การแยกองค์ประกอบของไขสังเคราะห์โดยการแยกลำดับส่วนแบบชะที่อุณหภูมิสูงขึ้น (ทีอาร์อีเอฟ)

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SEPARATION OF SYNTHETIC WAX COMPONENTS BY TEMPERATURE RISING ELUTION FRACTIONATION (TREF)

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A Thesis Submitted in Partial Fulfillment of Requirements
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ประยุกต์ใช้ TREF (Temperature Rising Elution Fractionation) เพื่อศึกษาการแยกองค์ประกอบของไขสังเคราะห์ห้าเอสเทอร์สายโซ่ยาวจากกรดไขมัน (กรดดีคาร์โนอิค, กรดพาลมิติค, กรดสเทียริค, กรดโอลีอิค และ กรดลิโนลีอิค) และแอลกอฮอล์ไขมัน (สเทียริลแอลกอฮอล์) ศึกษาสมบัติทางความร้อน , สัณฐานวิทยา และการละลายของเอสเทอร์ทั้งห้าเพื่อเลือกเอสเทอร์ที่เหมาะสมเพื่อเตรียมไขสังเคราะห์สำหรับการแยกด้วยเทคนิคนี้

ใช้ของผสมของปริมาณที่เท่ากันของสเทียริล โอลีเอท, สเทียริล พาลมิเทท และสเทียริลดีคาร์โนเอทเป็นไขสังเคราะห์สำหรับงานวิจัยนี้ ได้ศึกษาปัจจัยต่างๆ ที่มีผลต่อการแยกด้วยเทคนิคนี้ได้แก่ตัวทำละลาย (นอร์มัล-เฮกเซน, ไอโซโพรพานอล และของผสม 1:1 ของเฮกเซนและไอโซโพรพานอล), ตัวรองรับเฉื่อย (ทรายขนาด 510 μm, 710μm และปราศจากตัวรองรับ) และอุณหภูมิ

สามารถแยกองค์ประกอบของไขสังเคราะห์นี้โดย TREF โดยการใช้ไอโซโพรพานอล เป็นตัวทำละลาย, ทรายขนาด 510 μm เป็นตัวรองรับเฉื่อย และที่อุณภูมิ 10 ^{o}C, 30 ^{o}C และ 50 ^{o}C

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต

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TAVEEPORN SIAMSAKUL: SEPARATION OF SYNTHETIC WAX COMPONENTS BY TEMPERATURE RISING ELUTION FRACTIONATION (TREF). ADVISOR: ASST. PROF. PREECHA LERTPRATCHYA, Ph.D., 127 pp.

TREF (Temperature Rising Elution Fractionation) was applied to study the separation of wax components. Five long chain esters were synthesized from a fatty alcohol (stearyl alcohol) and fatty acids (decanoic acid, palmitic acid, stearic acid, oleic acid and linolaic acid). Thermal property, morphology and solubility of the esters were studied in order to select the suitable esters to make the synthetic wax for this separation technique. The mixture of equal amounts of stearyl oleate, stearyl palmitate and stearyl decanoate was used as the synthetic wax in this work. A number of factors affecting the separation by TREF technique including solvent (*n*-hexane, isopropanol and mixed hexane and isopropanol 1:1), inert support (510 µm, 710 µm and without inert support) and temperature profile were studied.

The components of the synthetic wax were successfully separated by TREF using isopropanol as the solvent, 510 µm sand as the inert support at 10 ^{o}C, 30 ^{o}C and 50 ^{o}C

Field of Study : Petrochemisity and Polymer Science Student's Signature……………………

Academic Year : 2012 Advisor's Signature……………………

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LIST OF ABBREVIATIONS

m.p.	Melting point
^{o}C	Degree Celsius
c.c.	cubic centimeter
g	gram(s)
TREF	Temperature Rising Elution Fractionation
DSC	Differential scanning calorimetry
FTIR	Fourier Transform Infrared Spectroscopy
^{1}H-NMR	Proton nuclear magnetic resonance
T_m	Melting Temperature
T_c	Crystallization Temperature
$CDCl_3$	deuterochloroform

