, by employing in situ generated performic acid to produce epoxidised neem oil. The increase in relative conversion of oxirane (RCO%) following Taguchi S/N ratio analysis demonstrated that epoxidation of neem oil using Amberlite IR-120H was successfully achieved. Research into renewable resources can be further diversified through the epoxidation of non-edible oils, which are more cost-effective than edible alternatives. The epoxidation process was investigated by varying formic acid molar ratio, catalyst loading, temperature, and stirring speed. The highest RCO achieved was 52% under optimal conditions, with experimental and simulated values closely aligned (difference of ~0.20), as confirmed by the kinetic study.

The use of non-edible vegetable oils for epoxidation has significant implications for green technology, as it offers an alternative to petroleum-based products. Employing performic acid as the oxidising agent and Amberlite IR-120H as the catalyst proved successful in the epoxidation of neem oil. The effects of varying parameter levels, as discussed, provide valuable data for future research. Maximum RCO was obtained when the formic acid/neem oil molar ratio was 1:1, hydrogen peroxide/neem oil 4:1, the reaction temperature maintained at 70 °C, and stirring speed at 300 rpm, with 1 g of catalyst loading. FTIR spectroscopy confirmed the presence of the oxirane ring at 1240 cm^{-1}, while the minimal disparities between kinetic simulation and experimental results further validated the feasibility of neem oil epoxidation. This study highlights the impact of different parameter levels on the epoxidation rate of neem oil.

Dihydroxystearic acid (DHSA) has emerged as a valuable industrial product owing to the renewable and eco-friendly nature of neem oil. In this study, oleic acid from neem oil was epoxidised using in situ generated performic acid, followed by hydrolysis to produce DHSA. The highest hydroxyl value (129.4 mg KOH/g) was achieved under optimal conditions: a water/neem oil ratio of 2:1, water addition for 90

minutes, and a reaction stop time of 120 minutes. ANOVA results indicated that reaction stop time was the most significant factor influencing the hybrid hydrolysis of epoxidised neem oil. A strong correlation between experimental and simulated values under optimal conditions was observed, with only a ~0.20 difference in kinetics. While the study objectives were achieved, further research could explore additional value-added applications of epoxides after ring opening, particularly in the production of DHSA for the lubricant, polymer, and adhesive industries, where its properties may offer substantial performance advantages.

Overall, the numerical kinetic models provided a satisfactory representation of the experimental trends for neem oil epoxidation and DHSA formation, suggesting their applicability for process analysis. The rate constants obtained provide valuable insight into the reaction mechanism, particularly the rapid yet reversible formation of performic acid and the comparatively slower but consistent hydrolysis of epoxidised neem oil to DHSA. Minor discrepancies between simulation and experimental profiles, especially in oxirane content, were attributed to idealised model assumptions that excluded heat loss and mass transfer limitations inherent in practical systems. Nonetheless, the integration of genetic algorithms with MATLAB's Runge–Kutta solver established a reliable framework for predicting reaction behaviour and optimising process conditions. DHSA produced in this study are supported by kinetic interpretation.

The highest relative conversion of oxirane (RCO%) was achieved when using pure neem oil as the raw material. However, since oleic acid is one of the main contributors to oxirane formation only makes up about 40% of neem oil, the maximum RCO% reached was around 52%. This suggests that by mixing neem oil with additional oleic acid, the overall oleic acid content could be increased, potentially leading to a higher RCO% in future studies.

- Highest relative conversion of oxirane achieved by using neem oil purely as raw material can be mixed with oleic acid for future study. This is because, oleic acid is the main component in the neem oil that contribute to the RCO%. Since oleic acid contain in neem oil is only 40%, explaining why RCO% conversion is at 52%. And if neem oil is mixed with oleic acid, total percentage of oleic acid would increase, hence higher RCO% conversion could be achieved.

For a greener and safer epoxidation process, acetic acid is a better alternative to formic acid. It is less toxic, less corrosive, and poses fewer health risks, while also reducing epoxide ring opening and improving oxirane yield.

