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I am submitting herewith a thesis written by Vinay Kumar Mannam entitled “Lipase-Catalyzed Synthesis of Monoundecenoyl, Di-*oligo*(Ricinoleyl) Triacylglycerol and Chemical Conjugation of Acid-Functionalized Poly(Ethylene) Glycol Monomethyl Ether to *Oligo*(Ricinoleic) Acid to Enable the ‘Green’ Preparation of Amphiphilic Star Polymers” I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science with a major in Biosystems Engineering.

Douglas G.Hayes

Major Professor

We have read this thesis
and recommend its acceptance:

X Philip Ye

Svetlana Zivanovic

Accepted for the Council:

Carolyn R. Hodges
Vice Provost and Dean of the
Graduate School

(Original signatures are on file with official student records.)

Lipase-Catalyzed Synthesis of Monoundecenoyl, Di-*oligo*(Ricinoleyl) Triacylglycerol and Chemical Conjugation of Acid-Functionalized Poly(Ethylene) Glycol Monomethyl Ether to *Oligo*(Ricinoleic) Acid to Enable the 'Green' Preparation of Amphiphilic Star Polymers

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DEDICATION

To my mom, dad, sister

and Harshitha

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ABSTRACT

Chemo-enzymatic synthesis of unimolecular polymeric micelles (UPMs) is proposed for their potential utilization as drug delivery vehicles in dilute aqueous solutions. The UPMs are star polymers consisting of a hydrophobic core and hydrophilic corona. The core consists of multiple *oligo*(ricinoleic acyl) chains extending outwards from enzymatically synthesized triacylglycerols that contain one undecenoic acyl group and two *oligo*-(ricinoleic) acyl groups, joined together via free radical polymerization of the terminal double bond of undecenoic acyl groups. The corona consisting of poly(ethylene) glycol monomethyl ether (MPEG) will be conjugated to hydroxyl end groups of *oligo*(ricinoleic acyl) chains of the core molecule.

The objectives of this thesis will enable successful synthesis of UPMs with the structure given above. The first objective is the synthesis of 2-undecenoyl, 1,3-*oligo*(ricinoleyl) triacylglycerol. The second objective is conjugation of carboxylic acid derivative of poly(ethylene) glycol monomethyl ether (MPEG) to the hydroxyl end groups of the core's *oligo*-(ricinoleic acid) chains, to enable the attachment of hydrophobic core and hydrophilic corona. A focal point of the work has been utilizing enzymatic catalysis in solvent-free systems, enabling the 'green' synthesis of the final product. Qualitative and quantitative analysis of the time course reaction and the reaction products were performed using analytical and preparative thin layer chromatography, high performance liquid chromatography, gel permeation chromatography, and proton nuclear magnetic resonance.

Candida antarctica lipase-catalyzed synthesis of triundecenoin via transesterification between undecenoic acid vinyl ester and glycerol, an important component of Objective.1, has been successfully achieved, at a > 95% yield. For the second sub objective, *the Rhizomucor miehei* lipase-catalyzed interesterification of triundecenoin and ricinoleic acid, has occurred at approximately 50-60% conversion yielding 1,3-ricinoleyl, 2-undecenoyl triacylglyceride(TAG) as the main product. As an alternative approach, the 1,3-selective *Rhizomucor miehei* lipase-catalyzed interesterification of undecenoic acyl groups with castor oil, had occurred at a similar yield (50-60% conversion). The third sub-objective, formation of di-*oligo*(ricinoleyl), 10-undecenoyl triacylglycerol was achieved using *Candida antarctica* lipase catalyzed esterification

between ricinoleic acid and the triacylglycerol formed through Objective 1.2. Studies on conjugation of the terminal –OH group of ricinoleic acid and *oligo*-ricinoleic acid with poly(ethylene) glycol monomethyl ether (MPEG) were successful with 40-50% conversion.

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CHAPTER 1

INTRODUCTION

1.1– Polymeric drug delivery

Drug delivery systems came into light from the time therapeutics started having greater impact in treating many diseases. These systems made the usage of drugs more effective and safe. Drugs have been traditionally administered directly in entire dosage *viz*., injections near to the target tissue or affected part of the body, leading to reduced effectiveness, or adverse side effects on the patients' health. Direct administration of drug has yielded problems ranging from fluctuation in drug levels to poor patient compliance.[1] Concentration, duration and bioavailability of pharmaceutical agents is difficult to control; thus, controlled delivery of drugs became more important as the conventional delivery was unpredictable and inefficient. Controlled delivery of drugs came into light in early 1960s when polymeric devices for administering drugs were developed. Drug delivery systems made therapeutics a more sought after solution for curing diseases. The field had tremendous growth, with widespread interest by both industry and research in government and academic laboratories. [12]

Polymers have special place in drug delivery systems.[3] Most of today's drug delivery systems are designed with polymers. They enable sustained delivery with localized and stabilized inception of the drug. [39] The key advantages of polymer drug delivery systems are summarized below:

1. Localized delivery of drug: The product can be implanted directly at the site where drug action is needed and hence systemic exposure of the drug can be reduced. This becomes especially important for toxic drugs which are related to various side effects (such as the chemotherapeutic drugs).
2. Sustained delivery of drugs: The drug encapsulated is released over extended periods and hence eliminates the need for multiple injections. This feature can improve patient compliance especially for drugs for chronic indications, requiring frequent injections (such as for deficiency of certain proteins).

3. Stabilization of the drug: The polymer can protect the drug from the physiological environment and hence improve its stability *in vivo*. This particular feature makes this technology attractive for the delivery of labile drugs such as proteins.

Drug release via polymeric drug delivery takes place *viz* diffusion and/or polymer degradation. Many reviews were presented on the polymeric drug delivery mechanisms, [40, 8, 10] all of which emphasized the effect of polymer chemistry and macromolecular properties, shape of polymer, and type of excipients added etc., Polymers used in drug delivery are often biodegradable because poorly degradable polymers often require surgery for their removal ~~P~~olymeric drug delivery vehicles exist as nanostructurally-tailored spheres, gels, films, or cylinders to enhance release kinetics. Polymers are also generally modified with suitable excipients to enhance the drug delivery and polymer degradation.

As evident from the numerous applications of polymeric drug delivery vehicles, they are increasingly important for the pharmaceutical industry. Emphasis to produce more effective and diverse materials is a focus of polymer-related research worldwide, as a new drug come into market day after day to enhance and sustain therapeutic drug uses by manufacturing good drug delivery vehicles.

1.2– Star polymers

Controlled polymerization techniques have brought vast improvements in capability to synthesize macromolecules with precise architecture and high functionality, which has strongly impacted synthesis of macromolecules for drug delivery, such as block copolymers, dendrimers, and star polymers. [25] The characteristics of these polymeric classes are well reviewed. [27]

Star polymers are macromolecules having a core branching point with branches extending from the core. They have unique architecture, with linear arms covalently attached to central core. Unimolecular polymeric micelles are star polymers consisting of covalently bound amphiphilic polymer chains. Two major approaches are currently being employed for the synthesis of star polymers. The first consists of growth of linear polymer chains outward from a

multifunctional moleucule such as pentaerythritol. [14] The second approach is the initial synthesis of linear chains capable of subsequent chain extension. Then, the reactive terminal groups of the chains are cross-linked together, or conjugated to a multifunctional molecule. [28]

1.3- Advantages of unimolecular polymeric micelles in drug delivery:

The current work presented in the thesis focuses on synthesis of star polymers that contain a hydrophobic core and a hydrophilic corona connected together to form unimolecular polymeric micelle. A core-first method is adopted in which polymer chains are grown from a multi-functional initiator, and later covalently attaching hydrophobic molecule (e.g., poly(ethylene glycol)) to complete the corona.

The self assembly of amphiphilic copolymers in aqueous media to form micelle with hydrophobic core domains surrounded by a hydrophilic corona provides a vessel that is particularly suited to pharmaceutical applications. The core is capable of solubilizing hydrophobic drug molecules, while hydrophilic corona stabilizes the particle in aqueous media. Unlike polymeric micelles, combination of rheological properties and ability to undergo controlled polymerization makes star polymers attractive candidate for variety of applications including drug delivery.

Polymeric micelles contain a lipophilic core which solubilizes hydrophobic molecules (e.g., drugs) and a hydrophilic shell that makes entire assembly water soluble. Conventionally, this property of micelles is used in drug delivery systems where micelles serve as drug reservoirs. However, the formation and stability of micelles is both temperature and concentration dependent. Micelles are thermodynamically unstable at concentration below their critical micelle concentration. This may lead to dilution of micelles after they are injected into bloodstream causing severe toxicity problems due to fluctuations in drug concentrations accompanied by breakdown of micellar structure into surfactant molecules.

In contrast to polymeric micelles, unimolecular polymeric micelles are structurally more stable as entire polymer behaves as a micelle compared to conventional polymeric micelles' structural dependence on concentration and temperature (Figure 1.1). Also, UPMs possess lower critical micelle concentration and thus higher stability when compared to surfactants. The higher stability enables longer half life of drug delivery vehicle in blood streams, and controlled release of drug avoiding side affects resulting from inconsistent delivery. These advantages make UPMs more favorable for usage as drug delivery vehicles in place of surfactants or other conventional polymer micelles.

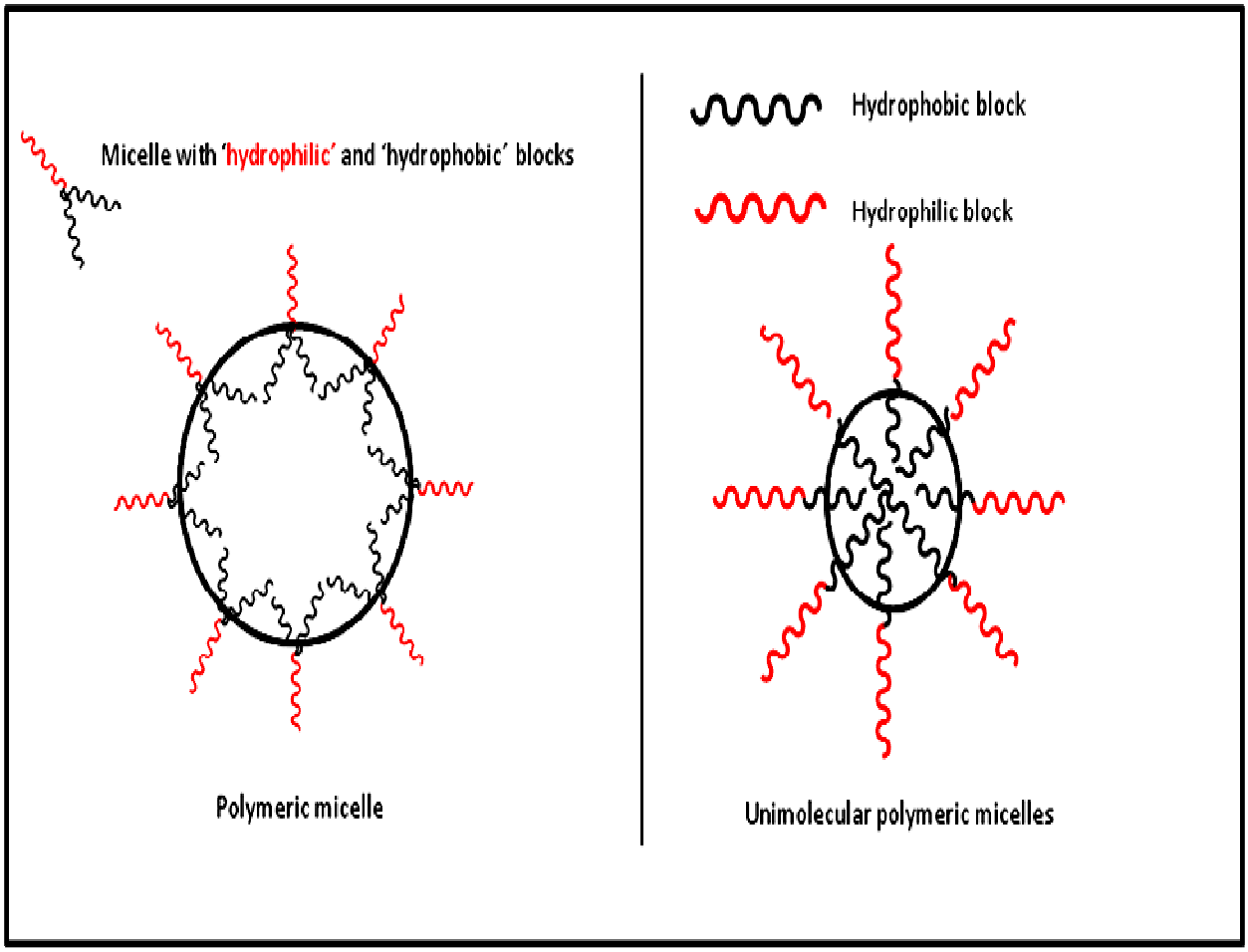


Figure 1.1. Structural comparison of conventional micelle and unimolecular polymeric micelle.

1.4- Proposed work:

The overall goal of the research described in this thesis is to develop technology to chemo-enzymatically produce UPMs to serve as vehicles for the delivery of hydrophobic drugs. In order to enable this goal, a chemical intermediate was enzymatically synthesized (Objective 1 of this thesis), which will serve as hydrophobic core upon its free radical polymerization. Also, to enable the covalent attachment of hydrophilic groups to the termini of the polymer chains extending outward from the hydrophobic core, to form the hydrophilic corona of UPMs, conjugation of carboxylic acid-functionalized poly(ethylene glycol) monomethyl ether ("MPEG-acid") to hydroxyl end groups of ricinoleic acid and its *oligo*mers was investigated (Objective 2 of this thesis).

The specific objectives consist of the following:

1. Enzyme- (lipase-) catalyzed synthesis of monoundecenoyl, di-*oligo* (ricinoleyl) triacylglycerol (TAG), a chemical intermediate which upon free radical polymerization~~,~~ will serve as the hydrophobic core of the star polymer.
    a. Step 1: 'random' lipase-catalyzed transesterification of glycerol and undecenoic acid vinyl ester, to yield triundecenoin
    b. Step 2: 1,3- selective lipase – catalyzed acidolysis of triundecenoin and ricinoleic acid, to yield a TAG mixture enriched in 2-undecenoyl, 1,3-ricinoleyl TAG.
    [As an alternative approach to Steps 1 and 2, 1,3-selective lipase-catalyzed transesterification of vinyl undecenoate and triricinolein (castor oil), to yield 1- (3-) undecenoyl, 2,3- (1,2-) ricinoleyl TAG, was investigated.]
    c. Step 3: 'random' lipase-catalyzed polymerization of monoundecenoyl, diricinoleyl TAG and ricinoleic acid.
2. Covalent attachment of carboxylic acid-functionalized poly(ethylene) glycol monomethyl ether (MPEG) to ricinoleic and *oligo*(ricinoleic acid)

1.5- Thesis overview:

The thesis has been organized as described below:

Chapter 2 presents background information on the current state-of-the-art for synthesizing biopolymers through enzymatic polymerization. Specific subtopics include 'lipase catalyzed synthesis of oligo-hydroxy fatty acids' and 'poly hydroxyl fatty acids'. Chemical characterization steps relevant to the reactions performed in this thesis are also reviewed.

Chapter 3 presents the materials and methods used in achieving Objectives 1 and 2, including chemical characterization techniques for analyzing the reaction products.

Chapter 4 contains the results and discussion pertaining to Objectives 1 and 2.

Chapter 5 consists of a summary of the results achieved and their significance, along with recommendations for future work relating to this thesis.

CHAPTER 2

LITERATURE REVIEW

2.1–Biopolymers

The use of polymers has become common in many fields, with wide range of applications. Interest in environment friendly and biodegradable polymers has caught attention in past two decades with raise in environmental conscience. Biobased materials have been employed for several different applications from chemicals to food products. [44]

Biobased polymers can be produced chemically or enzymatically using biologically produced monomers. [12] Lactic acid is an early example of biological monomers which can be produced by fermentation of corn. Polylactic acid (PLA) has been employed as a replacement for polyethylene terephthalate (PET). [33] Poly hydroxy fatty acids which can be obtained from variety of natural sources, including plants, are currently being used in cosmetics, paints, lubricants, and the food industry. [21] In addition to being environmentally-friendly and biodegradable, increasing prices for petroleum feedstocks have created tremendous opportunities for biobased polymers to compete with synthetics on a cost comparative basis. [44]

Industrial competitiveness has led to new methods for producing biopolymers. Advances in biocatalysis have resulted in development of new methods which can replace chemical protocols. Enzymatic synthesis of biopolymers provides the important advantage of reducing production costs due to lower energy consumption (e.g., near-ambient temperature and pressure). Enzymatic polymerization has been well received with increasing demand for sustainable production of polymers. [44]

The ability to synthesize wide variety of polymers with different functional groups and structure led biopolymers to variety of applications in industry and research. As introduced in the first chapter, the pharmaceutical and drug industries have primarily benefited through progress in biopolymers.