CHAPTER I

INTRODUCTION

Waxes are by products from industry separation process of plant oil such as rice brand oil, sunflower oil, soil bean oil and palm oil etc. They are mixtures of high molecular weight esters resulting from fatty alcohols and fatty acids. The ester moiety may contain 12-32 carbon atoms. Waxes are found in nature as coatings on leaves and stems of plants. The waxes prevent the plants from losing excessive amounts of water [1]. They are important constituents in many commercial products such as cosmetics, polishes, lubricants, surface coating, and many other applications. The chemical composition of waxes is complex. They usually contain a broad variety of molecular weight species and reactive functional groups, although some classes of mineral and synthetic waxes are composed totally of hydrocarbon compounds. Table 1 shows compositions of various waxes.

Table 1. Compositions of natural waxes from plants

Types of waxes	WE	FA moieties	FAL moieties
Sunflower-oil wax	C_{40}-C_{44}	C_{16}-C_{30}	C_{18}-C_{30}
Rice bran wax	C_{44}-C_{64}	C_{22}-C_{30}	C_{24}-C_{40}
Carcauba wax	C_{45}-C_{54}	C_{16}-C_{20}	C_{30}-C_{34}
Beeswax	C_{40}-C_{64}	C_{16}-C_{36}	C_{24}-C_{34}

WE = Wax Ester, FA = Fatty Acid and FAL = Fatty Alcohol

Due to their high molecular weights and similarities, separation of the constituents by conventional methods is difficult and expensive. Temperature rising elution fractionation (TREF) is a separation technique for fractionating polymers based on crystallinity. It has gained popularity over the past few years [2]. The TREF technique has found application mainly in the area of analysis of semi-crystalline polyolefins. It has not been applied for the separation of waxes. This research intended to separate components of synthetic wax by applying this TREF technique.

Several esters were synthesized by esterification of appropriate fatty acids and appropriate fatty alcohols. The resulting esters were mixed together and used as synthetic wax for this study.

1.1 The objectives of this thesis are:

1.1.1 To apply TREF technique for the separation of the synthetic wax components.

1.1.2 To study factors affecting the separation.

1.1.3 To find optimum conditions for the separation.

1.2 The scope of the thesis are:

1.2.1 To synthesize esters by esterification reaction, sulfuric acid as catalyst.

1.2.2 To characterize the obtained products from the reaction by using FTIR, NMR and GC.

1.2.3 To study thermal property, morphology and solubility in order to select appropriate esters with suitable properties to make the synthetic wax for the separation by TREF technique.

1.2.4 To study conditions affecting the separation by TREF.

1.2.5 To compare the TREF and Fractionation Crystallization for the separation of synthetic wax components.

CHAPTER II

THEORY AND LITERATURE REVIEWS

Waxes are a class of organic compounds consisting of long alkyl chains. Naturally occurring waxes are mainly esters of fatty acid and long chain alcohols. Typically, they melt above 45°C to give a low viscosity liquid. They are insoluble in water but soluble in non polar organic solvents.

2.1Classification of waxes [3].

Waxes are classified as follow.

1. Insect and animal waxes.
2. Vegetable waxes
3. Mineral waxes
4. Synthetic waxes

2.2.1 Insect and animal waxes

Beeswax

Beeswax has been used since ancient times as evidenced in the wall pictures of the Lascaux cave and in Egyptian mummies. Ancient Egyptians used it also for its adhesive and coating properties, and in ship building. Beeswax was used as a waterproofing agent and treatment for painted walls. This wax was valuable and exchanged as a form of currency. Beeswax is a natural wax produced in the bee hive of honey bees of the genus Apis. Beeswax is secreted by bees and is used to construct the comb in which bees keep their honey. Removing the honey and melting the comb in boiling water harvests the wax, the melted product is then filtered and cast into cake. The yellow beeswax cake can be bleached with oxidizing agents to white beeswax, beeswax favored in the cosmetic industry.

