

DEVELOPMENT OF POLYOLS FROM VEGETABLE OILS VIA OXIRANE RING OPENING TECHNIQUE FOR POLYURETHANE APPLICATIONS

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Abstract

Vegetable oils are a promising source of raw materials for the production of high functionality polyols. These polyols are widely used in the manufacture of high-quality products such as polyurethanes and poly(ether urethanes). In this study, a series of bio-based triglyceride vegetable oils, including *Sesamum indicum* (sesame oil), *Arachis hypogaeae* (peanut oil), *Azadirachta indica* (neem oil), *Jatropha curcas* (jatropha oil), and *Madhuca longifolia* (mahua oil), were used to prepare high molecular weight polyols. The effects of carboxyl to epoxy ratio on the ring opening of epoxy groups were investigated by using castor oil fatty diol. The resulting epoxidized oils and bio-polyols were characterized by Fourier Transform Spectroscopy (FT-IR), Proton Nuclear Resonance Spectroscopy (1H NMR), and Carbon Nuclear Resonance Spectroscopy (13C NMR). The molecular weight of the polyols was determined by Gel Permeation Chromatography (GPC), and the stability and decomposition of the polyols were characterized by thermogravimetric analysis (TGA). Rheometry was used to determine the viscosity of the polyols, and the flow rate of the polyols was derived from a power law model. The results showed that the bio-based polyols produced from vegetable oils exhibited excellent thermal stability, high molecular weight, and good rheological properties. This study provides valuable information for the development of bio-based polyols as an alternative to petroleum-based polyols. The use of vegetable oils in the synthesis of high functionality polyols could lead to a reduction in environmental pollution and provide a sustainable solution for the production of high-quality polyurethane materials.

1. Introduction

The demand for new materials has given rise to the extensive exploration of renewable resources, both in academe and in industry. Hence, the epoxidation is one of the most important functionalization reactions of the carbon-carbon double bond of fatty acids of vegetable oils. This functionalization can be achieved through

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environmentally friendly procedures such as catalysed chemical oxidation with hydrogen peroxide or by enzymatic oxidation. Epoxidised vegetable oils offer promising inexpensive renewable materials for many industrial applications because they share many of the characteristics of conventional petroleum-based epoxy thermosets. [1-3] Epoxidation is carried out by four different procedures: (i) epoxidation with peracids such as peracetic acid or perbenzoic acid in the presence of an acid catalyst; (ii) epoxidation with organic and inorganic peroxides, including transition metal catalysts; (iii) epoxidation with halohydrins using hypohalous acids and their salts and (iv) epoxidation with molecular oxygen.[4] Although, the most preferred acids for this purpose are formic and acetic acids because of their ready availability at a relatively low price in liquid form at room temperature. The order of reactivity of some of the peracids is *m*-chloroperbenzoic > performic > perbenzoic > peracetic.[1-5] The presence of electron withdrawing groups promotes the reaction. More work has been published on the synthesis of epoxidised oil by peracetic acid than of any other organic peracid. The presence of an inert solvent in the reaction mixture stabilized the epoxidation product and minimized the opening of the oxirane ring. The catalytic reaction of peroxyacetic /peroxyformic acid formation was found to be characterised by adsorption of acetic (or formic) acid and peroxyacetic/peroxyformic acid only on the active catalyst sites and the broad interest in epoxy oils has its origin in the versatility of the epoxy group towards a wide variety of chemical reactions and in producing useful properties in the finished products.

The epoxy group, often called the oxirane ring, is highly reactive and undergoes ring opening reactions. It cleaves readily to polyols by alcoholysis in the presence of alcohols/ thiols, and by hydrolysis in the presence of acid catalysts.[6] The main achievement is devoted to the preparation of polyol from plant sources due to its wide availability, economical and eco-friendly characteristics[7,11]. Several bio-based materials, including cellulose, starch, natural oil, and sugar, have been utilized as starting material for polyurethanes[17]. Vegetable oils are among the most promising, because they are an inexpensive, readily available, and renewable resource. [8-10]. The six common fatty acids are palmitic, stearic, oleic, linoleic, linolenic and ricinoleic acids [9]. The reactive sites are ester bonds, C=C, and hydroxyl group which exist in some oils. All the vegetable oils have been extensively studied for the production of polyols [6-11].

In this paper, we report on the development of polyols by the oxirane ring opening technique which can be prepare for polyurethane. The oxirane ring opening is an important class of organic equilibrium exchange reaction in which one epoxy ring opened by another fatty diol moiety. In the epoxidation of vegetable oils, the triglyceride reacts with methanoic acid followed by addition of peroxide and hence, the process is often called epoxidation. Several aspects, including the type of polyol preparation, the carboxyl to epoxy mole ratio and temperature have influence on the progress of the reaction. The castor oil fatty diol is obtained directly via saponification of castor oil, that is by treatment of the oil with alkali in the presence of HCl as a neutralizing agent. The preparation method follows, variety of vegetable oils are prepared to polyols. Such as, castor oil fatty diol (CaOD) and was used to ring- opening to epoxy groups in epoxidised sesame oil (ESO), epoxidised peanut oil (EGO), epoxidised neem oil (ENO), epoxidised jatropha oil (EJO) and epoxidised mahua oil (EIO). The effect of ring-opening duration, stoichiometry of CaOD and ESO, CaOD and EGO, CaOD and ENO, CaOD and EJO and CaOD and EIO and reaction temperature was maintained in correlation with the obtained sesame-castor polyol (SCOL), peanut- castor polyol (GCOL), neem- castor polyol (NCOL), jatropha- castor polyol (JCOL) and mahua- castor polyols (ICOL) structures and functionalities were characterized by FT- IR, ¹H NMR, ¹³C NMR and the viscosity of the polyols were characterized by Rheometry.