2.2–Enzymatic synthesis of biopolymers:

The use of biocatalysts to synthesize polymers is well understood and reviewed. [17] Their ability to limit the product distribution through their inherent substrate, regio- and stereo selectivity is prime factor. [23] Polymer synthesis using enzyme as catalyst mainly occurs in the following processes. In living cells (*in vivo*), enzyme systems catalyze the synthesis of many biopolymers via biosynthetic (metabolic) pathways. In "test tubes" (*in vitro*) enzymatic catalysis is achieved for the synthesis of polymers using single enzyme systems or single enzymes. *In vivo* enzymatic catalysis have advantages of high catalytic activity (high turnover), mild reactions under conditions (with respect to temperature, pressure, solvent, neutral pH, etc.), and excellent reaction control via stereo-, regio-, chemo- and enantio-selectivities. [11] Depending upon the catalyst and reaction conditions, these characteristics can also be realized *in vitro*. These goals are often difficult to achieve by chemical synthesis of polymers. [17]

Advances in nonaqueous enzymology have expanded the conditions in which polymer synthesis can be undertaken, which facilitate flexible design of reaction systems. Moreover, biobased catalysts are more environmentally-friendly than chemical catalysts, which often contain heavy metals. All of these characteristics make enzymes viable candidates for polymer synthesis.

2.3–Lipase –catalyzed synthesis of oligo-hydroxy acids:

Lipases are enzymes which comprise a subclass of esterases that catalyzes the hydrolysis of ester bonds in water insoluble lipid substrates. Lipases are known to catalyze polymer synthesis at relatively low temperature and pressure as compared with conventional methods and reduce undesirable side reactions.

Hydroxy fatty acids can be obtained from many natural resources as estolides and lactones, as presented in (Table 2.1). Estolides are linear *oligo*meric polyesters of hydroxy fatty acids, which occur mainly in seed oils (Ex: *Lesquerella* genus plants). Lactones are cyclic esters that contain one or more hydroxyl acyl chains, which mainly occur in homogenized milk, fruit,

tobacco, and insect pheromones. Studies showed that lipases are good catalysts to utilize hydroxyl fatty acyl substrates, enabling wide range of reactions. As reviewed elsewhere, [21] lipases catalyze several hydroxy fatty acids 1-substrate reactions. The location of hydroxyl group has greater impact on formation of either lactone or estolide upon biocatalytic esterification. Studies revealed that estolides are formed when hydroxy group is at carbon-3 (β-position), whereas lactones are formed when they are present at carbon-4(γ) and 5(δ).[23] For omega(ω)-hydroxy fatty acids, their concentration influences product distribution with a higher concentration of ω-hydroxy fatty acids resulting in estolides, and a low concentration producing their *oligo*-lactones. The studies implied the lactone formation is increased by lower substrate concentrations. The exceptions in the formation of estolides are presented in the cited review. [21] Omega-(ω-)C_{10} and ω-C_{11} hydroy fatty acids have resulted in estolides with molecular weights up to 20-35 kDa after few day of reaction period. [43] Ring opening polymerization of lactide (cyclic diester of lactic acid) with trimethylene carbonate resulted in unbranched polymers of molecular weights up to 21 kDa, which possess a high degree of crystallinity. [9]

From Table 2.2, ricinoleic and lesquerolic acids (*R*-12-hydroxy-9(*cis*) octadecaenoic and *R*-14-hydroxy-11(*cis*) eicosaenoic acid, respectively) mainly resulted in estolides upon lipase catalyzed reactions, significance for current study. Lipase catalyzed esterification reaction of hydroxyl fatty acids is operated at mild reactions conditions with very few side reactions. Also reactions are mostly carried out in solventless systems. Water content is known to affect the lipase catalyzed esterification. [44] To maximize yields and/or to minimize hydrolytic side reactions, it is desirable to operate lipase catalysis at lowest possible water content; thus, care needs to be taken to remove water generated during reaction.

Table 2.1. Hydroxy fatty acids, and their sources. [21]

Selected Hydroxy Fatty Acids (HFA) and Their Sources		
Common HFA name	Structure	Source
2-Hydroxy lauric	$12{:}0\text{—}OH^{2}$	*Azotobacter. agilis*
3-Hydroxy oleic	$18{:}1^{9c}\text{—}OH^{3}$	*Alcaligenes* sp.[a]
Ricinoleic	$R\text{-}18{:}1^{9c}\text{—}OH^{12}$	*Ricinus comminus* (Castor) seed
C_{18} ω-HFA	$18{:}0\text{—}OH^{18}$	Epicuticular wax (Pine trees)
Densipolic	$18{:}2^{9c,15c}\text{—}OH^{12}$	*Lesquerella densipila* seed
Dimorphecolic	$18{:}2^{10t,12t}\text{—}OH^{9}$	*Dimorphotheca sinatua* seed
α-Kamlolenic	$18{:}3^{9c,11t,13t}\text{—}OH^{18}$	*Mallatus phillipinensis* seed
Lesquerolic	$R\text{-}20{:}1^{11c}\text{—}OH^{14}$	*L. fendleri* seed
15-Hydroxy eicosaenoic	$S\text{-}20{:}5^{5c,8c,11c,13t,17c}\text{—}OH^{15}$	*Laminaria* sp. (Brown algae)
Phellonic	$22{:}0\text{—}OH^{13}$	Cork
Ipurolic	$14{:}0\text{—}OH^{3,11}$	*Ipomoea purga* (plant tubers)
Dihydroxyhexadecanoic	$16{:}0\text{—}OH^{9,16}$	Coffee or papaya (Cutin)
Ustilic	$16{:}0\text{—}OH^{15,16}$	*Ustilago zeae* (mold)
(+)-Threo-9,10,18-trihydroxyoctadecenoic	$18{:}1^{12c}\text{—}OH^{9,10,18}$	*Chamaepeuce afra* seed

Table 2.2. Effect of chain length and hydroxy group position in formation of estolides and lactones through lipase catalyzed esterification. [21]

Carbon length	-OH position	Product
3	3(β)	Estolide
4–8	4(γ)	γ-Lactone
5	5(γ,δ)	Estolide
6–16	5(δ)	δ-Lactone
6	6(ε,ω)	Estolide
8,11	ω1	ω-Lactone/polyolides
10–20	ω	ω-Lactone/polyolides[a] Estolide[b]
13–15	ω6	Lactone and estolide
18	12	Estolide
20	14	Estolide

Although the water content decreases the yield of lactone formation, it doesn't have any effect on formation of estolide as the reaction is strongly driven in forward direction. [21] Thus, the main advantages of lipase catalyzed esterification of hydroxy fatty acids is its mild operating conditions, which decreases degradation of starting materials or other side-reactions; and, the ability to operate in solvent free systems makes this approach environmental-friendly. [21]

Ricinoleic acid, obtained primarily from castor oil (Table 2.1) and its derivatives have numerous applications in food, cosmetic and chemical industries. [34] Estolide formation ricinoleic acid is catalyzed successfully by random lipases or non 1,3-positional selective lipases. Lipase such as *Rhizomucor miehei,* which is a 1,3 – selective has failed to catalyze ricinoleic acid. A random selective lipase can readily utilize all three positions (both primary and secondary hydroxyl position) of triacylglycerol as conjugation sites, while a 1,3 – selective can only use primary hydroxyl positions of triacylglycerol. The lipase selectivity is graphically presented in Figure 2.1. Here, *Candida antarctica* lipase (CAL) is given as example for "random lipase" and *Rhizomucor miehei* lipase (RML) as 1,3-selective. The ability of random lipase to utilize both primary and secondary hydroxy groups is used to form *oligo*mers of ricinoleic acid with average molecular weight as high as 10 kDa. [4, 24]

Although condensation polymerization of hydroxy fatty acid is possible without biocatalysis. [44] An increase of temperature is known to increase rate of reaction and degree of polymerization for lipase catalyzed reactions up to the limit where enzymic denaturation occurs. Thus, optimization of water activity and temperature will enhance reaction~~s~~ rate, yield, and degree of polymerization.

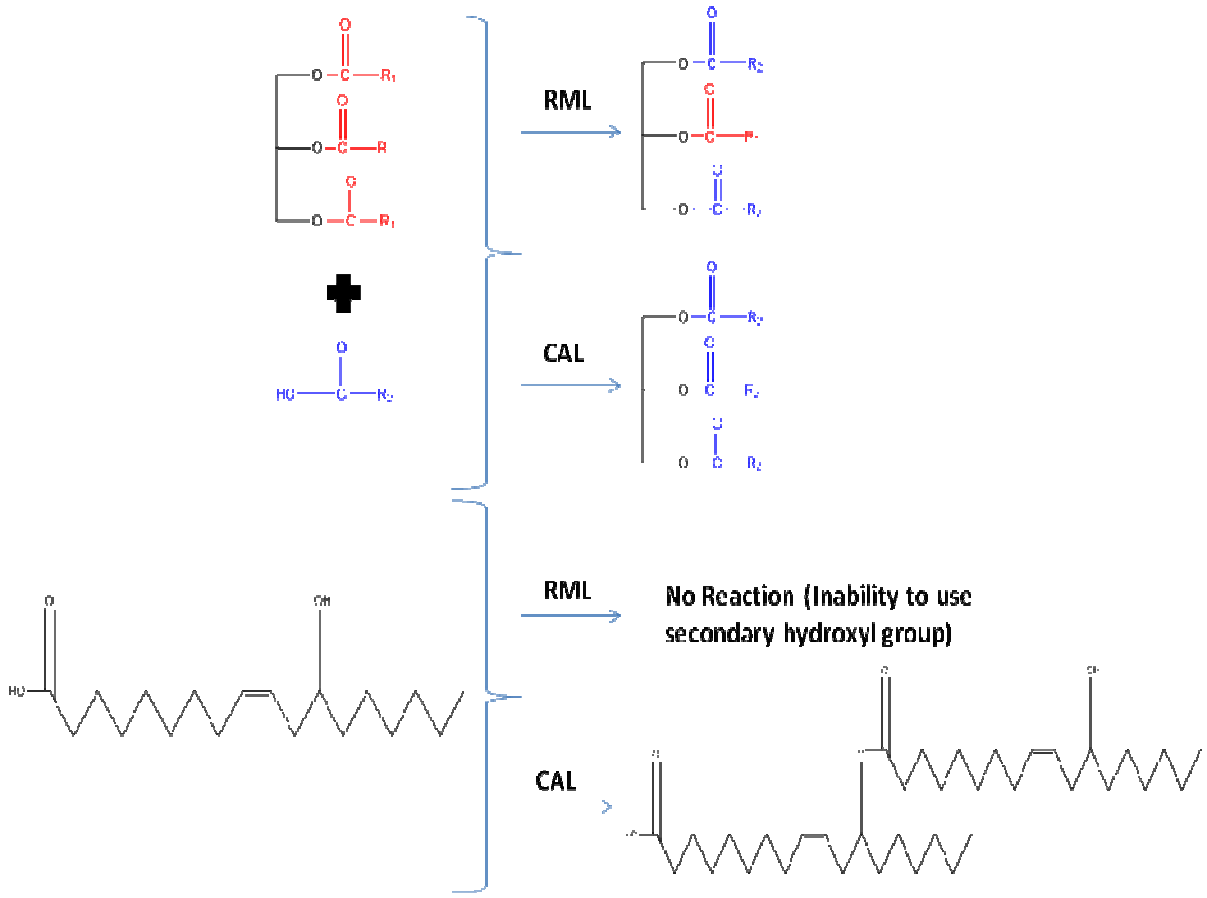


Figure 2.1 Selectivity of *Candida antarctica* lipase (CAL) and *Rhizomucor miehei* lipase (RML), examples of 1,3-seelctive and "random" lipases, respectively, towards acidolysis of triacylglycerols and condensation polymerization of hydroxy fatty acids.

2.4–Enzymatic synthesis of polyhydroxy fatty acid esters:

Ricinoleic acid is successfully esterified using CAL to produce di- and poly hydroxy fatty acid esters. [20] Star polymers have been synthesized by biocatalysis using polyol cores such as pentaerythritol (Figure 2), trimethylopropane, and dimer diol using poly (ricinoleic) acid as acyl donor by Hayes and co-workers. [4] Reactions were conducted at 70^0C using the 'random lipase' CAL (Figure 2), producing star polymers of 4850 ± 440 Da average molecular weight with 78% esterification of pentaerythritol hydroxyl groups. [4]

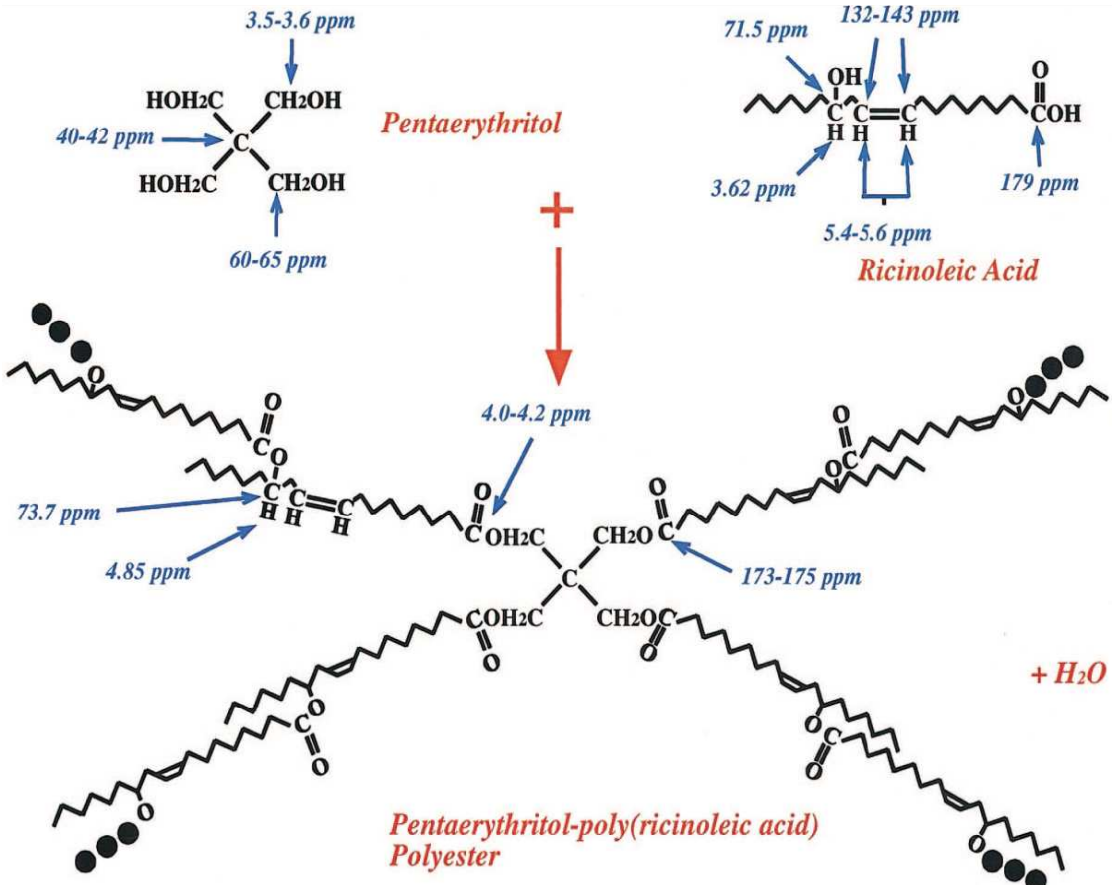


Figure 2.2. Lipase catalyzed synthesis of star polymers using poly (ricinoleic acid) and Pentaerythritol as acyl donor and acceptor, respectively. [4]

Further modification of the star polymers via conjugation of hydrophilic groups such as acid-functionalized poly(ethylene glycol) (through either chemical of biocatalytic synthesis) to the free hydroxyl groups on the terminus of *oligo*(ricinoleic) acid chains may produce a UPM that is a possible candidate for drug delivery (Section 1.3). [9] Similar molecules have been already chemically synthesized and tested which had 11% weight loading and sustained preliminary in vitro release tests. [35] The chemo-enzymatic approach under development by Hayes and co-workers, to which the work described in this thesis strongly contributes, is distinct in its 'green' synthetic approach.

2.5–Analysis and chemical characterization of star polymers

Molecular weight and molecular weight distribution of synthesized polymers are generally analyzed using ^{1}H-(proton) Nuclear Magnetic Resonance (NMR), Gel Permeation Chromatography (GPC), normal and reverse phase – High Performance Liquid Chromatography (HPLC) [36] ^{1}H- NMR provides number of different types of hydrogen present in the molecule, and also relative number of different types of hydrogens. From this information using ^{1}H- NMR the degree of polymerization of the reaction, along with percent esterification of polyol hydroxyl groups can be calculated. [4] GPC separates the analytes on the basis of size and is used for separation of polymers in particular. GPC is helpful to determine molecular weights, by using retention time vs molecular weight of product obtained from calibration of known molecular weight standard under similar condition and also accurately measure polydispersity index (PDI). HPLC separates the analytes which are carried by a mobile phase (solvents system) through interaction with a stationary phase (column). Normal-phase (NP-) HPLC separates the analytes based on absorption to stationary phase by polarity, while reversed-phase (RP-) HPLC separates on the principle of hydrophobic forces. For RP-HPLC, retention time is longer for molecules which are more non-polar, while polar molecules elute more readily, where as for NP-HPLC, polar molecules elute at longer retention times, while non-polar elute readily. [26] HPLC is thus, helpful in determining the product distribution, and percent conversion of the reactants. Specifically, NP-HPLC is useful to separate triacylglycerols from partial glycerides where as RP-HPLC is useful to distinguish different triacylglycerols in the analytes.

2.6. Summary

Enzymatic synthesis of biopolymers and their applications are receiving renewed interest. Advances in nonaqueous enzymology facilitate flexible design of systems that are required for polymer synthesis with easy product separation and enzyme re-use. Syntheses of poly (hydroxy) fatty acids by lipase catalyzed polymerization reactions are found to be more favorable than using chemical routes and environmentally friendly. Conjugation of *oligo*(hydroxy acids) and polyols to produce star polymers as described in the literature was

reviewed. The star polymers are potentially valuable bio-lubricants due to their desirable physical properties. Their conjugation to hydrophilic groups such as acid-functionalized polyethylene glycol may lead to the formation of biobased UPMs, which are potential drug delivery vehicles.