Beeswax typically has a melting point of 62-65^{o}C, a viscosity of 1470 mm^2/s at 98.9 ^{o}C, an acid number of 20 and a saponification number of 84. Beeswax is esters of fatty acids and alcohols with various long chains. The main compositions are palmitate and oleate esters of long-chain $C_{30\text{-}32}$ alcohols. One of the major components of beeswax is myricyl palmitate which is used in constructing their honeycombs. The major applications of beeswax are in the cosmetic and in pharmaceutical industries.

Spermaceti wax

Spermaceti is oil in the head of Sperm whale or bottlenose whale. It was separated from the oil by the process called wintering. It congealed into waxy white crystalline solid. The components of Spermaceti are mainly cetyl palmitate and esters of other fatty acids and fatty alcohols. It has a melting point of about 44 °C. It is used as a component in creams, cosmetics, candles, pomades, fabric finishing and lubricant.

2.1.2 Vegetable waxes

Carnauba

Carnauba wax is a hard wax produced by Brezillian palm which grows in semi-arid Northeast of Brazil. It is separated from the leaves of the palm trees. It has many applications and it is widely used in leather, candies, pills, cosmetics and casting.

Carnauba has high melting points about 84 ^{o}C, a viscosity of 396 mm^{2}/s at 98.9 ^{o}C, acid number of 8 and saponification number of 80.

Candelilla

Candelilla is found in the plants in Mexican states and Texas. It has a melting point of 70 ^{o}C, an acid number of 14 and a saponification number of 55. Its major applications are in pharmaceuticals, cosmetic, glazing agent and foods.

Japan wax

Japan wax is a by-product from lacquer manufacture. Japan wax obtained from the berries of a tree native to Japan and China, it has a pale yellow, insoluble solid. Major components of Japan wax are triglycerides, and primarily tripalmitin. It has a melting point of 53 ^{o}C, an acid number of 18 and a saponification number of 217. It is used in candles, additive to resins, polishes, lubricants, and also used in food.

Rice-Bran Wax

Rice bran wax is a by-product from processing of rice bran oil refinery. Rice bran wax has been used in the cosmetic industry, such as cold cream, pharmaceutical, food industry and polymer leather. It is comparable with Carnuba wax.

Rice bran wax has high policosanol which is a long chain alcohol with 20-36 carbon atoms that can reduce the capture of platelets and reduce harmful to cholesterol in the lining of blood vessels and various tissues. Major components of rice bran wax are acid lignoceric, behenic acid and alcohol. It can be applied to the skin in cream formulations. It acts as an emulsion thickener, gelling agent and antioxident. Rice-bran wax is widely used in cosmetics, lipstick and lip balm, cream, ointment bases, bath and body lotions and mascara.

Sunflower wax

Sunflower wax is a crystalline, high melting-point substance. It is obtained from the winterization process of sunflower oil. Sunflower wax compose mainly of components of long chain saturated $C_{42\text{-}60}$ esters. It is used as an emulsifying and gelling agent. The advantage properties of sunflower wax are light color and very low odor.

Jojoba wax

Jojoba wax is obtained from the jojoba plant found in the tropical rain forest in Fort California, Arizona and Mexico. The Jojoba wax is extracted from the seeds of the jojoba. The major components are high molecular weight monoester with $C_{36\text{-}46}$, monoethylenic acids, eicosenol and docosenol. Jojoba wax is mainly used in cosmetics industry because it helps slow down moisture loss and helps to improve flexibility and elasticity to feeling soft and slow down dry skin.

Bayberry Waxes

Bayberry wax is derived from the bayberry (myrtle) shrub. The main components are esters of lauric, myristic and palmitic acid and alcohol. It has a melting point of 45 ^{o}C, an acid number of 15, a saponification number of 220, and an iodine number of 6. The special characteristic of Bayberry wax is its aromatic odor. It is mostly used in the manufacture of candles and other products that require unique odor.