- For greener epoxidation, acetic acid is the better choice. Formic acid is more corrosive and hazardous and exposure can cause severe burns and lung injury, and the permissible exposure limit is only 5 ppm. Acetic acid, while still corrosive, is less toxic and has a higher allowable exposure of 10 pp. Moreover, formic acid tends to promote ring-opening of the epoxide, leading to lower oxirane yields. By using acetic acid you minimise health hazards and waste formation, making the process safer and more environmentally friendly.

In the epoxidation of neem oil, using water for ring opening leads to epoxide degradation and lower oxirane content. Replacing water with an alcohol like methanol or ethanol can prevent this, producing more stable and valuable alkoxy hydroxy compounds instead.

- For epoxidized neem oil, hydrolysis by water converts the oxirane ring into a diol, which effectively destroys the epoxide and lowers the oxirane content. If you instead use an alcohol such as methanol or ethanol as the nucleophile, the epoxide opens to give a β-alkoxy alcohol rather than a 1,2-diol. This "alcoholysis" route produces useful alkoxy-hydroxy derivatives and avoids the rapid ring degradation caused by water. Therefore, replacing water with an alcohol in the ring-opening step can preserve epoxide functionality and yield more stable, value-added products.

Amberlite IR-120H can remain the main solid acid catalyst, but adding a small amount of sulfuric acid can boost its acidity and improve epoxide yield. Using only a low concentration helps maintain stability and keeps the system easy to filter and reuse.

- Continue to use Amberlite IR-120H as the primary solid acid catalyst, but consider impregnating or co-dosing it with a low level of sulfuric acid to enhance its acidity. Keep the sulfuric-acid concentration modest (around 2 % of the aqueous phase) to avoid excessive oxirane ring opening and maintain the

advantages of a heterogeneous system. This approach can improve epoxide yield while still allowing the catalyst to be filtered off and reused.

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APPENDICES

APPENDIX 1

Calculation For Mole and Mass of Reactants Used

The crude oleic acid consists of 75% oleic acid (OA), 12.2% linoleic acid (LA), 6.5% palmitic acid (PA) and 7.5% stearic acid (SA). Oleic acid (C18:1) has one unsaturation (double bond), linoleic acid (C18:2) has two unsaturation while both palmitic acid (C16:0) and and stearic acid (C18:0) has no unsaturation on their respective hydrocarbon chain.

Unsaturation mole fraction, $f_{DBi} = xi \times Dbi$ (where DB = number of double bond)

$f_{DBOA} = 0.75 \times 1 = 0.75$, $f_{DBLA} = 0.122 \times 2 = 0.244$, $f_{DBPA}16:0 = f_{DBSA}18:0 = 0$

f_{DBOA} in crude OA = $f_{DBOA} / (f_{DBOA} + f_{DBLA}) = 0.75/(0.75 + 0.244) = 0.7545$

IV of crude oleic acid = 97.87

IV of oleic acid fraction = $0.7545 \times 97.87 = 73.84$ g I_2/100 g sample

Mole = mass/molecular weight

Mole of POA = 100 g/282.47 g/mol = 0.3540 mol

Mole of POA fraction is 0.3540 for 100 g basis of crude oleic acid. Subsequently, the mole and mass needed for other reactants are listed in Table A.1.

Table A.1: Mass of Reactant Used

Reactant	Unsaturation Molar Ratio	Mole	Mass (g)
Neem oil	1	0.354	100
Formic acid	0.5: 1	0.177	8.147
	1:01	0.354	16.29
	1.5: 1	0.531	24.44
	2.0:1	0.708	32.59
Hydrogen peroxide	0.5: 1	0.177	6.01
	1:01	0.354	12.06
	1.5: 1	0.531	18.04
	2.0: 1	0.708	24.07

APPENDIX 2

Standard Method for Oxirane Oxygen Content Determination (AOCS Tentative Method Cd 9-57)

This method is used to determine oxirane oxygen, i.e., oxygen contained in EPOA. Under the prescribed conditions of this test method, the oxygen is titrated directly with hydrogen bromide in acetic acid. This method is applicable to epoxidized fatty substances and epoxy compounds in general.

Detailed experimental procedure for performing this analysis as follows:

1. Apparatus

   Burette assembly, titration flask with one-hole rubber stopper, 50 mL Erlenmeyer flask and a magnetic stirrer with round Teflon-covered magnetic stirring bar.