CHAPTER 3

MATERIALS AND METHODS

3.1–Methodology overview:

The main goal of the research is to chemo-enzymatically synthesize Unimolecular Polymeric Micelles, or UPMs, consisting of a hydrophobic core and a hydrophilic corona to serve as drug delivery vehicles. The molecules' hydrophobic core consists of linear polymer chains covalently attached to a multifunctional molecule that serves as a hub The corona of UPMs consists of hydrophilic moieties such as poly(ethylene glycol) that are conjugated with functional groups on the termini of the core's linear polymer chains

Kelly and Hayes developed a synthetic convergent approach to form star polymers which can serve as the core of UPMs, where poly(ricinoleic acid) is first synthesized in bulk to serve as acyl donor and then polyol acyl acceptor such as pentaerythritol is added to form star polymers, by immobilized lipase catalyzed reactions conducted in solvent-free systems. [4] The main idea of synthesizing star polymers by enzymatic polymerization is extension to the procedure earlier developed by Hayes and his group. While Kelly and Hayes developed a first generation of star polymers of ricinoleic acid with molecular weights upto 5 kDa, the current approach utilizes lipase-catalyzed polymerization of hydroxyl fatty acids to synthesize the core molecule of proposed star polymer. The triacylglyceride molecule, which upon free radical polymerization can serve as hydrophobic core molecule of proposed star polymer were synthesized. Specifically, Objective 1 is to enzymatically produce mono-undecenoyl, di-ricinoleyl TAG. This molecule can undergo free radical polymerization via the terminal double bond of the undecenoic acyl group, to form a core molecule that would contain several *oligo*(ricinoleic acid) chains extending outward from a central "hub."A sub-objective will be to prepare triundecenoin by 'random' lipase catalyzed esterification using glycerol and undecenoic acid (Objective 1.1). Subsequently, ricinoleic acid will be attached to the 1- and 3- positions of triundecenoin by 1, 3-selective lipase catalyzed acidolysis (Objective 1.2), [22] which is to be subsequently esterified to ricinoleic acid by a 'random' lipase to produce 2-undecenoyl, 1,3-

oligo(ricinoleyl) TAG (Objective 1.3). (An alternative approach to achieve Objective 1.2, castor oil, approximately 90% triricinoate, was transesterified with undecenoic acid vinyl ester using RML to produce 2,3-(1,2)-ricinoleyl and 1-(3)-undecenoyl TAG). Objective 2 of the proposed research is to develop approaches to covalently attach acid-functionalized poly(ethylene glycol) monomethyl ether to the hydroxy end groups of ricinoleic acid and its oligomers, to enable unimolecular polymeric micelle (UPM) formation. For all objectives, chemical characterization will be a key component of the research plan.

3.2.1 –Synthesis of vinyl undecenoate

Undecenoic triacylglycerol was produced via lipase-catalyzed transesterification of 3 mole equivalents of undecenoic acid vinyl ester and one mole equivalent of glycerol (Figure 3.1). Undecenoic acid was chemically esterified with vinyl acetate to produce vinyl undecenoate, to activate the acyl donor with a vinyl alcohol leaving group. Since vinyl undecenoate is not commercially available, the acyl donor was first produced using vinyl acetate. Vinyl alcohol from the reaction irreversibly tautomerizes to acetaldehyde, an isomer disabling it from further interacting with reaction medium, making it a very good leaving group

***3.2.1.1* –Materials:**

10-Undecenoic acid (98% pure) was purchased from Sigma-Aldrich (St. Louis MO). Vinyl acetate, hexanes (HPLC grade), acetic acid (glacial, ACS certified) and potassium hydroxide (flakes, technical grade) were purchased from Fisher Scientific (Suwanee, GA). The homogenous catalyst: palladium acetate (nitrogen-flushed) and diethyl ether (anhydrous, ≥99.7%, 1 ppm BHT as inhibitor) were purchased from Acros Organics (Fairlawn, NJ). Silica gel flexible TLC plates (2.5 x 7.5 cm) of 60 A mesh size with thickness 200 microns were purchased from Selecto Scientific (Suwanee, GA).

***3.2.1.2* –Synthesis procedure:**

Undecenoic acid was converted to its vinyl ester form by using the following method, as outlined in Figure 3.1. [34] A mixture of 10-undecenoic acid (86.5 mg, 1 mmol), vinyl acetate (15 ml, 1 mmol), palladium acetate (11.2 mg), and KOH (1.68 mg) were placed in 20 mL glass

scintillating vial and stirred at 300 rpm and at room temperature (RT) for 24 hours in a closed environment. The mixture was then filtered through a filter paper using vacuum filtration and the residue was washed with vinyl acetate (10ml). Vinyl acetate was than evaporated at 25°C in a vacuum oven to obtain the final product.

3.2.1.3 – Characterization of the reaction product:

Small aliquots were taken out periodically for monitoring the course of reaction with time using thin layer chromatography (TLC) using flexible TLC plates. Hexane/diethyl ether/acetic acid (70/29/1, v/v/v) was used as developing solvent, and iodine vapors for detection. Characterization of final product was also carried out using normal-phase high performance liquid chromatography (HPLC) equipped with an evaporative light scattering detector (ELSD), Model MK III from Alltech Associates, a division of W.R. Grace (Deerfield, IL). An isocratic solvent system, consisting of hexane: diethyl ether: acetic acid (70/29/1, v/v/v) was employed at a flow rate of 1 ml/min, along with a 'Sunfire' silica column (5µm, 4.6 x 250 mm) from Waters (Milford, MA)

3.2.2 –Synthesis of Triundecenoin (Objective 1.1)

3.2.2.1 –Materials:

Novazym SP 435, immobilized *Candida antarctica* B lipase, or CAL produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous acrylic resin, a commercial product manufactured by Novozymes, Inc. (Franklinton, NC), was purchased from Sigma-Aldrich. Glycerol (ACS certified), silica gel (selecto scientific) of particle size 32-63 micron, molecular sieves (grade 514, type 4A), tertiary butyl alcohol (ACS certified), and the organic solvents used for characterization (HPLC grade acetone, hexane, acetonitrile, and diethyl ether) were obtained from Fisher.

***3.2.2.2* – Synthesis procedure:**

Undecenoic acid vinyl ester (Section 3.2.1) acyl donor and glycerol acyl acceptorwere used to produce triundecenoin catalyzed by CAL (Figure 3.1). The main concern associated with reaction is that acyl donor and acceptor are not miscible. Biphasic reaction media will lower the enzyme activity. Some common approaches to improve the miscibility of glycerol are reviewed in the literature. [22] Polar solvents like *tert*-butanol help to improve the miscibility of medium. [44] Silica gel is used to disperse glycerol into medium. [6] The suspended silica gel – glycerol particles act as reservoir for glycerol in the medium and prevent adsorption of non-solubilized glycerol by lipase leading to a mass transfer barrier for the enzyme and hence to a lower biocatalytic turnover. Using the approaches in cited literature, [6] undecenoic acid vinyl ester was reacted with glycerol using following method. Initially equal amounts of glycerol (15 g, 1mmol) and silica gel (15 g) were mechanically mixed until homogeneity was achieved. The free-flowing dry powder was stored in desiccator. The absorbed glycerol was than mixed with vinyl undecenoate (42 mg, 3mmol) at a 3:1 stoichiometric ratio along with *tert*-butanol (75ml) as polar solvent and 10% (w/w) CAL. The later is an effective 'random' enzyme capable of utilizing all three hydroxyl groups of glycerol. Molecular sieves of size- 4 A were added at regular intervals of 6h in equal amounts of 2mg during the time course of reaction. The reaction was carried out at room temperature under continuous stirring at 300rpm. The time course of reaction was continuously monitored using TLC until the reaction was stopped after 48h. The product was first filtered out by vacuum filtration through filter paper to remove silica gel, enzyme, and molecular sieves. Subsequently, product was rinsed in *tert*-butanol and solvent was evaporated away.

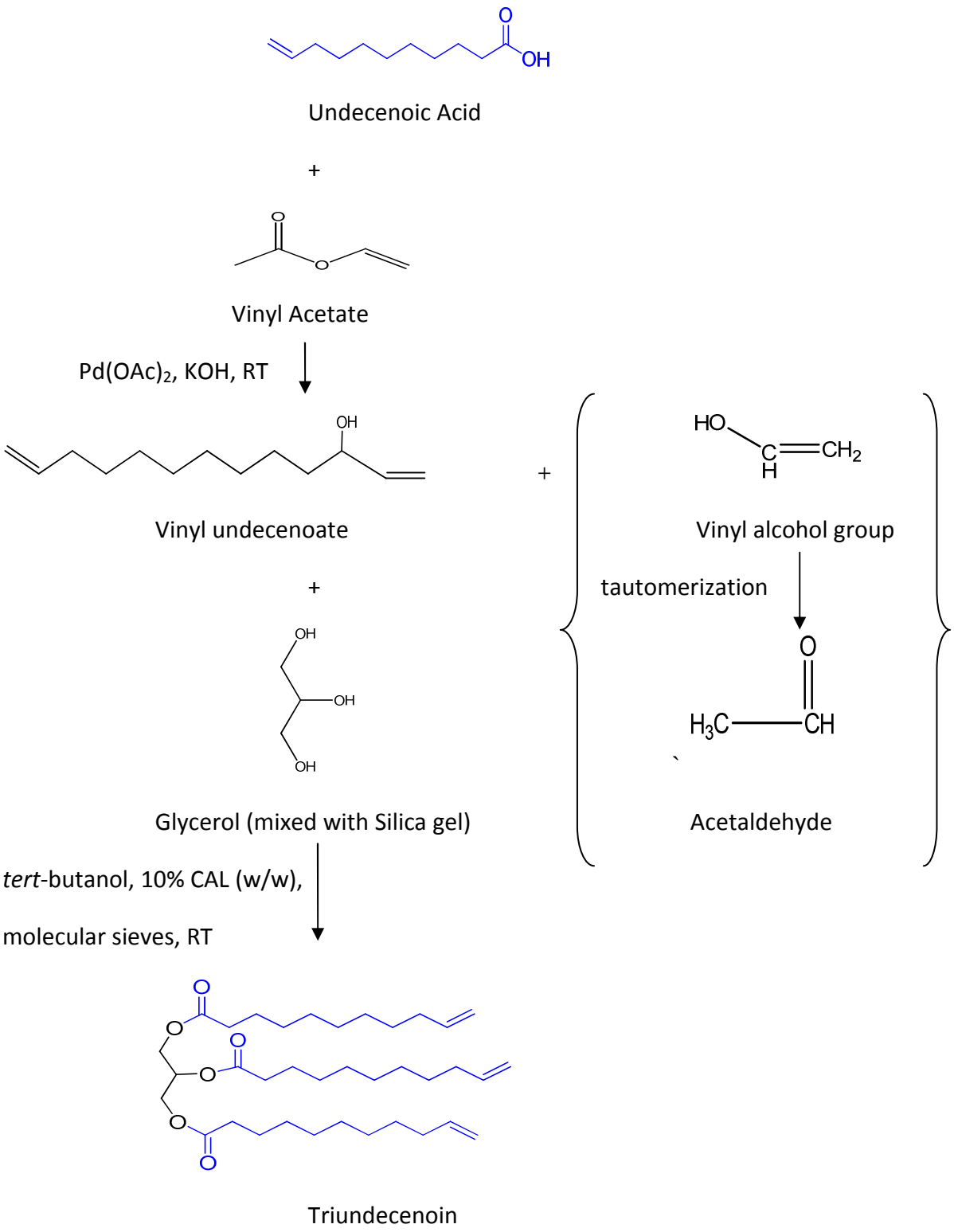


Figure. 3.1. Lipase-catalyzed synthesis of triundecenoin (Objective 1.1)

3.2.2.3 – Characterization of the reaction product:

The progress of undecenoic acid vinyl ester-glycerol transesterification reaction was monitored using chromatographic techniques. By thin layer chromatography (TLC), the formation of triacylglycerol was identified using similar approach as described in section 3.2.1.2. Standards used for TLC analysis included triolein, castor oil (to represent trihydroxyacyl TAG), and a mixture of triunsaturated triacylglycerols (tricaprin, tricaprylin, trilaurin, trimyristin, tripalmitin) to represent non-hydroxylacyl TAG. Reversed-phase (RP-) HPLC was performed for separation of product using a 40min gradient method as reported in literature. [5] It utilized a 4.6mm x 250mm x 5µm Microsorb MV C18 column (Varian Inc., Walnut Grove, CA), and a gradient elution system for mobile phase at 0.8ml/min consisting of acetone and acetonitrile (70/30 v/v) held for 2minute, followed by an 18 minute gradient stage during which, the solvent system was changed linearly to 100% acetone, followed by a 5 minute hold using 100% acetone, and a 2 minute period in which the solvent system is changed linearly back to initial condition. Both solvents were pumped using solvent delivery system pumps (Alltech) separately using solvent delivery system pumps. The volume of samples injected was 20µL, at concentration of 1 mg/ml. An ELSD was used for signal detection.

3.2.3 – Synthesis of 2-undecenoyl, 1,3- ricinoleyl TAG (Obejective 1.2):

3.2.3.1 –Materials:

Lipozyme IM, immobilized *Rhizomucor miehei* lipase (RML), purified 1, 3-specific lipase from *Rhizomucor miehei* produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism, a commercial product from Novozymes, Inc, and sodium metal (stick, dry ACS reagent) were purchased from Sigma-Aldrich. Ricinoleic acid,(Fluka, technical grade), originally containing 90% ricinoleic acid, 8% C_{18} mono and dienes, and 2% saturates was purchased from Sigma-Aldrich. All solvents used are of HPLC grade and are purchased from Fisher. Sodium metal (stick, dry ACS reagent) is purchased from Sigma-Aldrich.

3.2.3.2 –Synthesis procedure:

The next step after synthesizing triundecenoin (Section 3.2.2) was interesterification of ricinoleic acid triundecenoin using a 1, 3-selective enzyme, RML [22], shown as in Figure 3.2. Alternatively, to synthesize a TAG molecule containing 2,3-(1,2-)ricinoleyl and 1-(3-) undecenoyl acyl groups, RML-catalyzed interesterification of castor oil (triricinolein, 1mmol) and undecenoic acid vinyl ester(1mmmol) (Section 3.2.1) was employed. Triundecenoin (63.8 mg, 1mmol) was added to ricinoleic acid (59.8 mg, 2mmol) along with 10%(w/w) of Lipozyme IM (RML). The reaction was carried out at 70° C with continuous stirring of 300rpm in closed environment. . Alternatively, to achieve Objective 1.2., 2,3-(1,2-)ricinoleyl and 1-(3-) undecenoyl TAG was synthesized through interesterification of castor oil (triricinolein, 50 mg, 1 mmol) and undecenoic acid vinyl ester(132.5 mg, 1 mmmol) (Section 3.2.1) catalyzed by RML (5 mg) under the same reaction conditions as described above.

3.2.3.3 –Characterization of the reaction product:

Characterization of 2-undecenoyl, 1,3- ricinoleyl TAG is carried out along similar lines to that of triundecenoin synthesis (Section 3.2.3). RP-HPLC (Section 3.2.3) was used to detect formation of new triacylglycerols in the product. In addition, a normal phase HPLC method [26] using the Sunfire® silica column and hexane: diethyl ether: acetic acid (70/29/1, v/v/v) as mobile phase at 0.8ml/min for 15 minutes was also performed as described in section 3.2.2.2. Preparative thin layer chromatography was used to isolate TAG from free fatty acids and partial glycerides. A scored silica gel chromatographic plate (“Uniplate” from Analtech, Newark, DE) of 250 microns thickness and 10x20 cm size was used with hexane: diethyl ether: acetic acid (70/29/1, v/v/v) as developing solvent. The plate was scored to create two sections, one for the development of standards on the left-hand side of the plate and a second for the separation of reaction mixture (Figure 3.4). A 25x25x10 cm chamber is used for development. A mixture of iodine crystals and silica gel powder was applied to the left-hand section of the plate for detection of the standards, to identify R_f ranges where products from the separation of reaction mixture should be collected. Samples were extracted from sections of the TLC stationary phase

that contained product, removed from the glass plate via a razor blade, using acetone, which was removed by evaporation using a rotary evaporator.