2.1.3 Mineral Waxes

Montan Waxes

Montan wax is obtained from solvent extraction of coal and lignite, which is a huge industry in Germany. It is a very hard wax with high concentration of saturated fatty acids and alcohols. The composition of Montan wax are mixtures of long chain esters (C_{24-30}), long-chain acids and long chain alcohols, ketones, and hydrocarbons

This wax typically has a melting point of 80 ^{o}C, an acid number of 32, and a saponification number of 92. It is used in carbon-paper ink and as plastics lubricants.

Petroleum Waxes

Petroleum waxes are also called paraffin wax. Unlike most natural waxes which are esters, paraffin waxes are hydrocarbons. These waxes are mixtures of saturated n-parafin and isoalkanes, naphthalenes, and alkyl- and naphthalene-substituted aromatic compounds. There are used in adhesives, in cosmetics, in foods, such as chewing gum and cheese wrapping, in soap, in coatings, shoe polish, etc.

Ozokerite and Ceresin Waxes

Products were from Poland, Austria and former USSR. Ozokerite wax which is used as an alternative to the petroleum wax by microcrystalline waxes with paraffin were blended together in order to used increasing the physical properties.

Waxes in nature are classified in several types with vary components based on the sources shown in Table 2.1. Each wax has different benefit application. Palmitic acid, stearic acid, oleic acid and linoleic acid have been widely used in cosmetic and pharmaceutical industries.

Table 2.1 the components of fatty acid in natural waxes

Fatty acid	Percentage Sunflower wax	Percentage Rice Bran Oil wax	Percentage Carnauba wax	Percentage Bees wax
Myristic (14:0)	-	4.7	-	-
Palmitic (16:0)	2.6	6.3	0.7	7
Stearic (18:0)	2.3	1.4	1.9	7
Oleic (18:1)	-	4.9	-	36
Linolenic (18:2)	7.5	4.0	-	-
Arachidic (20:0)	46.7	1.3	9.1	-
Behenic (22:0)	25.8	8.4	9.8	4
Lignoceric (24:0)	7.5	24.4	28.4	-
Cerotic (26:0)	6.6	43.9	13.5	12
Montanic (28:0)	6.8	0.1	21.2	11

Palmitic acid is widely used to produce soap, cosmetics, food stuffs and to add texture in foods by using its sodium salt. Sodium palmitate is used as an additive in organic products.

Stearic acid is used in the manufacture of detergents, soaps and cosmetics such as shampoo and shaving cream products besides esters of stearic acid with ethylene glycol. Glycol stearate and glycol distearate are used as an ingredient in shampoos, soaps, cosmetics and other products.

Linoleic acid has been very popular in the beauty products because of its beneficial properties on the skin [4]. It can also be used in the oil paints and varnishes.

The application of oleic acid is its sodium salt. A key ingredient in a variety of soaps. It can be used in drug to treat lung and used to test new drugs. It is also used as a mixture in spray products.

Table 2.2 components of fatty alcohol in natural waxes.

Fatty alcohol	Percentage Sunflower wax	Percentage Rice Bran Oil wax	Percentage Carnauba wax	Percentage Bees wax
Stearyl (18)	0.2	3.3	-	-
Arachidyl (20)	2.3	1.9	1.0	4
Behenyl (22)	15.6	11.3	8.4	-
Lignoceryl (24)	34.8	26.7	19.2	42
Cerotyl (26)	26.8	14.4	9.5	15
Montanyl (28)	9.2	3.9	12.4	9
Melissyl (30)	3.9	12.1	12.3	14
Domelissyl (32)	2.2	-	17.8	16

Stearyl alcohol is more popular due to its benefits for the skin and moisturizers. It is used in skin care products. It is also used as an ingredient in lubricants, resins, perfumes and cosmetics. It is most widely used in coatings, hair shampoos and hair conditioners. The stearyl alcohol molecules can form a layer thickness coated on the surface water to reduce evaporation of water in the pool.