2. Experimental details

2.1. Materials

Vegetable oils such as, sesame, peanut, neem, jatropha, mahua oil (approximately six oxirane rings per triglyceride) was purchased from local source, Magnesium sulfate (MgSO₄), hydrogen peroxide, methyl ethyl ketone (MEK), and ethyl ether were purchased from Fisher Scientific Company (Fair Lawn, NJ). Hydrochloric acid, sodium hydroxide, sodium bicarbonate, formic acid and dibutyltin dilaurate (DBTDL) were obtained from Sigma-Aldrich (Milwaukee, WI). All materials were used as such without further purification.

2.2. Synthesis of vegetable oil :

2.2.1. Synthesis of the Epoxidized Vegetable Oils

Epoxidized vegetable oils with different epoxy groups were prepared according to a method previously reported.[1] Briefly, sesame oil and formic acid (the molar ratio of these two is 1:4.12) were charged into a 500 mL flask at 50 °C under vigorous stirring. Then, hydrogen peroxide (50%, the molar ratio of hydrogen peroxide to double bonds in triglyceride is 1.8:1) was added slowly using syringe over a 4 h period. The reaction was continued at 50 °C for another 4 h. Then, sodium bicarbonate was added to neutralize the solution and diethyl ether was added, resulted in two layers. The organic layer was washed with distilled water until the solution became neutral. Epoxidized sesame oil (ESO) were obtained after drying with MgSO_4 and filtering, organic solvent is removed by rotary evaporator, and the resulted product was dried in a vacuum oven overnight. The same procedure was followed for the synthesis of epoxidized vegetable oils namely such as, EGO, ENO, EJO and EIO respectively.

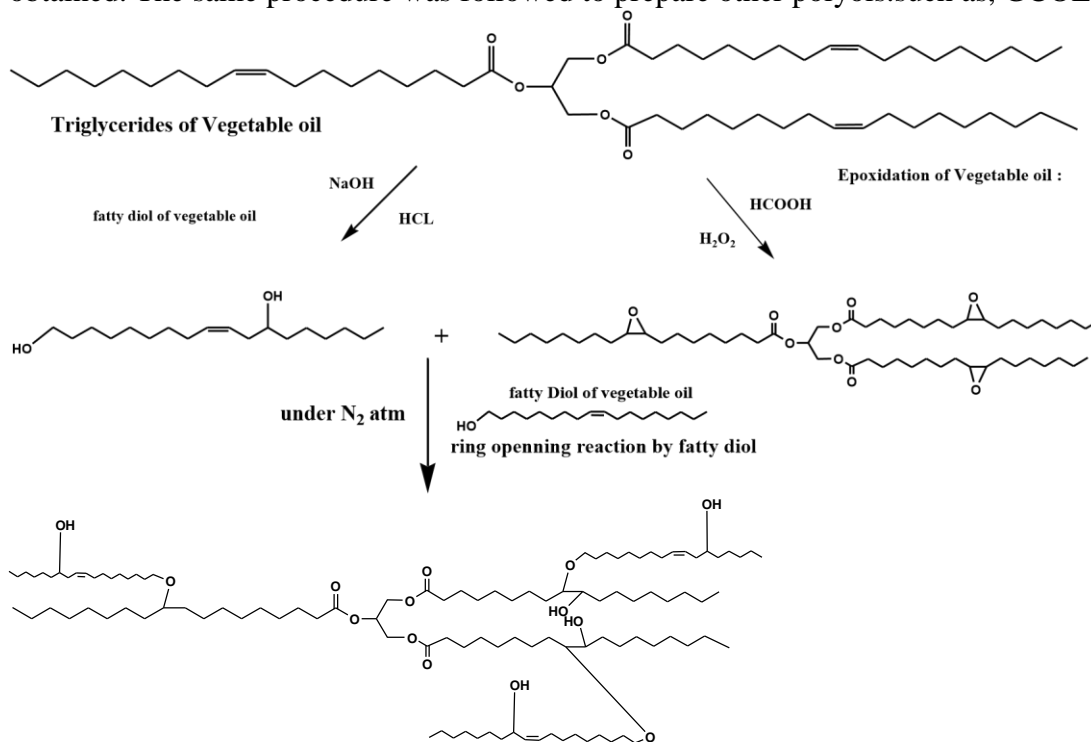
2.2.2 Synthesis of castor oil diol

CaOD was prepared using a reported procedure. Firstly 100 ML of THF was added to LiBH_4 in a 1000 ml three-neck flask at 0 °C. then the addition of castor oil 0.11 mol was dissolved in THF and then added to the LiBH_4 suspension with mechanical stirring. The reaction was maintained at 0 °C for 10 hrs.

The reaction mixture was poured into a 2000 ml beaker with ice water, followed by addition of HCl, until the solution was clear. After the extraction with 300 mL of ethyl acetate, the organic layer was purified by washing with water, drying over MgSO_4 , and filtering. Finally, the clear castor oil diol was obtained after removal of organic solvent using rotavapor and dried under vacuum. The yield of CaOFA was about 92 %, which was calculated value divided by theoretical value.

2.2.3. Synthesis of Polyols

The polyols were prepared by ring – opening reaction between epoxidized oils and CaOD fatty diol. The polyols were identified as sesame-castor oil polyol (SCOL). Several aspects, the CaOD and epoxidized oil (ESO) were mixed and kept at 150 °C – 180 °C in dry N_2 atmosphere. After the 6 hours, a yellowish viscous liquid was obtained. The same procedure was followed to prepare other polyols. such as, GCOL, NCOL, JCOL and ICOL.



Schematic Representation of epoxies and polyols from vegetable oils.

3. Characterization

A varian spectrometer Chemical structural studies were conducted using Bruker FTIR analyzer; ALPHAPlatinum FT-IR Spectrometer with ATR Platinum–Diamond sampling module. A Varian spectrometer (Palo Alto, CA) at 400 MHz was used to record the ^1H NMR and ^{13}C NMR spectroscopic analyses of the monomers of polyol and cross-linker CaOD fatty diol. The prepared polyols and CaOD fatty diol molecular weight distribution were measured using a Varian PL-gel permeation chromatography (GPC) 50 plus equipped with a differential refractive index (DRI)/viscometer which is a combined detector. The reference material tetrahydrofuran (THF) used as a eluent and the flow rate was fixed 1.00 mL min^{-1} . The viscosity of prepared polyols were determined using U/S portable rheometer at 25°C . The present communication deals with synthetic and thermal degradation properties of a newly synthesized polyols (SCOL and GCOL) characterized by thermal analysis.