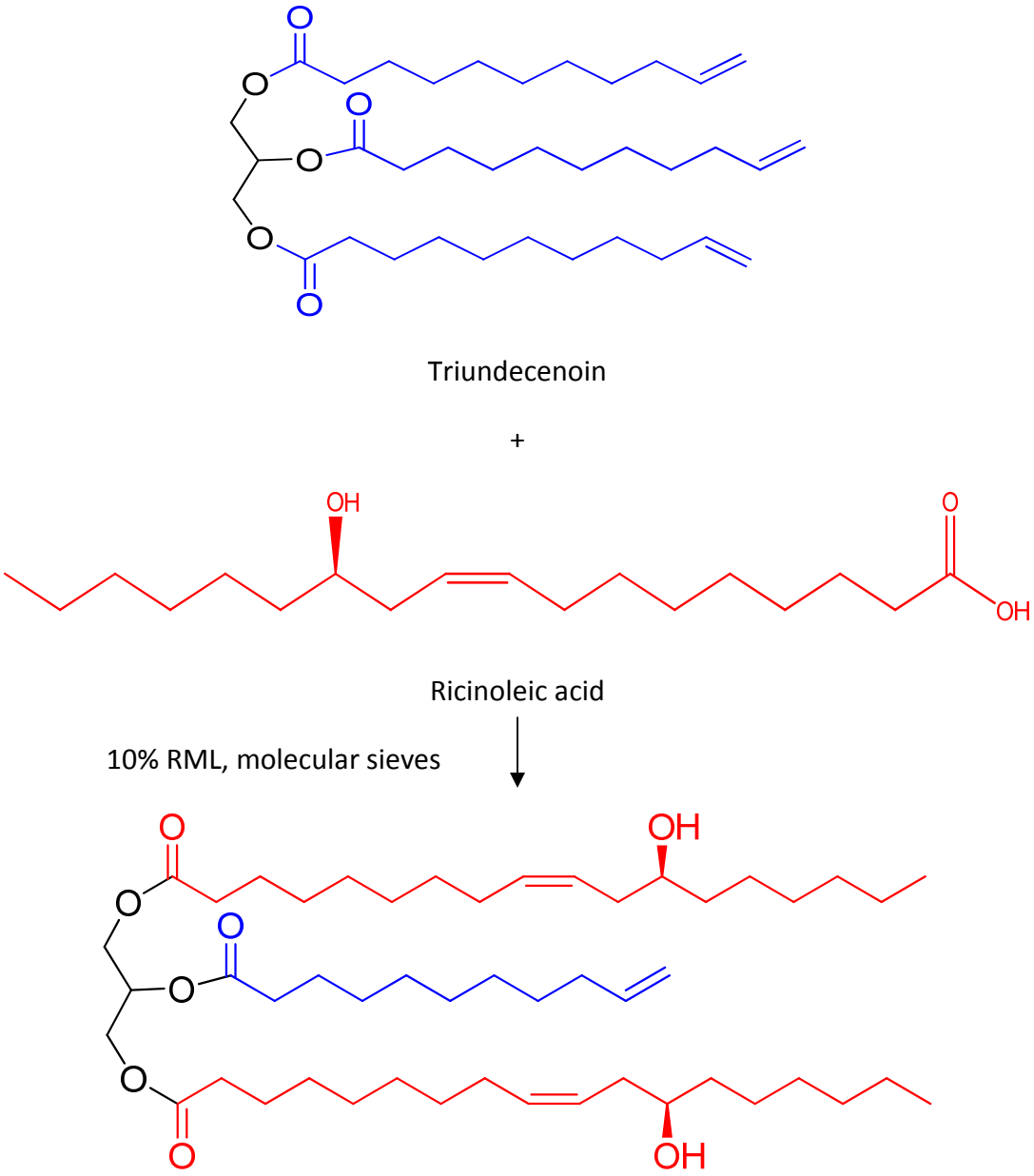


Figure 3.2. Lipase-catalyzed synthesis of 2-undecenoyl, 1,3- ricinoleyl TAG synthesis (Objective 1.2)

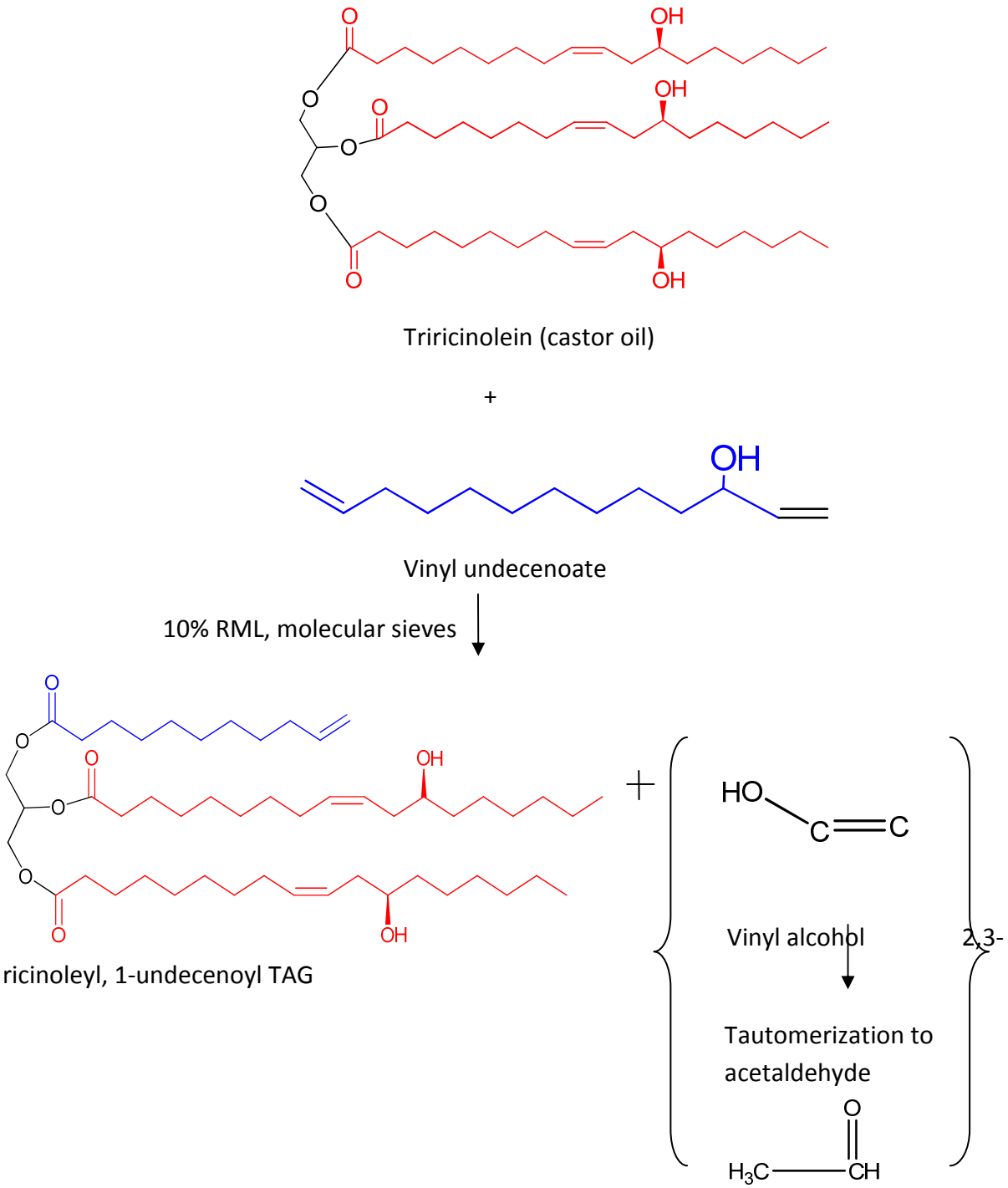


Figure 3.3. Lipase-catalyzed synthesis of 2,3-ricinoleyl, 1-undecenoyl TAG synthesis (an alternate approach to achieve Objective 1.2)

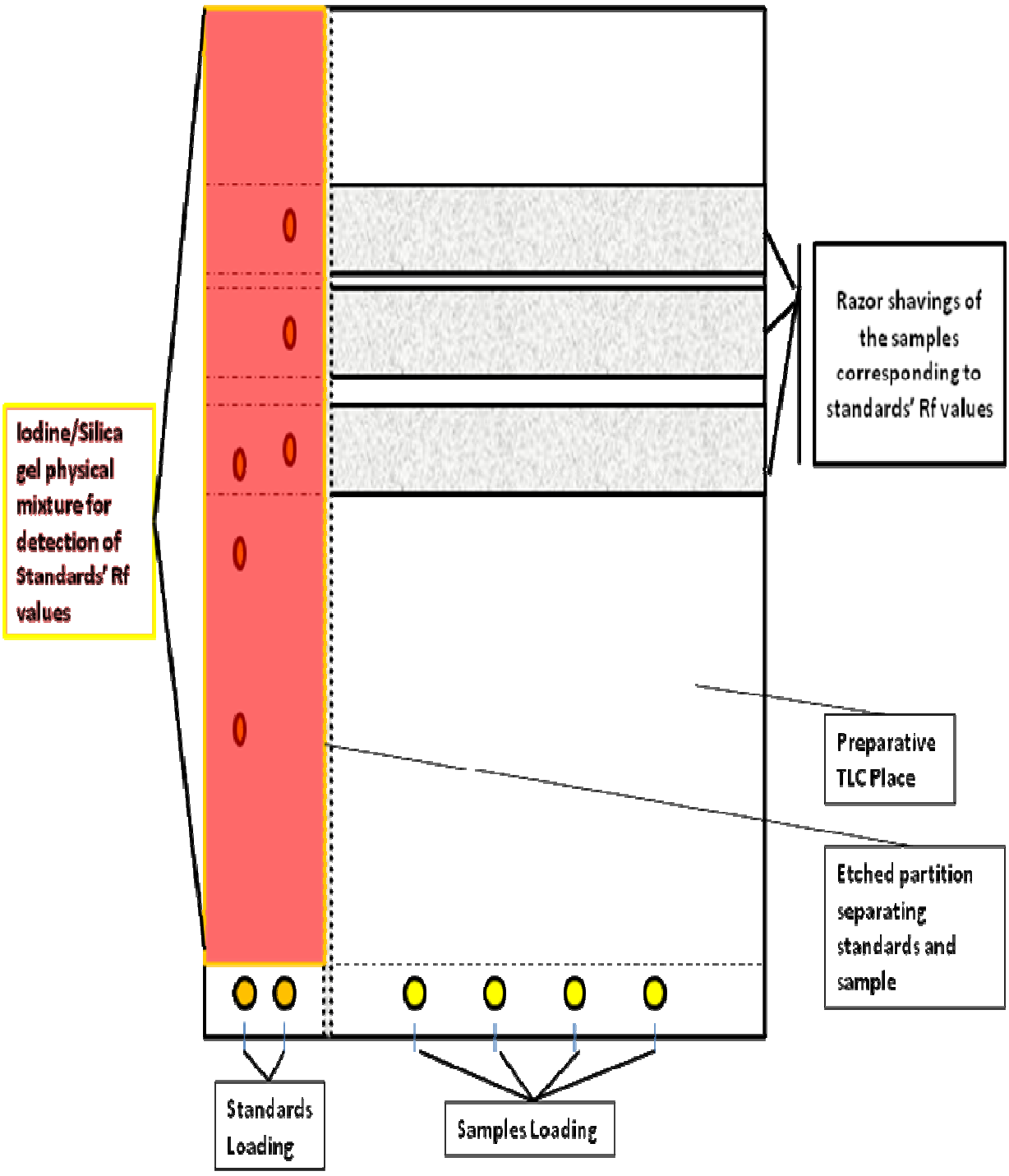


Figure 3.4. Schematic showing preparative TLC setup for separation of product samples

3.2.4. – Synthesis of monoundecenoyl, di-oligo(ricinoleyl) TAG (Objective 1.3)

3.2.4.1. –Materials:

Novazym SP 435, immobilized *Candida antarctica* B lipase, or CAL produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous acrylic resin, was purchased from Sigma-Aldrich. Ricinoleic acid,(technical grade), originally containing 90% ricinoleic acid, 8% C18 mono and dienes, and 2% saturates was purchased from Fisher Scientific. Chloroform-D (D, 99.8%) was obtained from Cambridge Isotope Laboratories (Andover, MA). All solvent are HPLC grade purchased from Fisher.

3.2.4.2. –Synthesis Procedure:

Formation of *oligo*(ricinoleic acyl) chains onto the TAG molecules derived from achievement of Objective 1.2 arms on the 2-undecenoyl, 1,3- ricinoleyl TAG was achieved using the scheme depicted in Figure 3.5. 2-undecenoyl, 1,3- ricinoleyl TAG (63.8 mg, 1 mmol) was added to ricinoleic acid (59.8 mg, 2 mmol) along with 10%(w/w) of CAL. The reaction was carried out at 70°C with continuous stirring of 300rpm in closed environment. A similar approach was employed for conjugation of *oligo*(ricinoleic acyl) chains to 2 (3-)-undecenoyl, 1,3 (1,2)- ricinoleyl TAG.

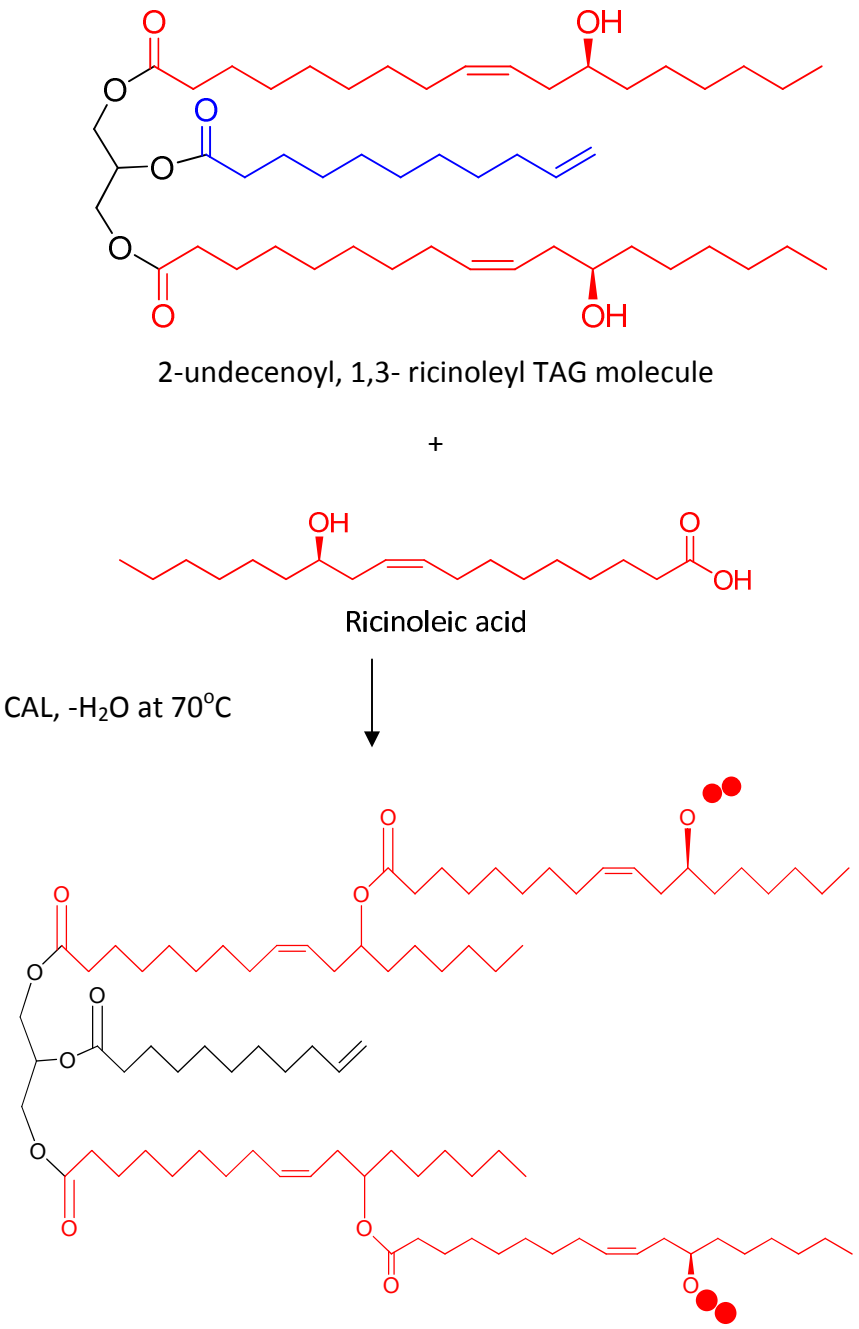


Figure 3.5. Lipase-catalyzed synthesis of 2-undecenoyl, 1,3- *oligo*(ricinoleyl) TAG (Objective 1.3)

***3.2.4.3.* –Characterization of reaction product:**

Gel permeation chromatography is used for characterization and molecular weight assessment of product. A 7.8 x 300 mm STYRAGEL® HR 4E GPC column from Waters (Milford, MA) is used with tetrahydrofuran (HPLC grade) as mobile phaseat 1 mL / min and ELSD detector is used for signal detection. The number-averaged molecular weights (M_n) and polydispersity index were calculated from the chromatographs obtained from GPC analysis using a semilog retention time-molecular weight calibration made from poly (ricinoleic) acid [38] (Table 3.1 and Figure 3.6). The chromatogram was integrated by dividing the underlying area into a series of trapezoids; then, the number- and weight averaged molecular weight ((M_n and M_w, respectively) were calculated using the following formulae:

$$M_n = \sum y_i MW_i$$

$$M_w = \sum y_i MW_i^2$$

where the subscript *i* refers to the *i*th trapezoid and MW*i* to the average molecular weight of trapezoid *i,* based on the average retention time and the corresponding molecular weight determined from the calibration. The polydispersity index, PDI, is then calculated from the ratio of the two:

$$PDI = M_w/M_n$$

Proton NMR Spectra of the sample were obtained for identification of the product's chemical structure using a 300 MHz Varian Mercury spectrometer and a VnmrJ software from Varian using a Linux-PC interface instrument control, data acquisition, and data processing. Spectra were prepared and integrated using Mestrec NMR processing software.