2.1.4 Synthetic waxes [3].

The synthetic waxes are manufactured for cosmetic applications. The structures of synthetic waxes have shorter chain than those of natural waxes. They are esters of alcohols and fatty acids with a straight or branched chain. Depend on the structural arrangement and chain length of substance, the ester are adjusted to provide different physical properties and types of emolience. Synthetic ester, for example cetyl palmitate and cetostearyl stearate are applied to increase the viscosity of emulsions in cosmetic. Branched chain esters, such as cetostearyl ethylhexanoate or isopropyl myristate, give product with good spreading properties. Furthermore, the choice of the ester influences both the solubility and spreadability of sunscreen agents and their ability to penetrate the skin.

2.2 Wax Crystals

Most polymers have semi-crystallinities. The characteristics of the crystallinity, more or less, are called ‘the degree of crystallinity’. The degree of crystallinity depends on molecular weight, molecular structure, secondary bonding between polymer chains and the physical processes acting with the polymer. For example, some polymers have high degrees of crystallinity due to the affect of linear structure and consistency which makes them easily arrange more uniformity. While some polymers have high degrees of crystallinity due to hydrogen bonding between the polymer chains [5].

Crystallization is an important factor for the success of separation by TREF technique. It has been observed that waxes crystallize in both plate and needle forms, depending on the conditions of crystallization.

Buchler and Graves attributed the differences in crystal system entirely to the presence of soft waxes, these are fatty ester, leaf waxes, candelilla wax and so on. It is now generally recognized that the rate of cooling affects merely the size of the crystals formed and not their type. Storage of small crystal waxes may result in the growth of larger crystals, but it can never cause a change in their type [6]. It was concluded that the plate-type crystals represent straight chain hydrocarbon and constitute the basic fraction of paraffin in crude oil. The needle malcrystalline types of crystals were probably the ‘soft wax’. Microcrystalline waxes are composed largely of these types, although high-melting paraffin waxes will sometimes be found to contain small amounts of needle, but the crystals are of extremely small or microscopic size. Types of wax crystals are shown in Figure 2.1

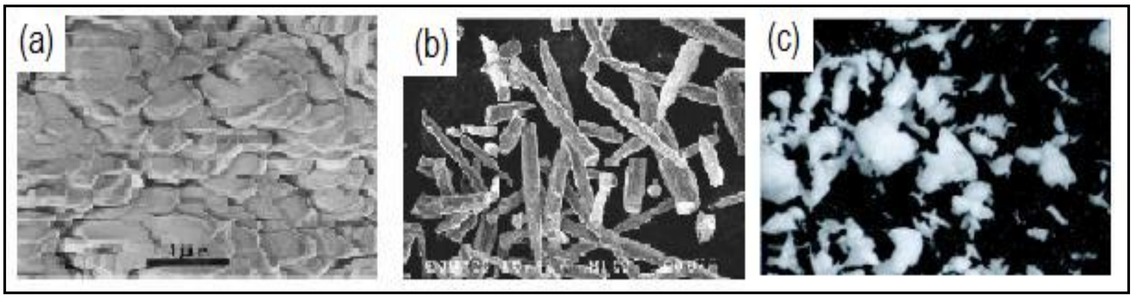

Figure 2.1 Micrographic types of wax crystals (a) plate-like crystals (b) needle-like crystals (C) microcrystalline crystals.

For synthetic waxes, Schaink and van Malssen explained the differences in shape and size of crystals depend on ratios of fatty acid and fatty alcohol. The fatty acid crystals are generally smaller than the alcohol crystals and crystal shape is more pronounced. The formation of plate crystals is not efficient with a 1:1 ratio of an acid and an alcohol. These crystals are smaller needles. It can concluded that the compositions of the waxes affect shapes of the crystals [7].

Gandolfo *et al.* explained effect of the temperature to crystallinity of synthetic waxes, as shown in Figure 2.2. Reduction in temperature affects the interfacial energy of the nuclei resulted in an increased nucleation rate. In Summary, with each temperature, the crystalline have different behavior [8].