4. Result and Discussion

4.1. Fourier Transform Infrared Spectroscopy

In this part, the prepared epoxy of vegetable oils and CaOD based different polyols structure and functionalities were investigated by FT-IR. The measurement of FT-IR were carried out in attenuated total reflectance mode using a thermoset scientific nicolet 6700 spectrometer. IR spectra were obtained at 4 cm^{-1} resolution between standard wave number ranging from 400 to 4000 cm^{-1} . [1,4-6]

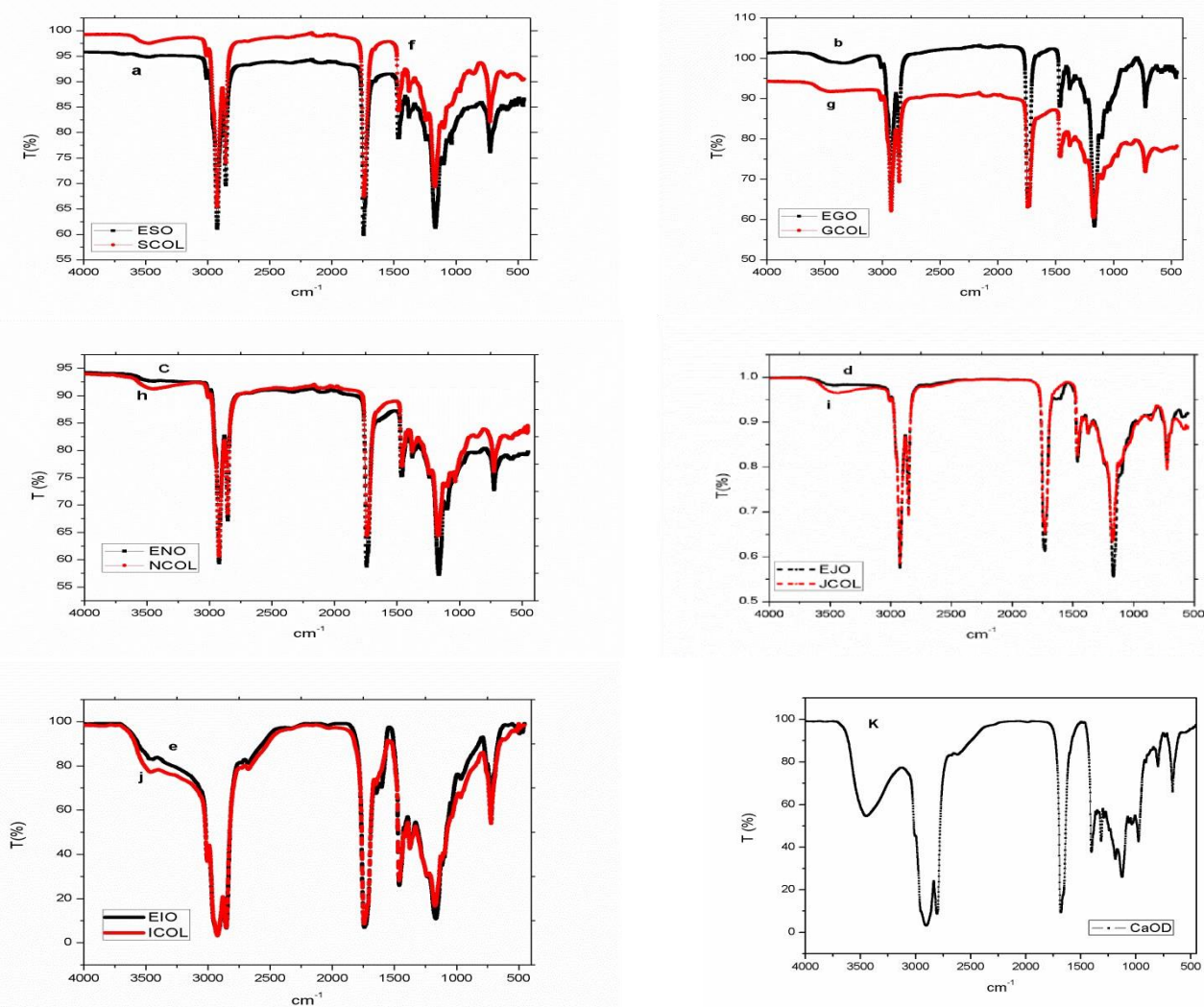


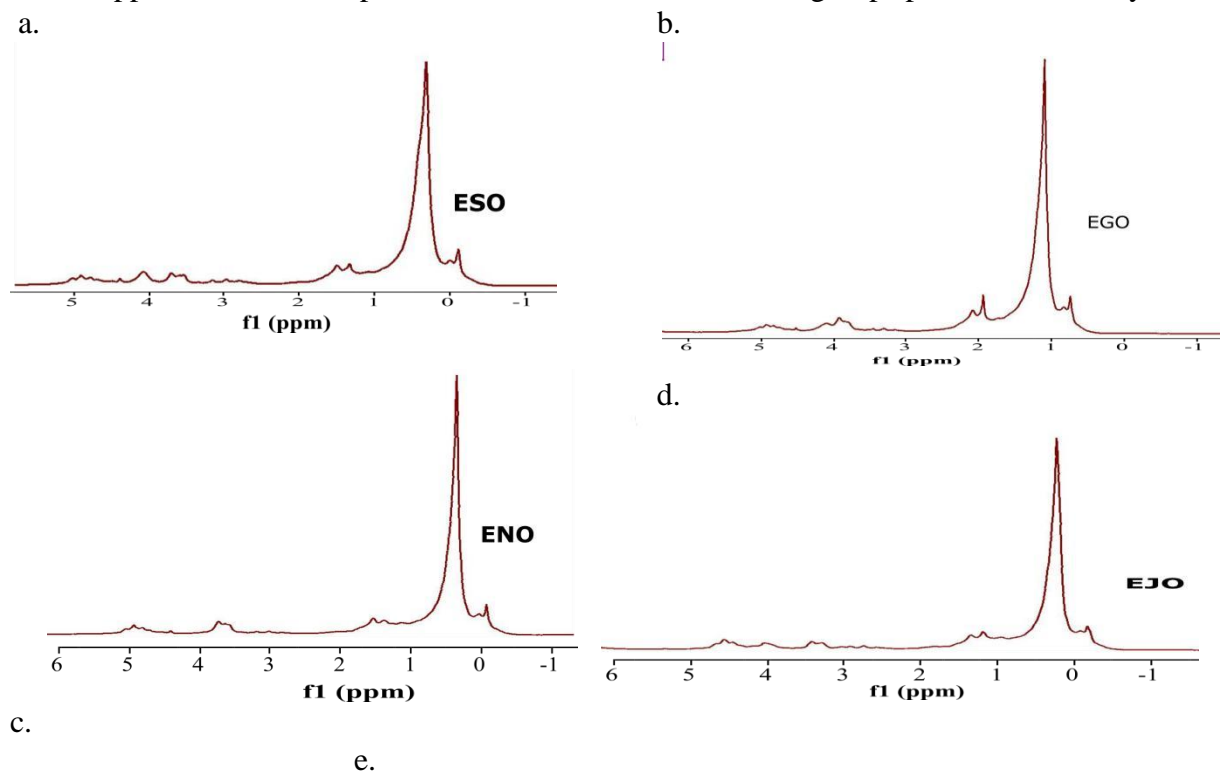
Figure. 1(a) to 1(e) shows the FT-IR spectra of epoxidised ESO, EGO, ENO, EJO and EIO, **Fig. 1(f) to 1(j)** shows, FT-IR spectra of polyols and **Fig. 1(k)** shows, FT-IR spectra of fatty diol (CaOD)

Fig. 1(a) to 1(e) shows the FT-IR spectra of epoxidised ESO, EGO, ENO, EJO and EIO. The FT-IR spectra reveals that the presence of epoxy group at 826 cm^{-1} , 824 cm^{-1} , 843 cm^{-1} , 837 cm^{-1} and 823 cm^{-1} respective epoxy oils. Fig. 1(k). shows, the broad peak between 3600 to 2500 cm^{-1} , which was assigned to overlapped signal from the -OH stretching of the hydroxyl and the carboxylic groups of fatty diol CaOD. The peak at 823 cm^{-1} was no longer observed for the fatty diols because most of the epoxy groups were reduced during the ring opening step[2-5]. Fig 1(f) to 1(h), shows the intensity of epoxy group at 826 cm^{-1} , 824 cm^{-1} , 843 cm^{-1} , 837 cm^{-1} and 823 cm^{-1} are disappeared while CaOD of fatty diol unit facilitate the ring opening of all the epoxy oils. The reaction of carboxyl to epoxy group ratio were increased because of which shows that most of epoxy groups were ring-opened by hydroxylated fatty diol CaOD. The -OH group is intrinsically present in CaOD fatty diol moiety