Table 3.1 Log molecular weight and retention time calibration from poly (ricinoleic) acid for molecular weight estimation from GPC analysis

Peak[a]	Retention time (minutes)	Molecular Weight (MW), Da[b]	log MW
R1	9.02	298	2.47
R2	8.76	579	2.76
R3	8.57	859	2.93
R4	8.36	1140	3.05
R5	8.21	1423	3.15
R6	8.01	1703	3.23

a. Peaks for *oligo*mers of ricinoleic acid obtained from GPC analysis. R1- R6 correspond to monomer, dimer, trimer, tetramer, pentamer, and hexamer, respectively

b. Average molecular weight of ricinoleic acid and their oligomeric units

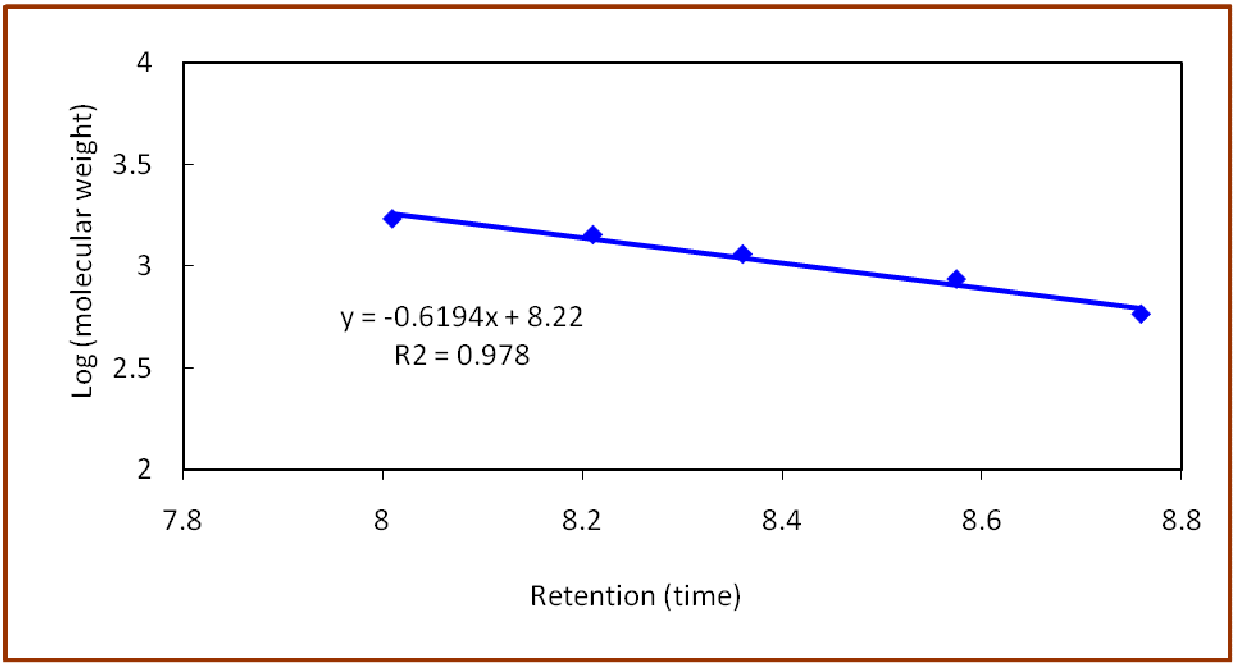


Figure 3.6. Calibration plot for Log molecular weight versus retention time of poly(ricinoleic) acid

3.3 –Overview of Chemical Conjugation of poly (ethylene) glycol monomethyl ether to oligo(ricinoleic acid) (Objective 1.2):

The methodology developed during the course of the earlier two steps (Sections 3.1 and 3.2) with ricinoleic acid and *oligo*(ricinolely) fatty acids can be finally used to covalently attach PEG derivative to synthesized hydrophobic core molecule. To achieve this goal was developed in this thesis with a series of studies on the conjugation of MPEG-succinimidyl ester to hydroxyl fatty acids. First, the PEG derivative was reacted with ricinoleic acid. Second, MPEG-acid conjugation with *oligo*(ricinoleic acid) to determine not only the extent of conjugation, but also to detect if hydrolysis of the internal ester and ether bonds of oligo(ricinoleic aicd) and PEG, respectively, occurred.

3.3.1 – Chemical Conjugation of acid functionalized poly (ethylene) glycol monomethyl ether to hydroxy end groups of ricinoleic acid (RA-MPEG$_n$):

3.3.1.1 –Materials:

MPEG- succinimidyl ester (2000 Da, MW) was purchased from Creative PEG works (Winston-Salem, NC). Ricinoleic acid, acetone, molecular sieves were obtained from vendors as mentioned earlier. Granular sodium hydroxide (ACS certified) was obtained from Fisher.

3.3.1.2 –Synthesis procedure:

Procedure: A stoichiometric ratio~~s~~ of MPEG Succinimidyl acid (201 mg, 1 mmol) and ricinoleic acid (30 mg, 1 mmol; pre-desiccated) was mixed in anhydrous acetone (10 ml) (Figure 3.6). After addition of molecular sieves, the reaction mixture was kept at room temperature under continuous stirring at 300rpm. [44] After 12h, the reaction was stopped final product was filtered to remove molecular sieves and leaving group (NHS) residue, and then the filtrate was placed in a rotary evaporator to remove acetone and recover the reaction product.

***3.3.1.3 –Characterization of the reaction product*:**

The resultant product obtained was characterized using HPLC, GPC and ^{1}H- NMR. Normal phase HPLC using isocratic method with mobile phase of hexane: diethyl ether: acetic acid (70:29:1), as described in Section (3.2.3.2) was used.

***3.3.2– Chemical conjugation of acid-functionalized poly (ethylene) glycol monomethyl ether to hydroxy end groups of oligo-ricinoleic acid* (oligo-RA-MPEG$_n$):**

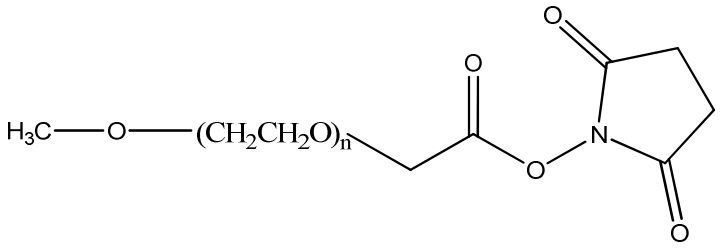
***3.3.2.1* –Materials:**

MPEG- succinimidyl carboxy methyl ester (2000 Da, MW) was purchased from Creative PEG works (Winston-Salem, NC). Ricinoleic acid, acetone, molecular sieves are obtained from vendors as mentioned earlier. Novazym SP 435, immobilized *Candida antarctica* B lipase, or CAL produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous acrylic resin, was purchased from Sigma-Aldrich

***3.3.2.2* –Synthesis procedure:**

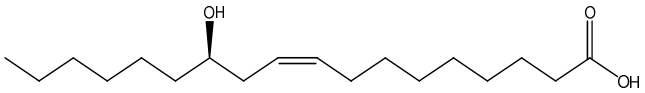
Initially ricinoleic was polymerized by lipase catalyzed polymerization using method mentioned in literature (Section 2.3). Ricinoleic acid (5 g) was polymerized using 500 mg of CAL at 70°C temperature in closed environment. An oligomer of ricinoleic acid with molecular weight of 1463 Da~~,~~ was obtained. The molecular weight of the *oligo*(ricinoleic acid) product was estimated by ^{1}H-NMR analysis by using percent esterification of hydroxyl acyl groups and average degree of polymerization to perform the calculation (discussed under results of Section 4.3 in Chapter 4). Subsequently, a stoichiometric ratio~~s~~ of MPEG-NHS ester (201 mg, 1 mmol) and *oligo*(ricinoleic acid) (200mg, 1 mmol; pre-desiccated) was mixed in anhydrous acetone (10 ml) (Figure 3.7). The reaction mixture was kept at room temperature and continuously stirred at 300 rpm. Molecular sieves were added to lower the water content. After 24 h, the reaction was stopped by filtering the mixture. The supernatant was placed in a rotary evaporator to

remove acetone and yield the final product. The resultant product was characterized using TLC, reversed-phase HPLC, and normal phase HPLC as described in Section (3.2.3.2).



mPEG Succinimidyl acid

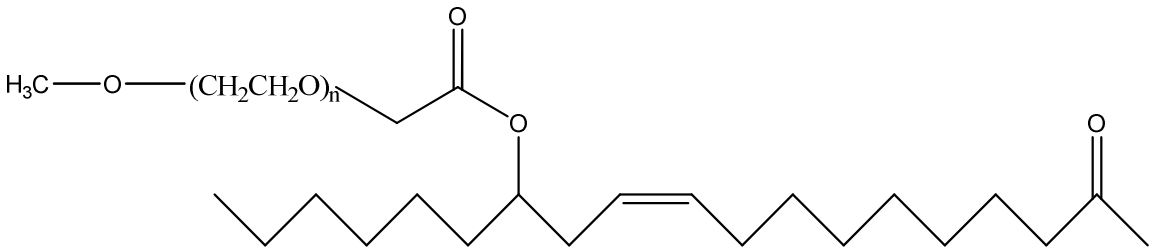
+



Ricinoleic acid

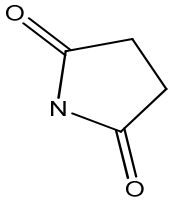
1. Acetone, molecular sieves, room temperature

2. NaOH (1N), 10ml



PEG-Ricinoleic acid

+



NHS (Succinimidyl) leaving group

Figure 3.7. Conjugation of acid-functionalized MPEG with ricinoleic aicd (Objective 2)

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1-Synthesis of Triundecenoin (Objective 1.1):

Synthesis of triundecenoin via the *Candida antarctica* B lipase-catalyzed transesterification between vinyl undecenoate and glycerol was monitored by thin layer chromatography (TLC). The final product was analyzed using TLC and reversed phase- (RP-) HPLC. Formation of triacylglycerol (TAG) as the major product was supported by TLC, with the product's major band possessing an R_f value comparable to tricaprylin standard, 0.71 (Figure 4.1). TLC analysis indicated a second band assumed to be free fatty acid, since its R_f value is near that of undecenoic acid (0.41).

The identity of RP-HPLC peaks was determined using equivalent carbon numbers (ECN) based on a retention time-carbon number relationship determined using a mixture of unsaturated TAG (tricaprylin, tricaprin, trilaurin, trimyristin, and tripalmitin) and castor oil (triricinolein) as standards. The RP-HPLC chromatogram of the product contained a peak with retention time of 5.14 min, near the retention times of triricinolein and tricaprylin (5.2 min and 4.65 min respectively) (Figure 4.2).The ECN calculated for this peak, C_n=33 is consistent with triundecenoin. (Where C_n is theoretical carbon number calculated from number of carbon numbers in the molecule minus twice the number of double bonds).

From the results of TLC and HPLC, based particularly upon the integration areas of the HPLC peaks (6640 mV s for triundecenoin, eluting at 5.14 min divided by the total integrated area, 7104 mV s), the yield of triundecenoin is 94%. Successive batch reactions of triundecenoin synthesis at a larger scale (10g of free fatty acid) have produced similar yields of 90-95%.

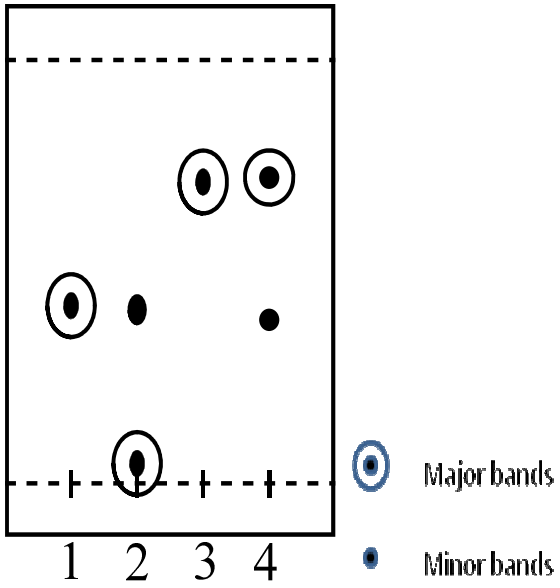


Figure. 4.1. Thin Layer Chromatography (TLC) analysis of triundecenoin synthesis (Objective 1.1), Lines 1-3 contain standards: 1. Undecenoic acid (R_f= 0.41); 2. vinyl undecenoate(R_f=0.05); 3. Tricaprylin (R_f=0.71). Line 4: Product from triundecenoin synthesis (R_f=0.71 for major band).

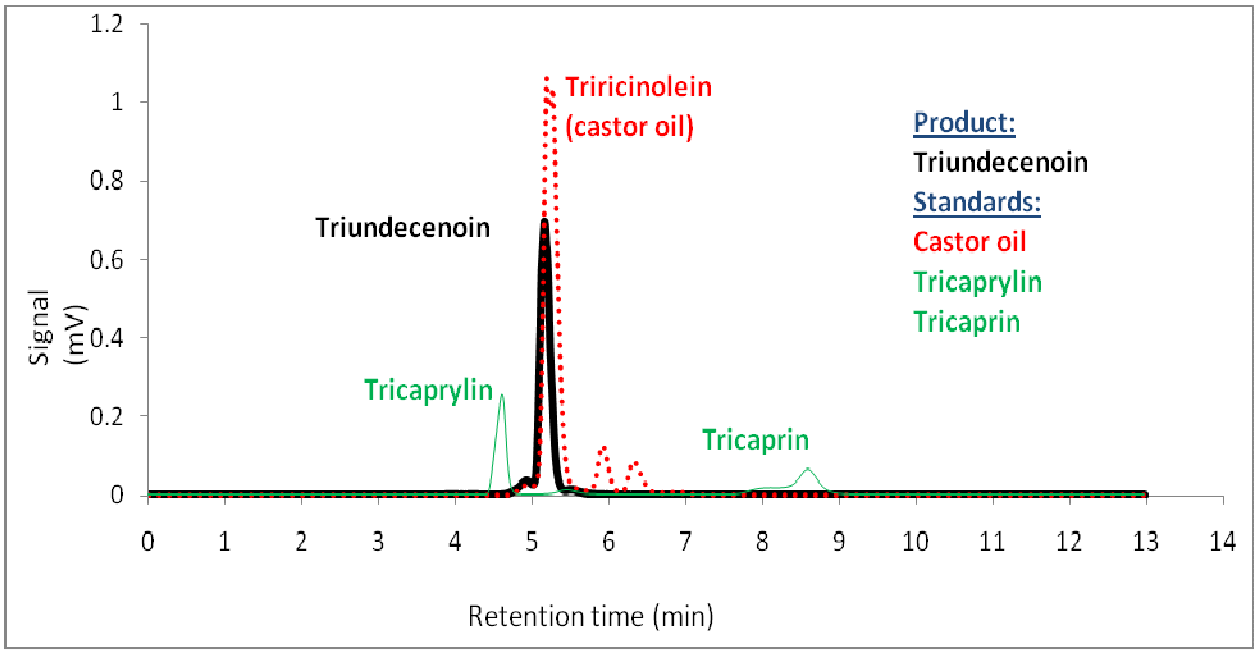


Figure. 4.2 RP-HPLC analysis of the product from CAL-catalyzed interesterification of glycerol and undecenoic acid vinyl ester

***4.2-Synthesis of 2-undecenoyl, 1,3- ricinoleyl TAG and of 2,3-ricinoleyl, 1-undecenoyl TAG (Objective 1.2)*:**

TLC was used to monitor the synthesis of the anticipated 2-undecenoyl, 1,3- ricinoleyl TAG from RML-catalyzed interesterification of triundecenoin and ricinoleic acid, and alternatively of the anticipated product, 2,3-ricinoleyl, 1-undecenoyl TAG, from the CAL-catalyzed transesterification of castor oil and vinyl undecenoin. ^{1}H-NMR was performed on the reaction mixture subsequent to lipase removal to determine the chemical structure of products that formed. Normal and reversed phase HPLC were also used to characterize the TAG-rich fractions purified by preparative TLC. TLC analysis indicates formation of TAGs that possesses a similar R_f value, hence a similar degree of polarity, as that of triundecenoin (Figure 4.3). The product is than separated of free fatty acids (R_f of 0.45) using preparative TLC (as described in Section 3.2.3.2). TAGs in the product sample are measured by weight and % of product formation is calculated (Table 4.1).

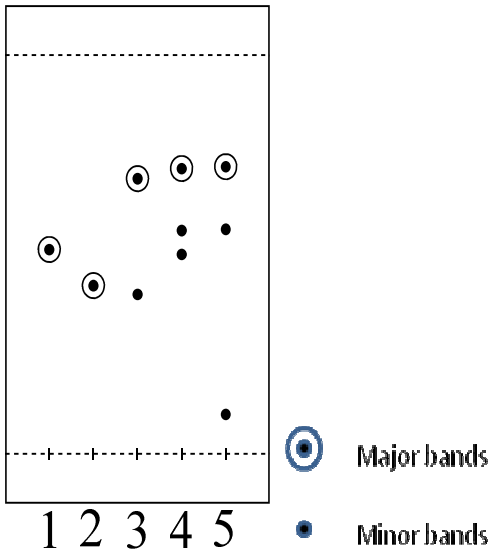


Figure. 4.3. Thin layer chromatography analysis of the reaction mixture for CAL-catalyzed synthesis of 2-undecenoyl, 1,3- ricinoleyl TAG (Lane 5; R_f=0.72). Lanes 1-4 are standards: 1. ricinoleic acid (R_f=0.43); 2. undecenoic acid (R_f=0.41); 3. triundecenoin (R_f=0.70); 4. castor oil (triricinolein, R_f=0.72).