2. Reagent and solutions

   1. Glacial acetic acid (A.C.S grade)
   2. Crystal violet indicator solution, prepared by dissolving 0.1 g of crystal violet in 100 mL of glacial acetic acid.
   3. 0.1 N HBr in acetic acid was prepared by diluting 30 to 32% HBr in acetic acid with glacial acetic acid to approximately 0.1 N. Hydrogen bromide solution normality was determined according to Equation A.1

$$\text{Normality} = \frac{\text{Weight of Phthalate (g)}}{0.2042 \times \text{titration (m)}} \qquad \text{A.1}$$

3. Procedure

   1. 0.3 to 0.5 g of sample weighed into a 50 mL Erlenmeyer flask. The sample then dissolved in acetic acid (10 mL). Subsequently, five drops of crystal violet solution were added into the Erlenmeyer flask.
   2. Teflon magnetic stirring bar was added into the Erlenmeyer flask,
   3. The solution was titrated under the of agitation with the standardized hydrogen bromide (HBr) solution to a bluish green end point that persisted at least 30 second.

APPENDIX 3

Method for Iodine Value Determination (C2.6:2004-MPOB)

1. Apparatus

   A 500 mL glass-stopper iodine flask, a 1000 mL glass-stopper volumetric flask, a 25 mL pipette, a 20 mL volumetric dispenser, a hot air oven and a magnetic stirrer.

2. Reagents and solutions

   1. Potassium iodine (KI) solution, prepared by dissolving 100 g of reagent grade KL in 1000 mL of deionized water.

   2. Reagent for sample dilution, prepared by mixing cyclohexane and glacial acetic acid at the volume/volume (v/v) ratio of 1/1

   3. Sodium thiosulphate ($Na_2S_2O_3.5H_2O$) 0.1 N, prepared from reagent grade sodium thiosulphate. In order to obtain the accurate concentration (in normality) of the sodium thiosulphate solution prepared, standardization was carried out. This was done by titrating potassium dichromate solution with the prepared sodium thiosulphate solution.

   4. Potassium dichromate solution was prepared by dissolving around 0.16 to 0.22 g of finely ground and dried potassium dichromate ($K_2Cr_2O_7$) in 25 mL of water which was added with 5 mL of concentrated hydrochloric acid and 20 mL of potassium iodide solution. It was done under influence of agitation until yellow color almost disappeared.

   5. 1 to 2 mL of starch indicator was added into the potassium dichromate solution and titration was continued until the blue color of the solution was disappeared. Normality calculated according to Equation A.1

$$\text{Normality} = \frac{20.394 \times \text{mass of } K_2Cr_20_{7\ (\text{in g})}}{\text{titration volume of } Na_2S_2O_{3\ \text{in ml}}} \qquad \text{(B.1)}$$

3. Procedures

   1. Around 0.2 (±0.001) g of sample was weighed into 500 mL flask and added with 15 mL of the prepared cyclohexane-glacial acetic acid mixture. T

2. The flask content was then stirred to ensure that the sample was completely dissolved.
3. Subsequently, 25 mL of Wijs solution was pipette into the flask containing the sample and then it was swirled. The flask was then stored in dark area for exactly 60 minutes at the temperature of 25 (± 5)^{0}C to allow reaction within the flask content.
4. After the 60 minutes' reaction time, the flask was quickly removed from dark area and the reaction was quenched (terminated) by adding with 20 ml of potassium iodide solution, followed by 100 ml of distillated water.
5. The flask content was then titrated with the standardized sodium thiosulphate solution. The titration with sodium thiosulphate solution was done under the influence of agitation until yellow color solution almost disappeared.
6. 1 to 2 mL of starch indicator was added into the potassium dichromate solution and titration was continued until the blue color solution disappeared.
7. The iodine value of crude oleic acid sample was also prepared and titrated exactly in the same manner as the crude oleic acid samples and calculated by using Equation A.2

$$\text{Iodine value: } \frac{(B-S)\times M\times 12.69}{m} \qquad \text{(B.2)}$$

Where,

B is the volume of sodium thiosulphate used for titration blank

S is the volume of sodium thiosulphate used for titration of sample

M is the molarity, mol/L of sodium thiosulphate solution

m is the numerical value of the mass of test potion, in gram

APPENDIX 4

Acid Value
Method for Determination of Acid Value (C2.7:2004 – MPOB)

1. Apparatus

   A 500 mL glass-stopper iodine flask, a 1000 mL glass-stopper volumetric flask, a 25 mL pipette, a 20 mL volumetric dispenser, a hot air oven and a magnetic stirrer.