is shown in the fig. 1(f) to 1(h). On the other hand, the SCOL, GCOL, NCOL, JCOL and ICOL polyol of all the -OH groups. The presence of broad peak appeared at 3421 cm^{-1} , 3480 cm^{-1} , 3498 cm^{-1} , 3462 cm^{-1} and 3356 cm^{-1} shows the -OH group of CaOD overlapped with all the epoxy vegetable oils.

4.2. Proton Nuclear magnetic resonance spectrometry (^1H NMR)

The polyols were prepared from epoxidised ESO, EGO, ENO, EIO and EJO oils, ring opened by CaOD. In the ring opening reaction, the polyols were synthesized from epoxidised oils and it is prepared by solvent/catalyst free method. fig. 3(a) shows the ^1H NMR spectra of CaOD fatty diol, their peak intensity at $\delta = 5.3\text{--}5.4\text{ ppm}$ corresponding to carbon-carbon double bond in CaOD, the terminal $-\text{CH}_3$ and internal long chain protons of $-\text{CH}_2\text{C}-$ signal at $\delta = 0.89\text{ ppm}$ and $\delta = 1.63\text{ ppm}$. [8,12,15]. The peaks for -OH protons of diol moieties of CaOD appeared at 3.56 ppm .

All the protons of triglyceride of the vegetable oil were also observed in the ^1H NMR spectrum. Fig.2(a) to 2(e) shows, a typical proton NMR spectrum of the prepared epoxidized oils. It was found that the chemical shifts value at $\delta = 2.22\text{--}2.65\text{ ppm}$ are due to the protons attached to the epoxy groups of the vegetable oils. The corresponding peaks at $\delta = 0.89\text{ ppm}$ are the terminal methyl groups of the fatty diols chains. The peaks at $\delta = 1.2\text{--}1.6\text{ ppm}$ are due to the protons of all the internal $-\text{CH}_2-$ groups present in the fatty diol chains.



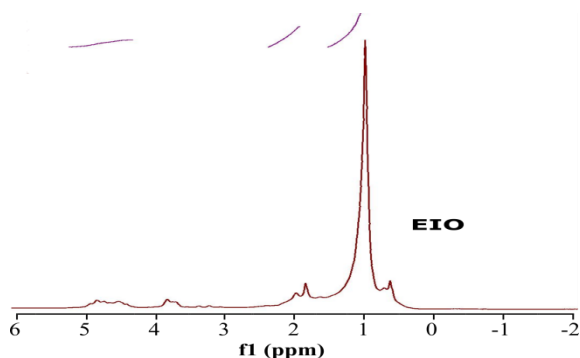
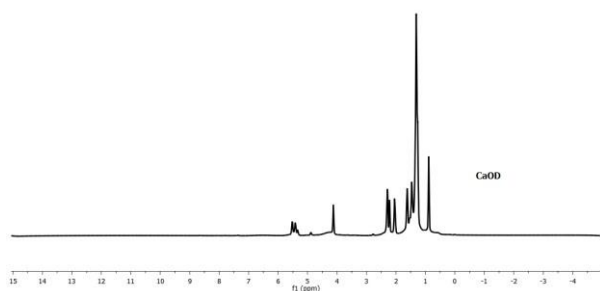


Figure. 2(a) to 2(e) ^1H NMR spectra of epoxidised vegetable oils.