Table. 4.1. Percent product recovery using Preparative TLC purification of TAGs formed through the reactions producing 1,3- ricinoleyl, 2-undecenoyl TAG and 2,3-ricinoleyl, 1-undecenoyl TAG.

Sample	R_f Range	% product recovery[a]
1,3-ricinoleyl, 2-undecenoyl TAG[b]	0.75-0.85	58.37
2,3-ricinoleyl, 1-undecenoyl TAG[c]	0.75-0.85	49.63

a. **Based on mass of recovered product divided by mass of product mixture placed on TLC plate initially**

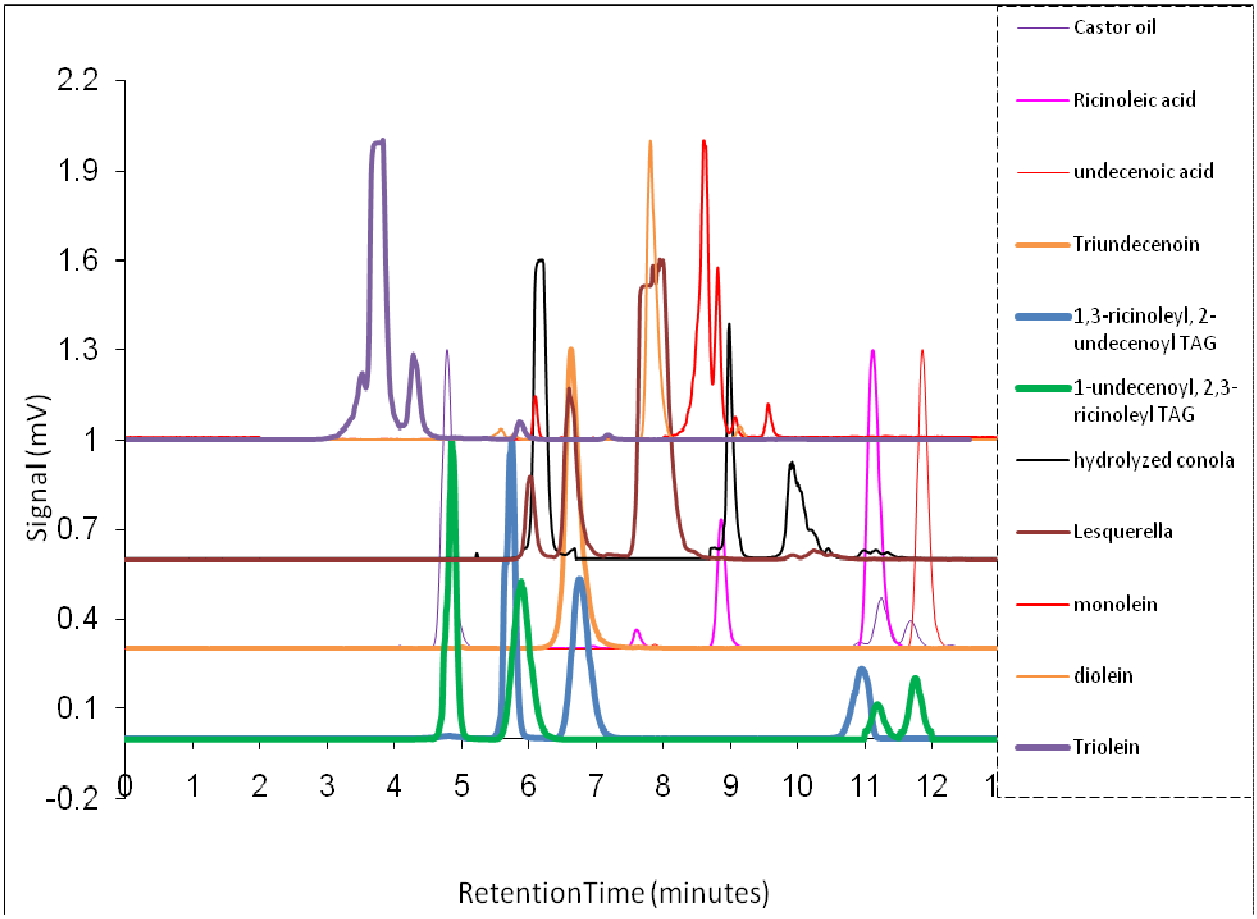
b. **RML-catalyzed interesterification of triundecenoin and ricinoleic acid**

c. **CAL-catalyzed transesterification of castor oil and vinyl undecenoin**

Normal phase HPLC analysis was performed upon the reaction mixture resulting from the synthesis of 2-undecenoyl, 1,3- ricinoleyl TAG and 2,3-ricinoleyl, 1-undecenoyl TAG, with identification of chemical species performed with the substrates castor oil, triundecenoin, undecenoic acid, and ricinoleic acid, and partially hydrolyzed conola oil, lesquerella oil, monoolein, dioelin, and triolein as standards (Figure 4.4).

The major product peaks for both reaction mixtures, for the synthesis of 2-undecenoyl, 1,3- ricinoleyl TAG and 2,3-ricinoleyl, 1-undecenoyl TAG, occurred at retention times (5.75 and 5.95 min respectively) that differed from castor oil and triundecenoin (4.83 and 6. 85 min respectively), indicating formation of new TAG species. Also, the eluted peaks of new TAGs doesn't correspond to mono and di-hydroxylacyl triacylglycerols of the lesquerella or diolein. Chromatogram for final reaction product of 2-undecenoyl, 1,3- ricinoleyl TAG consist of triundecenoin (6.8 min), ricinoleic acid (10.9 min) along with new TAG corresponding to product formation. Chromatogram for final reaction product of 2,3-ricinoleyl, 1-undecenoyl TAG consist of triricinolein (4.8 min), undecenoic acid (11.4 min) along with new TAG corresponding to product formation. Also, both reaction product chromatograms are devoid of any estolides. From normal phase HPLC chromatograms, the % conversions of product for both TAGs are 53% and 47% respectively for 2-undecenoyl, 1,3- ricinoleyl TAG and 2,3-ricinoleyl, 1-undecenoyl TAG, based on integration of peak areas (373 mV s for 2-undecenoyl, 1,3- ricinoleyl TAG eluting

at 5.75 min divided by the total integrated area of 703 mV s for the product and, and 421 mV s for 2,3-ricinoleyl, 1-undecenoyl TAG, eluting at 5.95 min divided by the total integrated area, 894 mV s of product). The results are conclusive to the % weight extractions obtained from preparative TLC (Table 4.1).



***Chromatograms for starting materials are offset to 0.3 mV, hydrolyzed conola and lesquerella are offset to 0.6 mV and mono-, di-, trioleins are offset to 1 mV to add clarity**

Figure. 4.4. Normal Phase HPLC of 2-undecenoyl, 1,3- ricinoleyl TAG and 2,3-ricinoleyl, 1-undecenoyl TAG

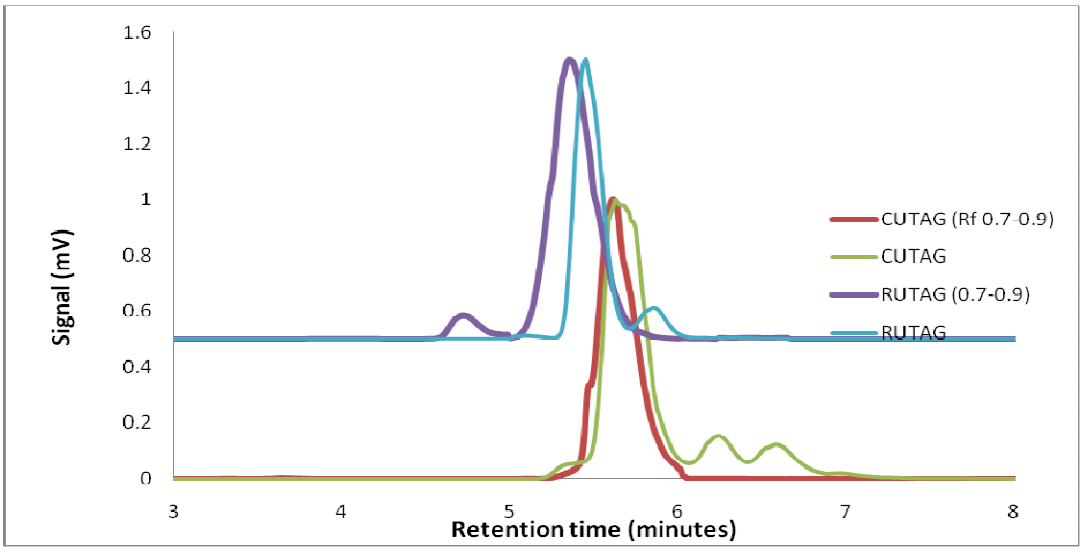
The purified TAG fractions obtained via preparative TLC were analyzed using RP- HPLC, to further identify their chemical structure (Figure 4.5). This identification is important for determining the yield of structured TAG product using the integrated peak areas for the chromatogram of the reaction product prior to preparative-TLC fractionation. The TAGs from reaction product elute at different retention times to that of triundecenoin and triricinolein (Table 4.5). From reverse phase HPLC, the % conversions of product for both TAGs are 60% and 65% respectively for 2-undecenoyl, 1,3- ricinoleyl TAG and 2,3-ricinoleyl, 1-undecenoyl TAG, based on integration of RP-HPLC peak areas (1453 mV s for 2-undecenoyl, 1,3- ricinoleyl TAG eluting at 6.02 min divided by the total integrated area of the product, 2466 mV s for 2,3-ricinoleyl, 1-undecenoyl TAG, eluting at 5.76 min divided by the total integrated area, 1865 mV s for).

Table 4.2 Retention times of the species from the chromatograms obtained by RP-HPLC for 2-undecenoyl, 1,3- ricinoleyl TAG (RUTAG) and 2,3-ricinoleyl, 1-undecenoyl TAG (CUTAG) with extracted fractions from preparative TLC (prep TLC) with R_f 0.7 to 0.9 as standards.

	Retention time (min)	
Species	Preparative TLC[a]	Reaction sample[b]
2-undecenoyl, 1,3- ricinoleyl TAG	6.12	6.02
2,3-ricinoleyl, 1-undecenoyl TAG	5.65	5.76
Triundecenoin	--	5.14
Triricinolein (Castor oil)	--	5.20

a. **Standards used from preparative TLC extractions for 2-undecenoyl, 1,3- ricinoleyl TAG and 2,3-ricinoleyl, 1-undecenoyl TAG with Rf 0.7 to 0.9**

b. **Unpurified reaction sample to calculate percent conversion**



***Chromatograms for 2,3-ricinoleyl, 1-undecenoyl TAG (CUTAG) are offset to 0.5 mV to add clarity**

Figure. 4.5. Reverse Phase HPLC of 2-undecenoyl, 1,3- ricinoleyl TAG (RUTAG) and 2,3-ricinoleyl, 1-undecenoyl TAG (CUTAG) with extracted fractions from preparative TLC (prep TLC) with R_f 0.7 to 0.9.

The yield of monoundecenoyl, 1,3-diricinoleyl TAG obtained from normal phase-HPLC and reversed phase-HPLC are close to the values obtained from preparative TLC (Table 4.1), at approximately 45-55%.

***4.3 – Synthesis of 10-undecenoyl, di-oligo (ricinoleyl) TAG (Objective 1.3)*:**

The products for *C. antarctica* lipase catalyzed esterification of 2-undecenoyl, 1,3-ricinoleyl TAG and ricinoleic acid, to yield 2-undecenoyl, 1,3- *oligo*-(ricinoleyl) TAG were analyzed using GPC and ^{1}H NMR for characterization and molecular weight assessment as described in Section 3.4.2.

^{1}H-NMR was carried out for triundecenoin, 2-undecenoyl, 1,3- ricinoleyl TAG; 2,3-ricinoleyl, 1-undecenoyl TAG, and mono-undecenoyl, di-*oligo*-(ricinoleyl) TAGs. Spectra show shift in the ppm position of the proton attached to C atoms containing the primary and secondary hydroxyls of the glycerol backbone between the substrate triundecenoin and the

product mixture, presumably enriched in 2-undecenoyl, 1,3- ricinoleyl TAG . A similar difference in the ^{1}H-NMR spectra occurred in the substrate and product for 2,3-(1,2-)ricinoleyl, 1- (3-) undecenoyl TAG synthesis. This change suggests a change in carbon chain length for the acyl groups contained in the TAG molecules of the product mixture of 2-undecenoyl, 1,3- ricinoleyl TAG and 2,3-(1,2-)ricinoleyl, 1- (3-) undecenoyl TAG.[35] (Figures 4.6 & 4.7). Also, the formation of monoundecenoyl, di-*oligo* (ricinoleyl) TAG from 2-undecenoyl, 1,3- ricinoleyl TAG and 2,3- (1,2-) ricinoleyl, 1- (3-) undecenoyl TAG alters the integrated areas for H atoms attached to the C atoms that contain the primary hydroxyl groups of the glycerol backbone relative to the substrate, due to change in carbon chain length of the acyl groups attached to the 1- and 3- glycerol position (Table 4.3).

Table 4.3. Peak areas for primary and secondary hydroxyl groups of glycerol back bone from ^{1}H-NMR spectra

Species	primary hydroxyls				secondary hydroxyls	
	C_1HO-		C_3HO-		C_2HO-	
	ppm	area	ppm	area	ppm	area
Glycerol	3.50	2.05	3.55	2.05	3.65	1.00
Triundecenoin	4.85	0.75	4.94	0.75	5.25	0.55
2-undecenoyl, 1,3- ricinoleyl TAG	4.15	1.11	4.25	1.03	5.25	0.33
2,3-ricinoleyl, 1-undecenoyl TAG.	4.15	3.60	4.25	1.03	5.25	0.24
2-undecenoyl, 1,3- *oligo* (ricinoleyl) TAG	4.15	1.56	4.24	0.56	5.25	0.32
2,3-*oligo* (ricinoleyl), 1-undecenoyl TAG	4.15	3.40	4.24	0.92	5.25	0.18

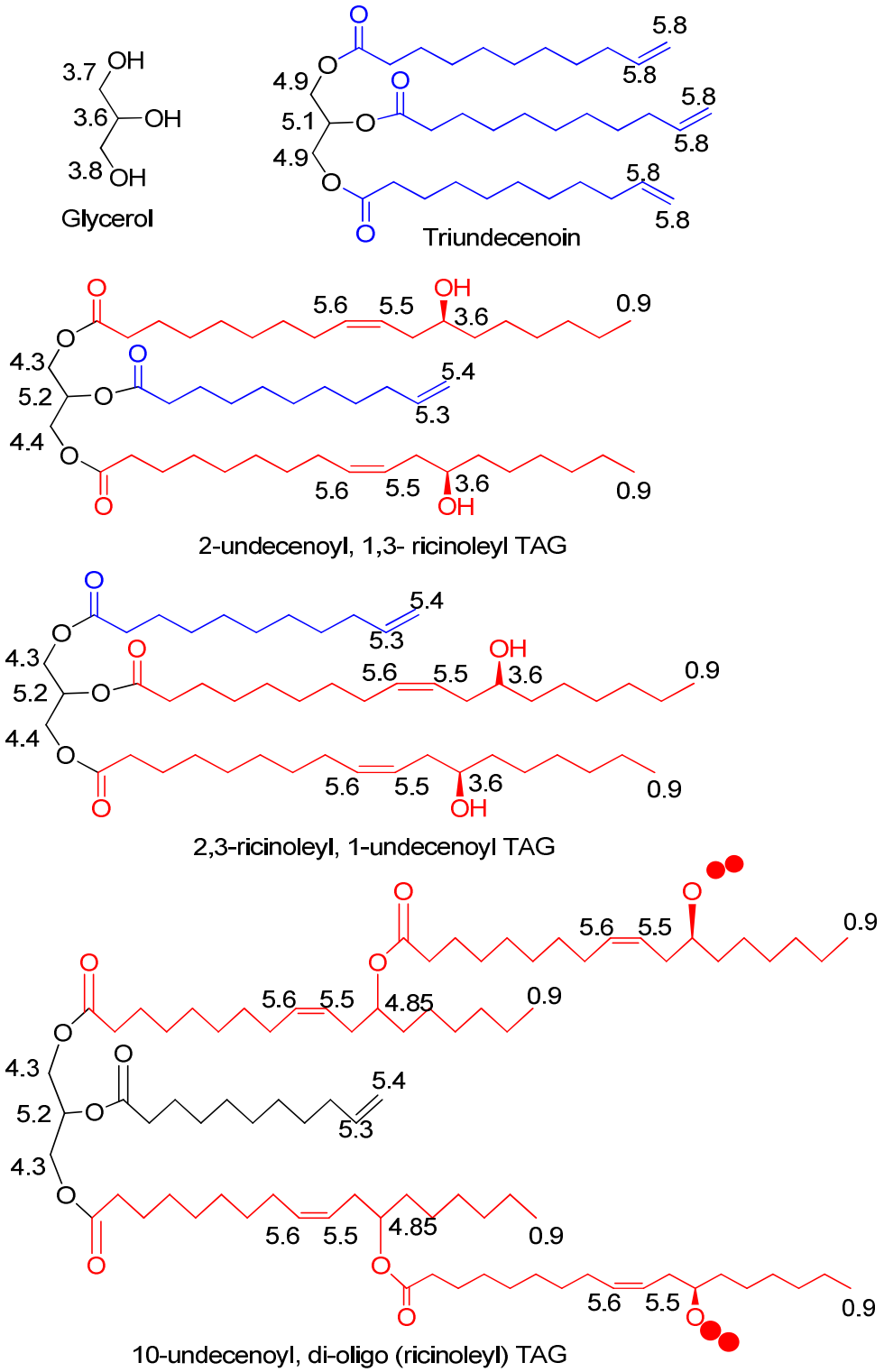


Figure 4.6 ^{1}H NMR ppm shift estimates for different protons in glycerol, triundecenoin, 2-undecenoyl, 1,3- ricinoleyl TAG, 2,3-ricinoleyl, 1-undecenoyl TAG and 10-undecenoyl, di-*oligo* (ricinoleyl) TAG