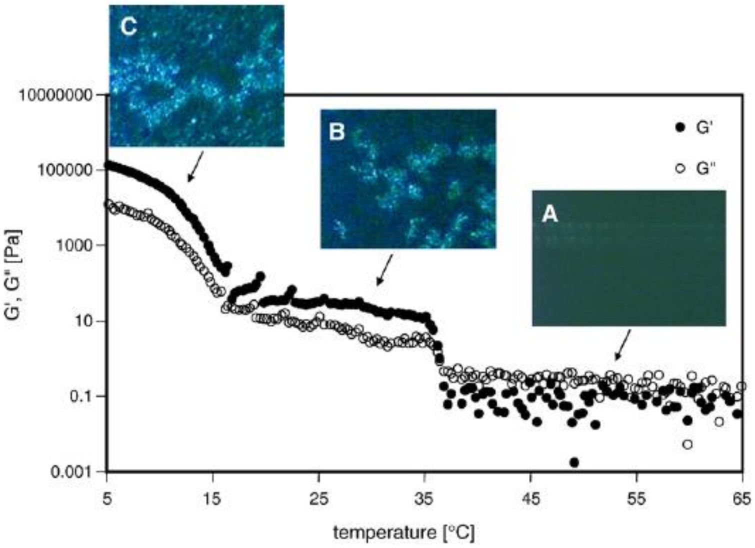

Figure 2.2 Rheological profile and polarized light micrographs of waxes at each temperature. (A) mixture at 60 °C, (B) after cooling to 30 °C, (C) after cooling at 5 °C.

Needle type waxes are much more soluble in various solvents than plate-type waxes of the same molecular weight. The malcrystalline type was intermediate between the two. Waxes of the same melting point, regardless of type, have about the same degree of solubility. Mixture of the plate, needle and malcrystallization was noted. It was found that the needle type was strong enough to impress its form on the plates if both types were allowed to crystallize simultaneously from the same solution. However, if crystallization was performed such that the needle type was kept in solution, either by high temperature or by excess of solvent, the plate type formed its characteristic crystals [6].

2.3 Solubility of Natural waxes

Solubility of natural waxes (melting at 20-50 ^{o}C) in *n*-heptane and *n*-butyl alcohol at various temperatures are shown in Figure 2.3. It shows that carauba waxes can be dissolved in *n*-heptane at 43 ^{o}C and can be dissolved in *n*-butyl alcohol at 54 ^{o}C. In summary, solubility of waxes depends upon type of solvent and temperature. However chemical composition is important to its solubility.

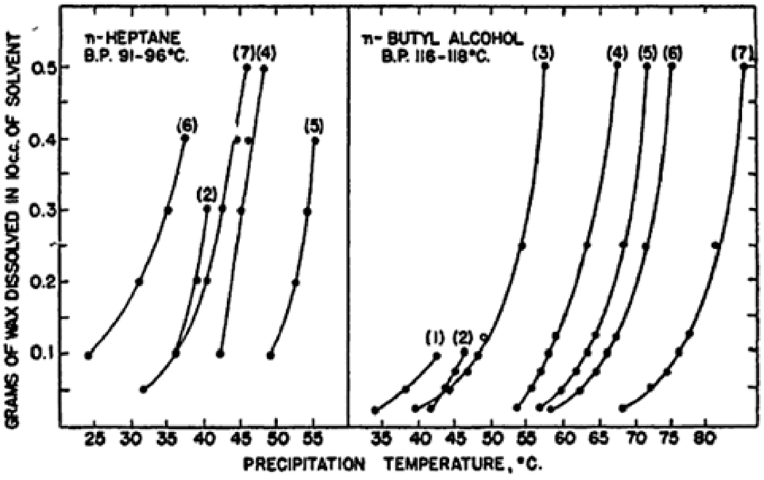

Figure 2.3 The relative of grams of wax dissolved in 10 cc of solvent and precipitation temperature.