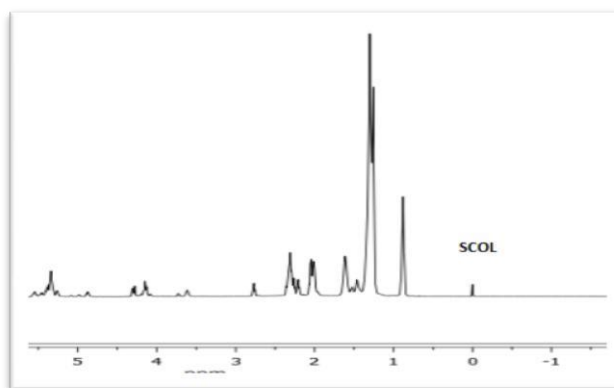
The prepared SCOL, GCOL, NCOL, JCOL and ICOL shown in the fig. 3(b) to 3(f). The chemical shift value observed at $\delta = 2.21$ ppm corresponds to the $-\text{CH}_2\text{COO}-$ proton. After the reduction reaction, the epoxy peak at $\delta = 2.22 - 2.65$ ppm disappeared. The chemical shifts value of the methylene proton of fatty diol (carbon-carbon double bond) signal shifted to 3.73 ppm.

The intensity of the proton of $\text{C}=\text{C}$ present in SCOL, GCOL, NCOL, JCOL and ICOL were slightly decreased, because of the oxirane ring opened by CaOD fatty diol, obtained peak at $\delta = 5.39$ ppm. The castor oil diol $-\text{OH}$ group overlapped with newly formed $-\text{OH}$ peak, the corresponding peak at $\delta = 5.56$ ppm. [9,15] On the other hand, the peak intensity at $\delta = 3.5 - 3.6$ ppm decreased, indicates the complete reduction of triglyceride. It results in the formation of primary $-\text{OH}$ groups backbone appear at $\delta = 5.56$ ppm. It leads to the conclusion that SCOL, GCOL, NCOL, JCOL and ICOL was successfully prepared and confirmed.[1-8].

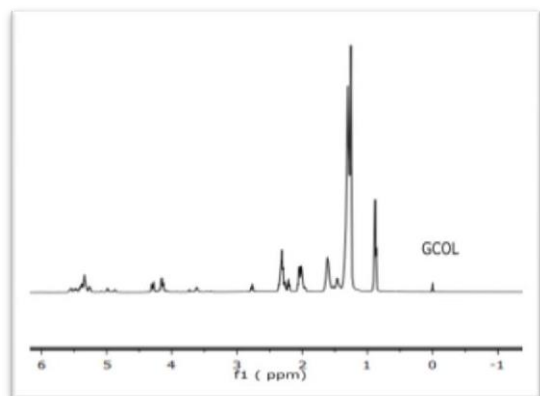
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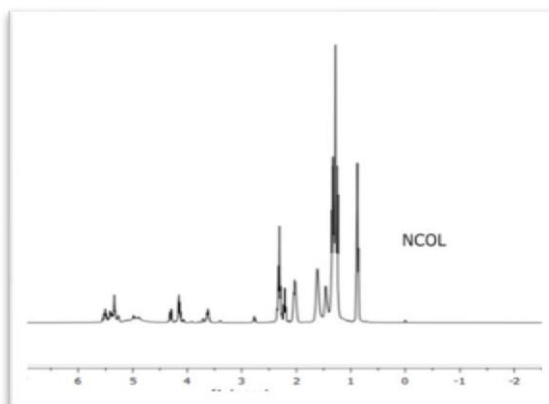
b.



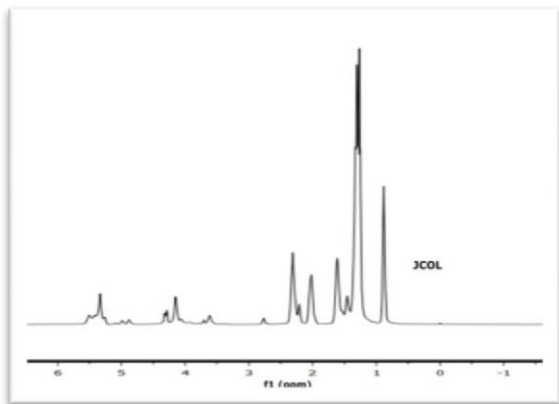
c.



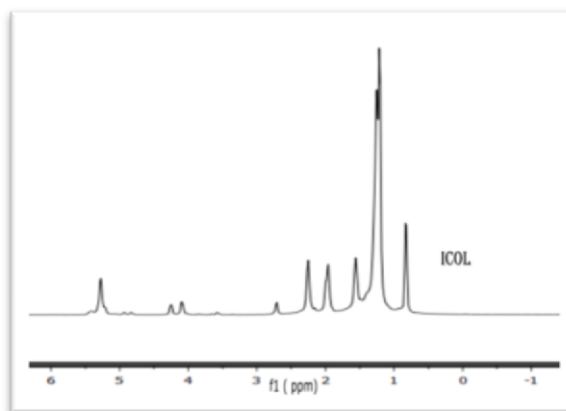
d.



e.



f.