2. Reagents and solutions

   1. 5g of sample was dissolved in 75 mL of hot neutralized alcohol. Agitation and further heating may be necessary to bring the fatty acid into solution.

   2. 0.5 ml of indicator was added and titrate immediately with 0.5M NaOH to the first pink color which persists for 30 s and based on Equation B.1

$$\text{Acid value: } \frac{56.1 \times V \times M}{m} \qquad \text{(C.1)}$$

   Where,

   V is the volume in milliliters of standards volumetric sodium hydroxide solution used

   M is the exact concentration in mole per liter of the standard volumetric sodium hydroxide solution used

   m is the mass in grams of the test portion.

APPENDIX 5

MATLAB Simulation

objFunction

```
function G = objFunc(k, Texp, Cexp, C0)

[~,Cspecies]=ode45(@(t,c) odeRK(t,c,k), Texp, C0);

C=Cspecies(:,6);

errAbs = C - Cexp;
errAbs=errAbs.^2;

absError=sum(errAbs);

[sizeExp,~] = size(Texp);

G=(absError/(sizeExp-2))^(0.5);
```

OptK

```
clc
clear all

filename = fullfile('C:\MATLAB\95.txt');
ExpData = load(filename, '-ascii');

Texp=ExpData(:,1);
Cexp=ExpData(:,2);

% Initial Concentration Values
C0(1)=2.9484;       % [FA]
C0(2)=4.4226;       % [HP]
C0(3)=0;            % [PA]
C0(4)=0;            % [H2O]
C0(5)=2.9484;       % [OA]
C0(6)=0;            % [EPOXIDIZED]

lb = [0, 0, 0, 0, 0];
ub = [15, 15, 15 , 0, 15];
intCon = 1:6;

[kOpt,errorMin] = ga(@(k) objFunc(k,Texp,Cexp,C0),6,[],[],[],lb,ub)

fid = fopen( 'Opt_K.txt', 'wt' );
fprintf(fid, '%f \n', [kOpt]);
fclose(fid);

fid = fopen( 'Min_Error.txt', 'wt' );
```

```
fprintf(fid, '%f', errorMin);
fclose(fid);

[T,C]=ode45(@(t,c) odeRK(t,c,kOpt), Texp, C0);
C_EPOXY=C(:,6);

plot(T,C_EPOXY,'k')
hold on;
plot(Texp,Cexp,'+')
```

odeRK

```
function dc_dt = odeRK(t,c,k)

r=zeros(3,2);

r(1,1)=k(1)*c(1)*c(2);
r(1,2)=k(2)*c(3)*c(4);
r(2,1)=k(3)*c(3)*c(5);
r(2,2)=k(4)*c(6)*c(1);
r(3,1)=k(5)*c(6)*c(4);
r(3,2)=k(6)*c(7);

dc_dt=zeros(7,1);

dc_dt(1)=-r(1,1)+r(1,2)+r(2,1)-r(2,2);
dc_dt(2)=-r(1,1)+r(1,2);
dc_dt(3)=+r(1,1)-r(1,2)-r(2,1)+r(2,2);
dc_dt(4)=+r(1,1)-r(1,2)-r(3,1)+r(3,2);
dc_dt(5)=-r(2,1)+r(2,2);
dc_dt(6)=+r(2,1)-r(2,2)-r(3,1)+r(3,2);
dc_dt(7)=+r(3,1)-r(3,2);
```

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LIST OF PUBLICATIONS:

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LIST OF INNOVATIONS

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2. Application of Epoxy Resin for Coating-Acoustic Panel- Virtual Asia Innovation Show 2022, Silver medal