(1) Bees wax, m.p. 60-62 ^{o}C,

(2) Montan, Bleached, m.p. 68 ^{o}C

(3) Candelilla, m.p. 68 ^{o}C

(4) Carnauba, Refined, m.p. 81.5 ^{o}C

(5) Carnauba No.1 Yellow, m.p. 83 ^{o}C

(6) Ozokerite, m.p. 65-67 ^{o}C

(7) Ozokerite, m.p. 76-78 ^{o}C

2.4 Temperature rising elution fractionation (TREF)

TREF is a high efficient technique to separate components of polyolefin. Typically, it is used for separating non-volatile materials [9]. This technique has been used to separate polymer on the basis of different crystallinities and solubilities. TREF is sensitive to molecular structure, chain crystallinity and dissolution temperature. Due to effect of different molecular structures to distinct crystallinities, dissolution temperatures are dissimilar. TREF method in the reverse order by using the differences of dissolution temperature due to the crystallinities is dissimilar of polymer.

TREF is composed of two sequential steps, namely, crystallization and elution. Samoth and Tantayanon studied effect of eluent polarity on crystallization of polymer. Thus a mixed solvent was applied to TREF technique in order to increase efficiency of the separation [10]. In the first step, the polymer was dissolved in appropriate solvent and then an inert support was packed into a column. The crystallization was carried out under controlled conditions by reducing the temperature slowly, until the crystals were completely. In the elution step, solvent was flowed through in to the column while temperature was increased appropriately.

TREF can be divided into two stages. The proposed mechanism of TREF is depicted as shown in Figure 2.4.

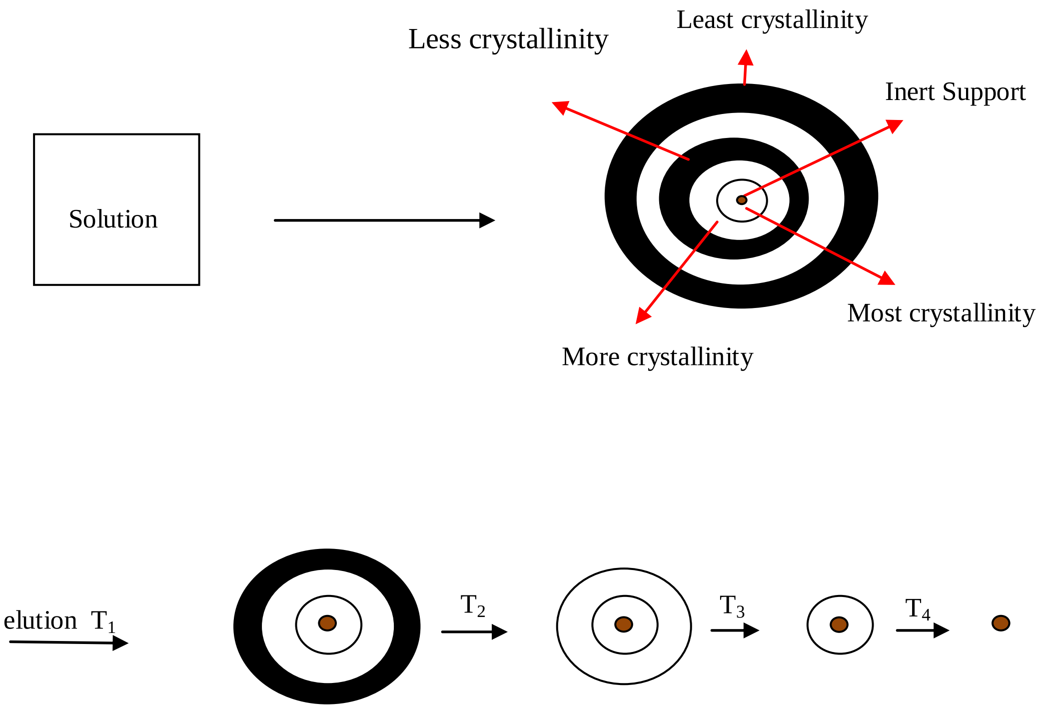

Figure 2.4 Mechanism of temperature rising elution fractionation.