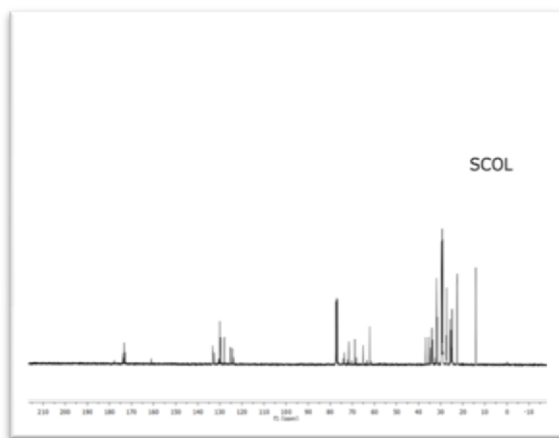


¹H NMR spectra of CaOD fatty diol in fig. 3(a) polyols in Figure . 3(b) to 3(f)

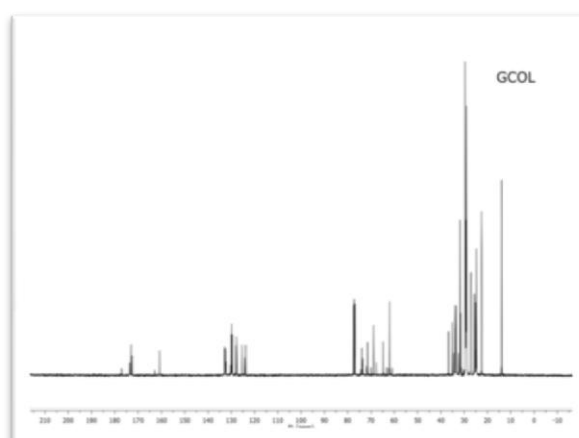
4.3. Carbon Nuclear magnetic resonance spectrometry (¹³C NMR)

The ¹³C NMR spectra of polyol SCOL, GCOL, NCOL, JCOL and ICOL were shown in the fig. 4(a) to 4(e). The chemical shifts value of -COOC- observed at $\delta = 172$ ppm and $\delta = 74$ ppm. The corresponding methylene (carbon-carbon double bond) peak appeared at $\delta = 131$ ppm, and the terminal and long chain carbon of polyol gives signal at $\delta = 14.0$ ppm. After the ring opening of the oxirane ring, the signals obtained at $\delta = 71$ ppm, $\delta = 34$ ppm and $\delta = 27$ ppm corresponds to C-C-C=C-C respectively. Finally, after the reduction reaction castor oil diol of CaOD hydroxyl (-OH) group overlapped with newly formed -OH group, the obtained peak at $\delta = 74$

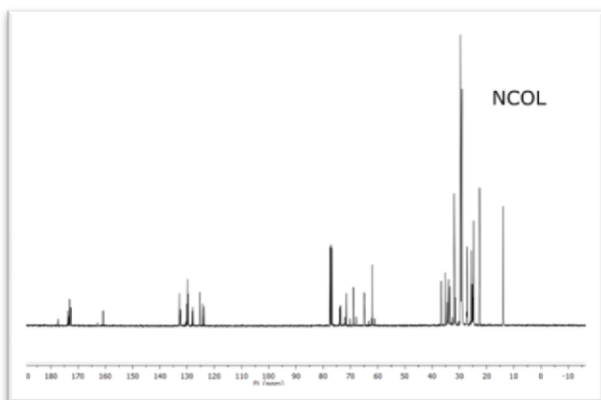
a.



b.



c.



d.

e.

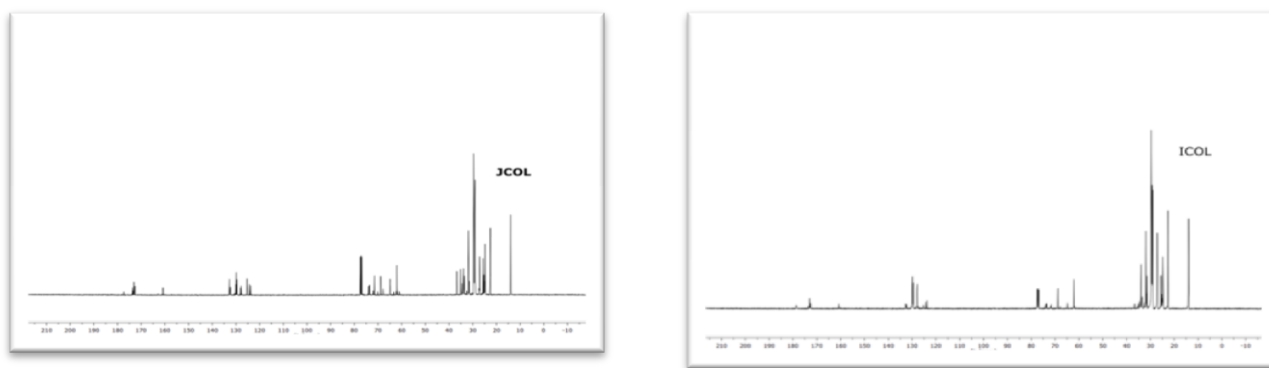


Figure. 4(a) to 4(e) ^{13}C NMR spectra of polyols.

ppm, the corresponding $-\text{C}-\text{O}-\text{CO}-\text{CH}_2-$ peak at $\delta = 67$ longer observed.[2-4,11-13]. It conclude that the SCOL, GCOL, NCOL, JCOL and ICOL was successfully prepared and again confirmed.

4.4. Gel permeation Chromatography

The GPC curve of polyol SCOL and CaOD fatty diol were shown in the fig.5. The reaction of carboxyl to epoxy group ratio increases (C. Zhang et al.), the epoxy group were totally ring opened by CaOD diol.[2,3] From GPC curve, comparison of CaOD fatty diol with SCOL, the polyol retention peaks were shifted to longer retention time and became broadend, which indicates that the molecular weight of the polyol increased. On the other hand, the obtained second peak shifted to shorter retention time. Considering there is another one $-\text{OH}$ group in CaOD, which the number was almost identical with carboxyl. It is deduced that the $-\text{OH}$ group in CaOD, as well as newly formed $-\text{OH}$ in epoxies, were also involved in the ring opening reaction and possibly connected with ESO molecules, leading to increasing molecular weight. [7,15-18]. The molecular weight values shown in the table 1. This can be explained, all the epoxidised vegetable oils fully ring opened by CaOD fatty diol. This leading to more connection of polyols and less oligomerization.