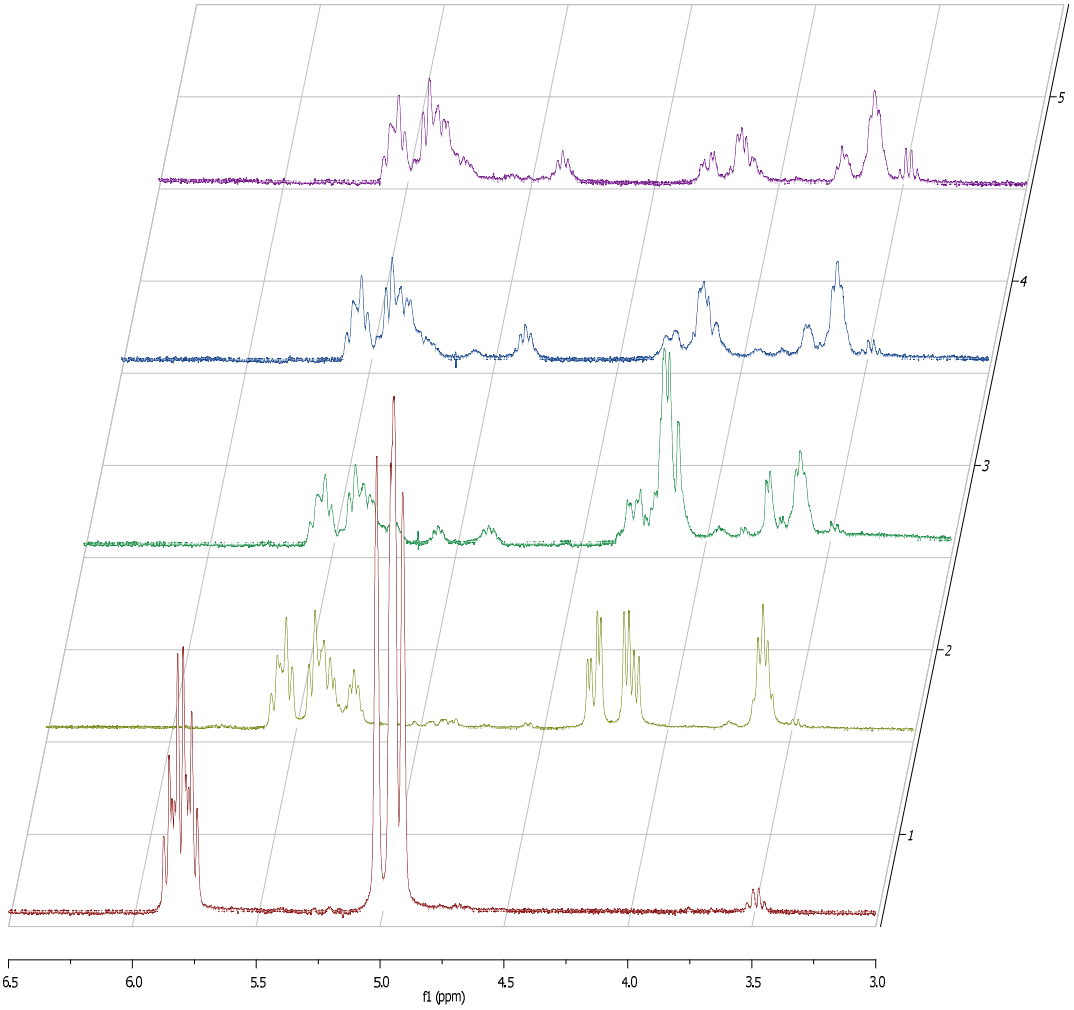


Figure 4.7. ^{1}H-NMR spectra for the region 3.0 to 6.5 ppm for reaction products: 1. triundecenoin. 2. 2-undecenoyl, 1,3- ricinoleyl TAG. 3. 2,3-ricinoleyl, 1-undecenoyl TAG. 4. 2-undecenoyl, 1,3-oligo (ricinoleyl) TAG. 5. 2,3-oligo (ricinoleyl), 1-undecenoyl TAG

From ^{1}H-NMR peak area calculations, the ratio of ricinoleic to undecenoic acyl groups in the product mixture formed from the synthesis of 2-undecenoyl, 1,3- ricinoleyl TAG and 2,3-(1,2-) ricinoleyl, 1- (3-) undecenoyl TAG corresponding to ricinoleic acid, and undecenoic acid were calculated (Table 4.4). There are 1.23 ricinoleyl groups per 0.5 undecenoyl groupsand 1.12 ricinoleyl groups per 0.5 undecenoyl groups for the former and latter product mixtures, respectively. The average number of acyl groups was calculated based on the H atoms

contained within the terminal CH_3 and H attached to C_{12} of ricinoleic acid, and the H atoms attached to C_{10} and C_{11} of undecenoic acid. The calculation of acyl groups based on the terminal CH_3 of ricinoleic acid disagrees with the other estimates, which can be possibly due to errors in the integration of the NMR peaks. Thus, the mole ratio of undecenoyl and ricinoleyl acyl groups as determined by NMR is 1:2, which is is in strong agreement with the ratio based on the relative amounts of substrates contained in the initial reaction mixture, based on gravimetric measurements. Also, it should be noted that the analysis was carried out for the reaction mixture itself (without any purification), which includes free fatty acids and possibly partial glycerides, along with TAGs.

GPC chromatographic peaks for the products shifted significantly towards lower retention times compared to the starting materials (Figure 4.8). The number-averaged molecular weights of starting materials and reactions products were calculated based on the semi-log retention time vs molecular weight calibration for ricinoleic acid oligomers (Section 3.2.4.2). The molecular weight of the ricinoleic acid reagent (487Da) is inconsistent with its actual value (298Da) due to the presence of short-chain ricinoleic acid estolides. Also, the molecular weight calculated for castor oil (triricinolein) (757Da) is inconsistent with its actual value (932Da), which is possibly due to the presence of fatty acid or partial glyceride impurities. Errors in the determination of the calibration plot, or the appropriateness of using *oligo*(ricinoleic acid) standards to calibrate the molecular weight of TAG can also be the underlying cause for the above-mentioned errors. The GPC results show an increase in number-averaged molecular weight for 2-undecenoyl, 1,3-oligo (ricinoleyl) TAGs and 2,3- (1,2-)ricinoleyl, 1- (3-) undecenoyl TAG (1743 and 1777 respectively) compared to the reaction substrates, 2-undecenoyl, 1,3- ricinoleyl TAG and 2,3-ricinoleyl 1-undecenoyl TAG (859 and 643 respectively) (Table 4.5).

Table 4.4. Average number of acyl groups from ricinoleic acid, and undecenoic acid from ^{1}H NMR spectra for 2-undecenoyl, 1,3- ricinoleyl TAG and 2,3-ricinoleyl 1-undecenoyl TAG

Synthesis of 2-undecenoyl, 1,3- ricinoleyl TAG							
		Ricinoleic acyl groups			Undecenoic acyl groups		
ppm	area (A)	proton (P)[a]	A/P[b]	Relative units[c]	proton (P)[a]	A/P[b]	Relative units[c]
0.9	5.44	3H, CH_3	1.81	1.43			
3.6	1.27	1H, CHOH	1.27	1.00			
4.15[d]	1.15						
4.24[d]	1.03						
4.85	0.04	1H, CHOR	0.04	0.03			
5.25[d]	0.63				1H, $CH=CH^2$	0.3	1
5.35	0.61				2H, $CH=CH^2$	0.3	1
5.45	1.25	1H, CH=CH	1.25	0.98			
5.55	1.25	1H, CH=CH	1.25	0.98			
	acyl groups via terminal CH_3			1.43			
	acyl groups via double bonds			0.98			1
	acyl groups via CHOH + CHOR			1.13			
Average number of acyl groups[e]				**1.23**			**0.5**
Synthesis of 2,3-ricinoleyl, 1-undecenoyl TAG.							
		Ricinoleic acyl groups			Undecenoic acyl groups		
Ppm	area (A)	proton (P)[a]	A/P[b]	Relative units[c]	proton (P)[a]	A/P[b]	Relative units[c]
0.9	5	3H, CH_3	1.67	1.11			
3.6	1.5	1H, CHOH	1.50	1.00			
4.15[d]	3.66						
4.24[d]	0.89						
4.85	0.19	1H, CHOR	0.19	0.13			
5.25[d]	0.62				1H, $CH=CH^2$	0.38	1
5.35	0.76				2H, $CH=CH^2$	0.38	1
5.45	1	1H, CH=CH	1.00	0.67			
5.55	1	1H, CH=CH	1.00	0.67			
	acyl groups via terminal CH_3			1.11			
	acyl groups via double bonds			0.67			1
	acyl groups via CHOH + CHOR			1.13			
Average number of acyl groups[e]				**1.12**			**0.5**

a. Type of acyl groups from NMR spectra.

b. Average area per proton.

c. Relative units for each peak area considering single acyl group.

d. 69Peaks corresponding to primary (4.15), secondary (5.25) and tertiary (4.24) hydroxyl groups of glycerol

e. Average number of acyl groups calculated from terminal CH3, and CHOR+CHOR for ricinoleic acid, and from double bonds for undecenoic acid

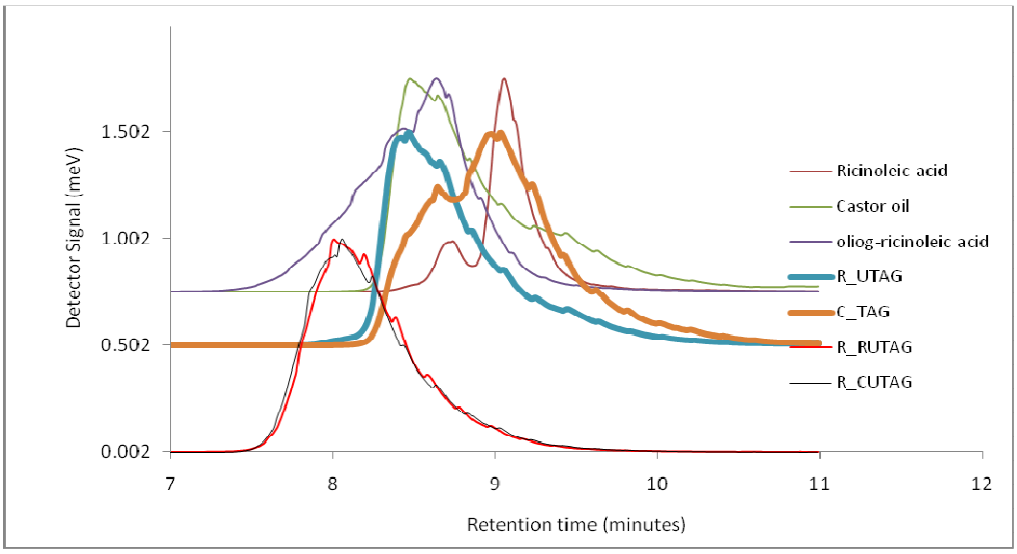


Figure. 4. 8. Chromatograms of GPC for reaction mixtures in synthesis of 2-undecenoyl, 1,3- ricinoleyl TAG (R_UTAG); 2,3-ricinoleyl, 1-undecenoyl TAG (C_UTAG); 10-undecenoyl, di-*oligo* (ricinoleyl) TAG from 2-undecenoyl, 1,3- ricinoleyl TAG and 2,3-ricinoleyl and 1-undecenoyl TAG (R_RUTAG and R_CUTAG respectively).

Table. 4.5. Estimation of number-averaged molecular weight, M_n, derived from GPC using semi-log retention time vs molecular weight calibration made from ricinoleic acid and *oligo* ricinoleic acid.

Sample	M_n	Polydispersity Index
Ricinoleic acid	487	1.14
Oligo-ricinoleic acid	1320	1.30
Castor oil	757	1.25
R_UTAG[a]	859	1.21
C_UTAG[b]	643	1.29
R_RUTAG[c]	1743	1.18
R_CUTAG[d]	1777	1.20

a R_TAG – Reaction mixture from synthesis of 2-undecenoyl, 1,3- ricinoleyl TAG.

b. C_TAG - Reaction mixture from synthesis of 2,3-ricinoleyl, 1-undecenoyl TAG.

c. R_RUTAG - Reaction mixture from synthesis of 2-undecenoyl, 1,3- *oligo* (ricinoleyl) TAG.

d. R_CUTAG- Reaction mixture from synthesis of 2,3-*oligo*(ricinoleyl), 1-undecenoyl TAG.

4.4. –Conjugation of carboxylic acid-functionalized poly (ethylene glycol) mono methyl ether (MPEG)- with hydroxyl end groups of ricinoleic acid, and oligo-ricinoleic acid (Objective 2):

Reactions involving conjugation of carboxylic acid-functionalized poly (ethylene glycol) mono methyl ether (MPEG) with the hydroxyl end groups of ricinoleic acid, and *oligo*-ricinoleic acid were analyzed using ^{1}HNMR, normal phase HPLC, and GPC.

^{1}H NMR was carried out upon the product from the reaction involving the conjugation of ricinoleic acid. Percent esterification of hydroxyl end groups of ricinoleic acid was estimated using the integrated ^{1}H-NMR areas of to the H atoms attached to ricinoleic acid's C_{12} carbon: CH_2OH (3.62 ppm) and CH_2O-MPEG (4.85 ppm) (Table 4.6). From the above results the conjugation of MPEG to ricinoleic acid has occurred successfully, with 58% of available hydroxyl groups being esterified.

For the conjugation of *oligo* (ricinoleic) acid and carboxylic acid-functionalized MPEG, the first step consisted of the polymerization of ricinoleic acid catalyzed by CAL. The average olecular weight of synthesized *oligo*(ricinoleic) acid was estimated from ^{1}H-NMR calculations to be 1463.32 Da, equivalent to a degree of polymerization of 5.35 (Table 4.7).

Table 4.6. Percent esterification of hydroxyl end groups of ricinoleic acid from ^{1}H NMR spectra.

ppm	Type	Protons	Area	A/p[b]	Relative units
0.9	3H, CH3	3	7.37	2.45	1.64
3.62	H, CHOH	1	1.49	1.49	1.00
4.85	H, CHO-(MPEG)	1	2.08	2.08	1.39
5.15	2H, CH=CH	2	5.37	2.68	1.80
Fraction of CHOx groups esterified[a] = 0.58					

a. Number of acyl groups from CHO-(MPEG)/total number of acyl groups from CHOH, and CHO-(MPEG)

b. Area-proton ratio, relative to the area/peak ratio of the 3.62 ppm peak

Table 4.7. Estimation of average molecular weight for *oligo*-(ricinoleic) acid from ^{1}H NMR spectra.

ppm	Type[a]	Protons(p)	Area (A)	A/p[b]	Relative units[c]
0.9	3H, CH_3	3	2.16	0.72	5.54
3.62	H, CHOH	1	0.13	0.13	1
4.85	H, CHOR	1	0.54	0.54	4.15
5.15	2H, CH=CH	2	1	0.5	3.85
Acyl Groups, via Terminal CH_3					5.54
Acyl Groups, via double bonds					3.85
Acyl Groups, via CHOH + CHOR					5.15
Fraction of CHOx groups esterified[d]					0.80
Acyl groups per free OH terminus[e]					5.35
Degree of polymerization[f]					5.15
Estimated average Molecular weight[g]					1463

a. Type of acyl groups from NMR spectra.

b. Average area per proton.

c. Area-proton ratio, relative to the area/peak ratio of the 3.62 ppm peak Number of acyl groups from CHOR/total number of acyl groups from CHOH, and CHOR.