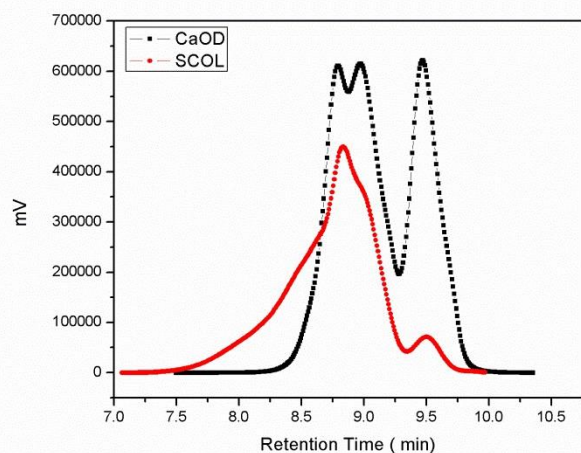


Figure 5. GPC Chromatogram of polyols.

Table. 1. polyol prepared from ESO and CaOD fatty diol

sample code	Viscosity (Pa s) at 25 °C	Number molecular weight	average Weight molecular weight	average PDI
SCOL CaOD	1.8	1594	2262	1.41
	2.2	485	492	1.02

PDI - polydispersity index

4.5. Rheometry analysis

The viscosity of prepared polyols were determined by using R/S portal rheometer at 25 °C for the shear rate varying from 1 to 100 s⁻¹. The polyols SCOL, GCOL, NCOL, JCOL and ICOL, and CaOD fatty diol plots (variation of shear stress against shear rate and viscosity against shear rate) shown in the Fig.(6a) and 6(b). However, the fig 6(a) shows, linear dependence of shear rate on shear stress. The rheological characteristics of polyols, such as the variation of viscosity against shear stress, temperature at constant stress and time, the variation of storage and loss modulus and loss factor against frequency, may be studied by the use of suitable rheometer under various conditions.

Table. 2. Power law method of polyols and CaOD fatty diol flow rate.

Sample code	K (pa s)	n (-)	R ²
CaOD	1.671	1.023	1.044
SCOL	1.419	1.011	1.049
GCOL		0.5855	0.6901
NCOL		0.2381	0.9997
JCOL		0.2539	0.999
ICOL		0.2027	0.9979
		0.2578	0.999
		0.0625	

Under a constant shear stress, viscosity will generally remain almost constant, indicating that vegetable oilbased polyols exhibit Newtonian behaviour. polyols with a higher molecular weight or higher polarity owing to the presence of other -OH functionalities have a higher viscosity than the cross-linker (fatty diol). This difference may also be a result of an increase in the number of -OH and the presence of newly formed -OH group (due to epoxy group were ring opened by cross- linker CaOD fatty diol). As with other polyols systems, the viscosity decreases with an increase in temperature when there is no curing agent present in the system. This decrease may be due to the increase in the molecular mobility of the chains as the kinetic energy of the system increases[9]. Because of increases - OH group and C=C intrinsically present in fatty diol moiety. [10] The power law method had a flow behavior index $n < 1$. The corresponding power law model values shown in table. 2. It indicates, the obtained bio-polyols were 'n' value decreases and 'K' value increased. In other hand, Under a constant shear rate, viscosity will generally remain almost constant, Thus, the fig. 6(b) shows apparent viscosity of a pseudoplastic substance increases with the decreasing their shear rate, this plots reveals that the prepared bio-polyols having higher molecular weight and it exhibits the Pseudoplastic Newtonian behaviour.[2,10,11]

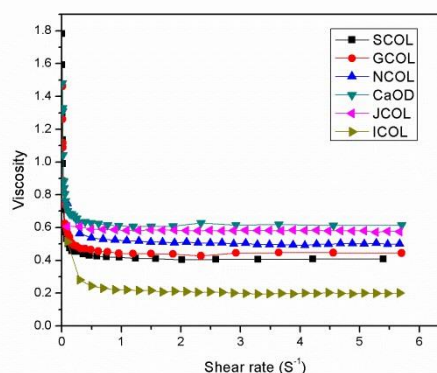
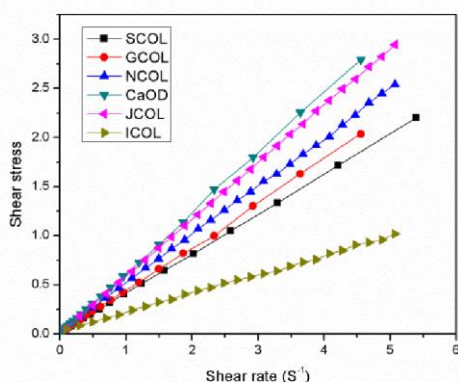


Fig. 6(a). Rheogram spectra of polyol and CaOD fatty diol polyol and CaOD fatty diol curves of Viscosity Vs sheat rate polt.

Fig. 6(b). Rheogram spectra of curves of shear stress Vs sheat rate polt.

4.6 Thermal Analysis

The thermal stability of SCOL and GCOL polyols was evaluated by thermogravimetry analysis (TGA) by heating rate $10\text{ }^{\circ}\text{C min}^{-1}$ under nitrogen atmosphere. The TGA curve are illustrated in fig.7(a) and 7(b). respectively. The stability of the reaction products of polyols such as SCOL and GCOL of $-\text{OH}$ group increases as the epoxidised oil content decreases. This may be due to the high degree of cross-linked density in the systems. This is fact that the degree of freedom available for segmental motion in the chains within the network structure is enhanced by the addition of cross-linker (fatty diol) to epoxies, the density of the $-\text{OH}$ moiety sufficiently increased.[21-22] In other hand, these two polyols depends strongly on fatty diol groups per unit volume and an increase in the initial weight loss was observed as a result of the increase in the amount of $-\text{OH}$ functionality, it reveals that the increase of diol moiety of $-\text{OH}$ group in both the monomers.[1-3,18-20] TGA curves of prepared higher $-\text{OH}$ content of polyols (oxirane ring opened by CaOD fatty diol), are shown in Figure 7(a) and 7(b). The corresponding TGA curves reveal two main degradation processes (Figure 7b & 7b). All monomers of polyols showed an initial weight loss.

(The first degradation of the both polyols (decomposition temperature ranges) was started from $185\text{ }^{\circ}\text{C}$ to $210\text{ }^{\circ}\text{C}$ corresponding to 94% weight loss (average value) that may be due to cleavage of recinoliec moiety ($\text{C}=\text{C}$ intrinsically present in fatty diol) of the cross-linker (CaOD), which dependence on the type of cross-linker ring opened by epoxies (such as ESO and EGO). The corresponding second degradation of both polyols (decomposition temperature ranges) was started from $380\text{ }^{\circ}\text{C}$ to $440\text{ }^{\circ}\text{C}$, which corresponds to epoxy oil fully decomposed.[22-25] This is due to the increase of the cross-linking density (castor diol) and increase of intermolecular attractions between epoxy and $-\text{carboxyl}$ group through $-\text{OH}$ bonding, polar-polar interaction, etc., which makes the structure more compact. Thermal stability of these two polyols (SCOL and GCOL) have slightly differ from the decomposition temperature. These results leads to assume that the other polyols such as (NCOL, JCOL and ICOL) could be have same decomposition ranges, and their stability also varied from each oils.

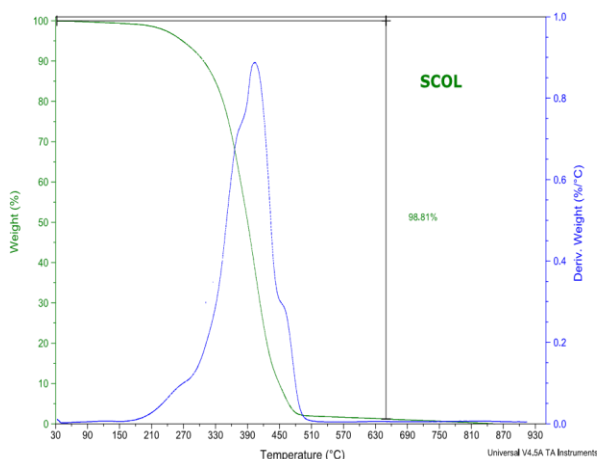


Figure 7(a) TGA plot of SCOL polyol

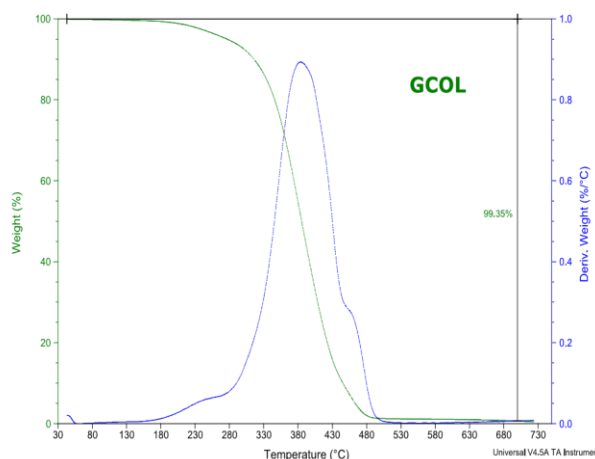


Figure 7(b) TGA plot of GCOL polyol

5. Conclusion:

A series of bio-based triglyceride vegetable oils such as *Sesamum indicum*, *Arachis hypogaeae*, *Jatropha curcas*, *Azadirachta indica*, *Madhuca longifolia* of high molecular weight polyols were successfully prepared. The obtained $-\text{OH}$ functionality of the polyols and CaOD functionalities were confirmed by FT-IR spectrometer. The different environment of hydroxyl group ($-\text{OH}$) in fatty diol chain and after the reduction reaction SCOL, GCOL, NCOL, JCOL and ICOL of polyols $\text{C}=\text{C}$ proton intensity slightly reduced, when the oxirane ring opened by CaOD, this leads to each polyol structure were again confirmed by ^1H and ^{13}C NMR spectrum. Furthermore, the carboxyl to epoxy groups increasing their cross-linking density of polyol moiety. This is due to epoxy oils successfully ring-opened by CaOD fatty diol. From the GPC, shorter and longer retention time of SCOL

chromatogram peak reveals to their molecular weight have been increased. The rheometry analysis, the cross-linker CaOD fatty diol reacts with epoxy group, number of -OH group increased in SCOL, GCOL, NCOL, JCOL and ICOL polyols, due to CaOD of the viscosity decreases with an increase in decreasing shear rate. On the other hand, the flow rate were determined by power law model, while ' n ' value decreased and ' K ' value were increased due to their flow rate of the viscous fluid is increased, this reveals to all the polyols acting as a pseudoplastic newtonian behavior. The resulting polyols stability were characterized by TGA analysis, their degree of cross-linking and decomposition of the polyols have been noted and the polyols (SCOL and GCOL) have close different decomposition, this reveals that the other polyols also assume to conclude their stability of the product.

The resulting polyols were used to prepare polyurethanes, poly (ether urethanes) and etc., polyurethanes can be fabricated by reactive liquid molding, a process in which a polyisocyanate or prepolymer is mixed with a polyol and either injected or cast into a mold. The reactive liquid mixture cures after injection to form a solid scaffold, biomedical applications, Intravascular devices.

6. Conflict of interests

The author stated that there is no conflict of interest regarding this manuscript preparation and work.

7. Acknowledgement

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