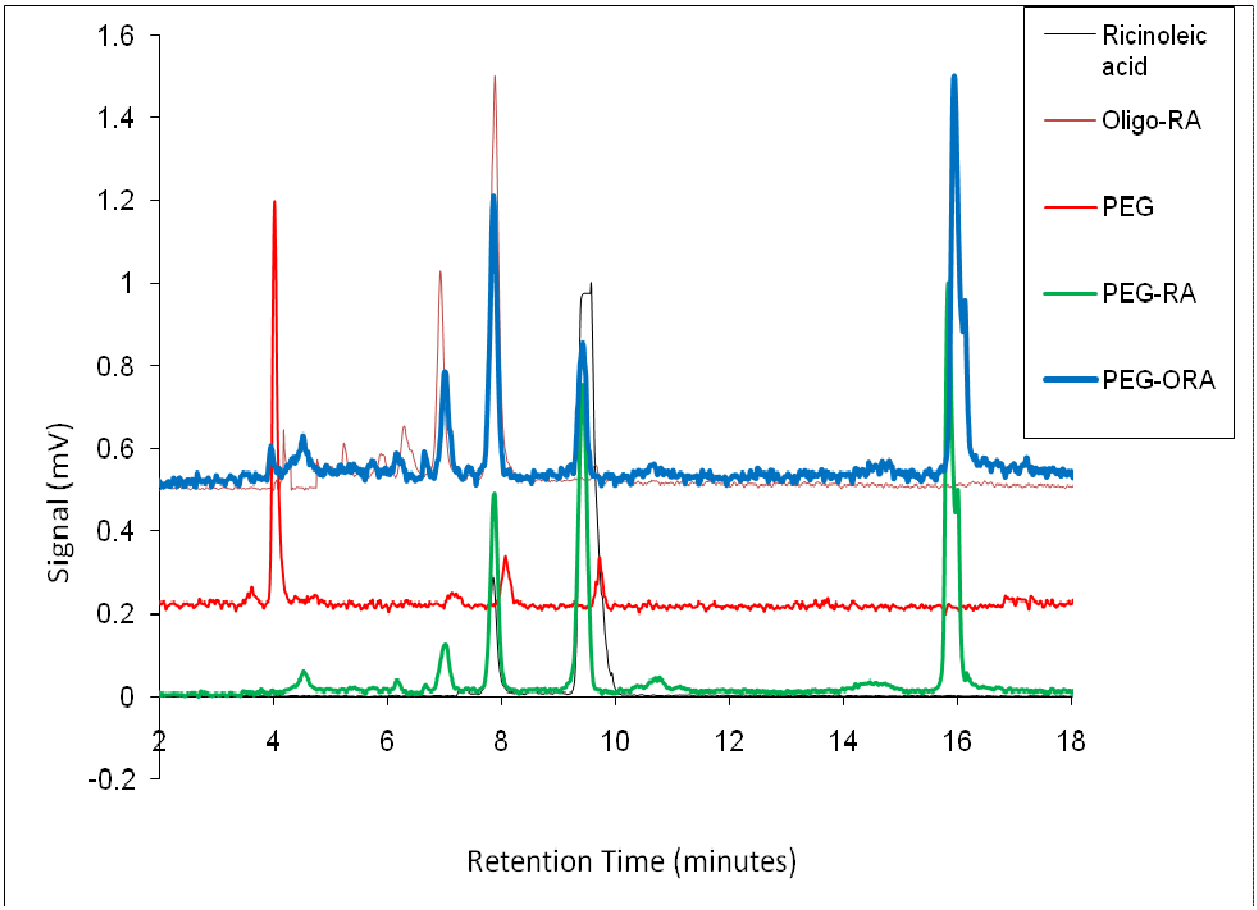
d. Average acyl groups from (total number of acyl groups from CHOR+CHOR) + (number of acyl groups from terminal CH3).

e. Average number of acyl groups for free OH terminus (degree of polymerization) = 1/(1-fraction of acyl groups esterified).

f. Based on average acyl groups per chain, molecular weight of sample = 18.02+(280.43)*degree of polymerization

Normal phase HPLC analysis of the reaction products and the starting materials for the conjugation of carboxylic acid-functionalized MPEG to hydroxyl fatty acids indicated product formation, evidenced by the formation of a peak with by elution of peaks' retention time near 16 min (Figure 4.9). The shift of retention time of the reaction product relative to unconjugated ricinoleic acid was expected, because MPEG conjugation increases the polarity, resulting in a longer retention of the product in the HPLC column stationary phase. The ratio of the integrated areas of unreacted ricinoleic acid and its oligomers for the product mixtures relative to the initial mixtures were nearly identical, for reactions involving both technical grade ricinoleic acid and *oligo*(ricinoleic acid). This result suggests that ricinoleic acid and each oligomeric species were equally effective acyl donors for the reactions, and that the hydrolysis of higher-molecular weight oligomers did not occur to a great extent. The percent conversion

was calculated from integrating the substrate and product peaks for the normal phase HPLC chromatograms (Table 4.8). The percent conversions for both ricinoleic acid and *oligo*(ricinoleic) acid were calculated to be 44% and 41%, respectively.



***Chromatograms for PEG is offset to 0.2 mV, PEG-ORA and ORA are offset to 0.5 mV to add clarity**

Figure. 4. 9. Chromatogramsfrom normal phase HPLC analysis of the conjugation of carboxylic acid-functionalized poly (ethylene glycol) monomethyl ether (MPEG) and hydroxyl end groups of ricinoleic acid and *oligo*-ricinoleic acid. The identification of peaks is discussed in the text.

Table. 4.8. Percent weight of PEG-RA[a] and PEG-ORA[b] in the final product from normal phase HPLC for conjugation of poly (ethylene glycol) mono methyl ether (MPEG) with hydroxyl end groups of ricinoleic acid, and *oligo*-ricinoleic acid

% weight of PEG-RA[c]	44.02
% weight of PEG-ORA[c]	41.26

a PEG-RA: Ricinoleic acid-poly (ethylene glycol) mono methyl ether (MPEG)

b. PEG-ORA: *Oligo*(ricinoleic) acid- poly (ethylene glycol) mono methyl eth

c Calculated from the relative peak areas (3670 mV s and 798 mV s corresponding to retention time 15.84 min and 15.94 min with a total peak areas of 8337 mV s and 1934 mV s of respectively) of PEG-RA and PEG-ORA chromatograms.

GPC analysis was performed on the samples to estimate molecular weight. Chromatograms of the final products for the conjugation of MPEG-acid and ricinoleic acid and *oligo*(ricinoleic) acid eluted at a lower retention time relative to the substrate (Figure 4.10). The corresponding molecular weights were calculated using semi-log retention time vs. molecular weight calibration made from *oligo* (ricinoleic acid) (Section 3.2.4.2). The results show an increase in molecular weight for the product mixtures (1600 Da and 1740 Da for conjugated ricinoleic acid and *oligo*(ricinoleic acid), respectively) relative to the molecular weight of the technical grade ricinoleic acid and its oligomers (477 Da and 1320 Da, respectively) (Table 4.9).

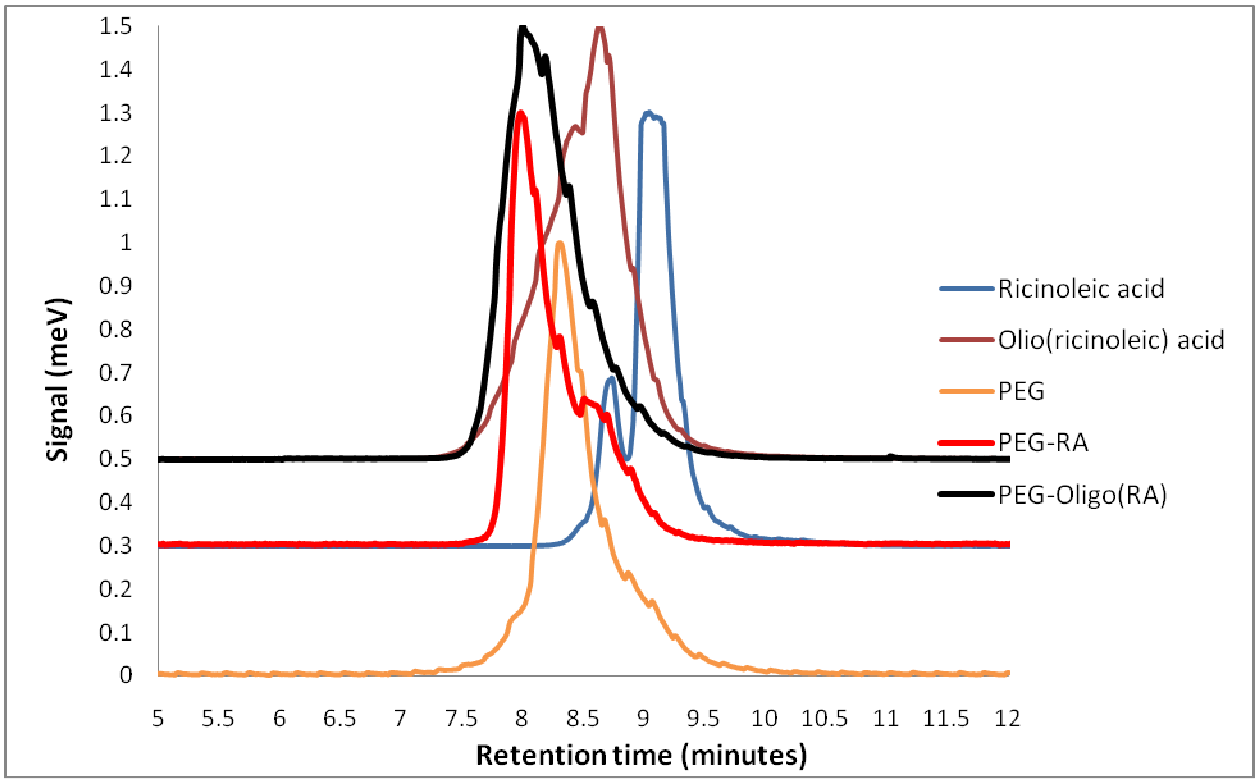


Figure. 4. 10. . GPC Chromatograms for molecular weight estimation of reactions of ricinoleic acid and *oligo*(ricinoleic) acid after conjugation with acid-functionalized poly (ethylene glycol) monomethyl ether (MPEG)

Table. 4.9. Molecular weight estimation of the reaction mixture, initially and for the final product, for conjugation of ricinoleic acid and oligo(ricinoleic) with acid-functionalized poly(ethylene glycol) mono methyl ether (MPEG)

Sample	MW	PDI
Ricinoleic acid	477	1.12
Oligo (Ricinoleic) Acid	1320	1.30
PEG[a]	1270	1.22
PEG-RA[b]	1600	1.16
PEG-ORA[c]	1740	1.18

a. PEG: Carboxylic acid-functionalized Poly (ethylene glycol) mono methyl ether (MPEG)

b. MPEG-Ricinoleic acid

c. MPEG-*Oligo*(ricinoleic) acid

4.5. – Summary:

The synthesis of a TAG that contains two *oligo*(ricinoleic acyl) and one undecenoic acyl group (Objective 1), and the conjugation of ricinoleic acid and its oligomers with an acid-functionalized MPEG have been achieved with moderate success. The analysis of reaction products was not trivial, requiring the simultaneous analysis of several different techniques that each provided different information: normal and reversed-phase HPLC, GPC, analytical and preparative TLC, and ^{1}H-NMR.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

Both Objectives 1 and 2 of this thesis have been achieved. Objective 1.1, "random" lipase-catalyzed synthesis of triundecenoin has been successfully completed, achieving a 90-95% yield, as calculated using reversed-phase HPLC. Formation of triacylglycerol (TAG) was confirmed by TLC and HPLC.

The objective 1.2 , 1,3-selective lipase-catalyzed synthesis of 2-undecenoyl, 1,3-ricinoleyl TAG and 2,3- (1,2-)ricinoleyl, 1- (3-) undecenoyl TAG has been achieved at 50-60% conversion, as calculated from preparative TLC. Formation of new TAG molecules was confirmed by normal phase and reversed-phase HPLC and ^{1}H-NMR.Formation of monoundecenoyl, di-*oligo*(ricinoleyl) TAG via "random" lipase-catalzyed esterification between ricinoleic acid and the products obtained from Objective 1.2 was performed and analyzed using GPC and ^{1}H-NMR (Objective 1.3). GPC results indicated an increase in molecular weight of the TAG, suggesting oligomerization of ricinoleic arms occurred.

Studies on conjugation of poly (ethylene glycol) mono methyl ether (MPEG) with hydroxyl end groups of ricinoleic acid, and *oligo*-ricinoleic acid are analyzed using normal phase HPLC, GPC and ^{1}H NMR. GPC results indicate change in molecular weight suggesting conjugation of PEG on hydroxyl fatty acids. Percent weight conversion of products were calculated to be 44% and 41% for ricinoleic acid and *oligo* (ricinoleic) acid conjugation reaction from normal phase HPLC.

Although UPMs were not synthesized directly in this thesis, technological methods developed will enable the synthesis to occur. For example, synthesis of 2-undecenoyl, 1,3-*oligo* (ricinoleyl) triacylglycerol can be used as a building block for synthesis of hydrophobic core with the units joined together via free radical polymerization (Figure 5.1). Also, the methodology developed for the successful conjugation of carboxylic functionalized poly (ethylene glycol) mono methyl ether (MPEG) with the hydroxyl end groups of ricinoleic acid and *oligo*-ricinoleic acid can be applied to the terminal hydroxyl groups of the hydrophobic core.

The results achieved are important for synthesis of UPMs towards their application as drug delivery vehicles. Studies have focused mainly on developing technologies to synthesize UPMs. Further work needs to be done on scaling up the reactions of Objectives 1 and 2 and achieving higher yields and conversion rates. During the course of the study, it has been observed that chemical characterization of the triacylglycerols using HPLC as an important challenge due to close resemblance of equivalent carbon number of triricinolein, triundecenoin, and mixed undecenoyl, ricinoleyl TAG. Chemical characterization for synthesized 2-undecenoyl, 1,3-*oligo* (ricinoleyl) triacylglycerol needs to be completed to determine the structure of the molecule. It is also suggested that further experiments be conducted on conjugating hydroxyl end groups of poly (ricinoleyl) star polymers with poly(ethylene glycol) mono methyl ether (MPEG) derivatives. Also, testing of star polymer derivatives for solubilization of hydrophobic drug models, and their dispersability and stability in aqueous solution, has to be determined to assess their suitability as drug delivery vehicles.

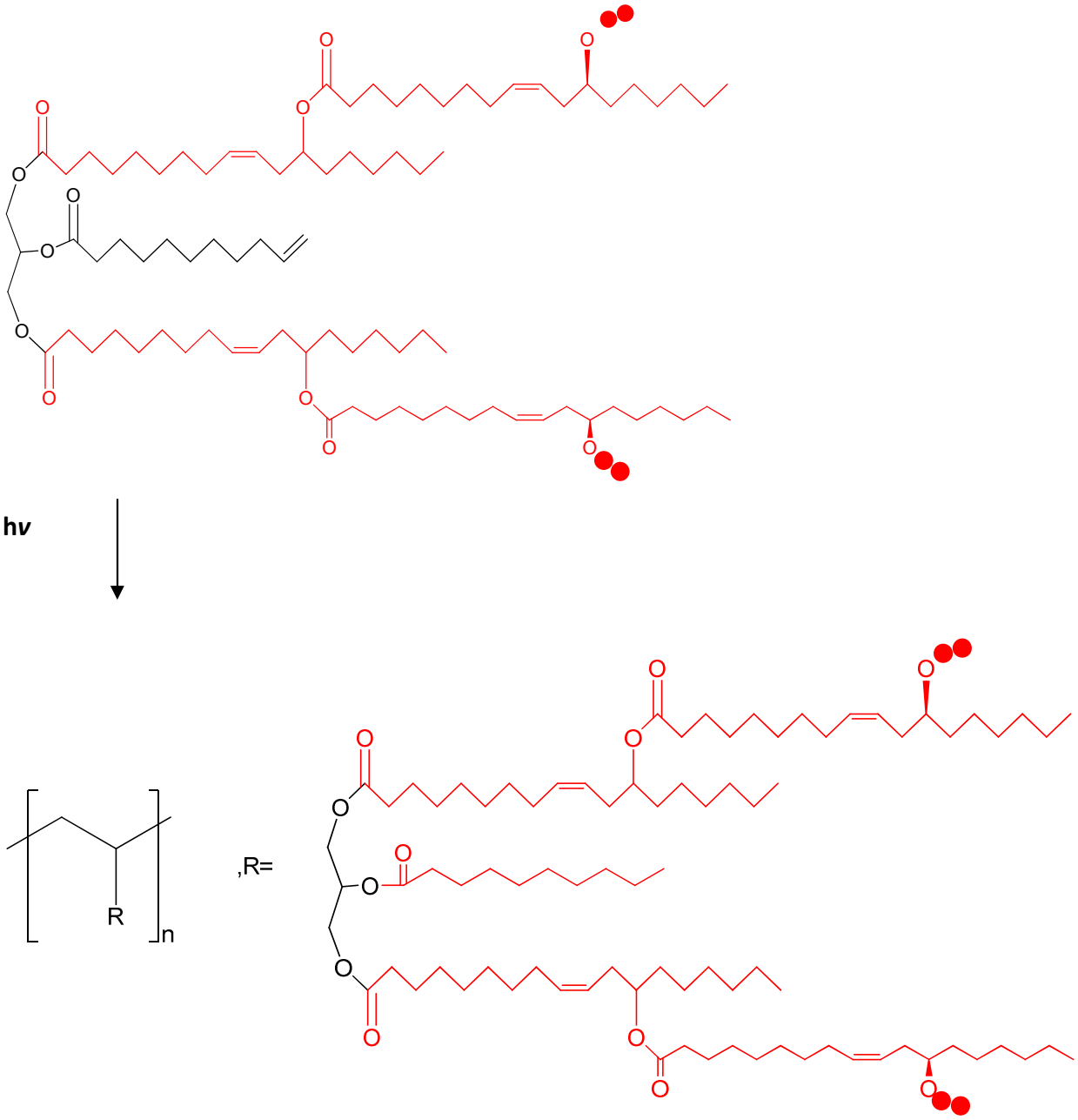


Figure 5.1. Free radical polymerization of 2-undecenoyl, 1,3- *oligo* (ricinoleyl) TAG

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Vita

Vinay Kumar Mannam was born in Kanigiri, of Andhra Pradesh State in India. His parents currently live in Hyderabad. He graduated from Acharya N G Ranga Agricultural University, Hyderabad in Summer-2007, with a Bachelor of Engineering degree in Agricultural Engineering. In the Fall of 2007, he came to United States to join Master of Science program at the University of Tennessee-Knoxville, Biosystems Engineering Department with research in the Bioprocess Engineering discipline under Dr. Hayes group.