The first step, the polymer is dissolved in a suitable solvent at high temperatures. The solution is loaded into a column containing an inert support, for example, sand, glass beads, silica gel, etc., and then the column is slowly cooled down to below dissolution temperature. The fraction precipitates from the solution and coats on the support in each layer depend on different crystallinities of polymer while temperature is decreased gradually. The fraction polymer with most easily crystallizes precipitates first and covers on the support in the innermost layer. On the other hand the fraction with least crystallinity precipitates last and covers on the outermost layer. This process is important and the key is a slow cooling rate because it ensures that the polymer factions precipitate according to the order of crystallinities. The slow cooling rate provides an optimal crystallizability separation which is independent of molecular weight.

The second stage is the elution of the polymer. The solvent is flowed through the column while the temperature is gradually increased thus eluting the fractions sequentially precipitated in the first stage. In this stage, the precipitated polymer is eluted with the solvent at increasing temperatures (continuously or stepwise). At lower temperatures, the fraction with less crystallinity (outermost layer) is eluted. With increasing elution temperature, the fraction of higher crystallinity (inner layer) is eluted. Thus the polymer is separated in fraction [2] .

2.5 LITERATURE REVIEW

2.5.1 Application of TREF

In 1988, Kulin *et al.* [11] studied fractionation of LDPE by using TREF and a liquid-liquid phase separation technique co-operatively. LDPE was separated due to different short chain branching and long chain branching. The results found the fractionation mechanism of TREF was depended on the short chain branching while the liquid-liquid method was depended on molecular weight. Nevertheless limitation of TREF is that it did not separate well for long-chain branching, due to the long-chain branches have little crystallinity.

In 2000, Usami *et al.* [12, 13] studied the composition of polyolefin blends by using TREF, Crystaf technique and DSC. This paper compares separation efficiency of each technique. The results found that separation by TREF are the best method comparing to the others. TREF can separate polyolefins with different branching components. The drawback of TREF is that time consuming and poor resolution when fractions are highly branched at low eluting temperatures. Crystaf technique used shorter analysis times, however disadvantage of Crystaf technique is in the separation of polypropylenes, it requires a super cooling. Separation polyolefins by using DSC is related to mechanical properties or the processing behavior. The melting curves depend on weight and heat of fusion in polymer. The polymer blend is analyzed by combination of the three methods, suitable with high degrees of super cooling such as polypropylene.

In 2000, P. Viville *et al.* [14] studied the molecular structure of polypropylenes which have different tacticity, as PP1 and PP2, produced from different heterogeneous Ziegler–Natta catalytic systems. They studied conditions of TREF. Increasing elution temperature, faction was separated as isotacticity and hight crytallinity and found PP2 has higher elastic modulus than PP1. TREF depends on crystallizability of each polymer. The fractions with a low tacticity and low crystallity could not be separated by TREF due to the low crystallizability of the polymers. This study allowed further identifies TREF fractions which were responsible for differences in rigidity.

In 2001, X. Junting *et al.* [15] studied separation component of olefins such as polyethylene and polypropylene, these polymers are semi-crystalline and distribution of the branch chain molecule, that affect the solubility. Important factor in TREF technique is crystallinity. Single and mixed solvent were used in order to increases crystallinity of polymer, resin products, LLDPE and comonomer. With 1-butene, 1-hexene and octane as solvents, they found that polymer with high crystallinity required elution temperature higher than that with low crystallinity. High density polyethylene (HDPE) and low density polyethylene (LDPE) were separated depended on their different length of their branching chains. HDPE has a much higher degree of crystallinity than that of LDPE so that elution temperature is higher than LDPE. Atactic PP and isotactic PP were also separated on the basis of their different stereoregularity, atactic isomer. The monomers are in a position to inverse. This polymer will be less crystalline than isotactic see in Figure 2.5 which have methyl groups on the same side of the chain, isotactic PP has the most crystallinity, the polymer is eluted a last at the highest temperature. TREF can be used to separate these polymers, they have similar molecular weight but difference of crystallinity at each temperature.

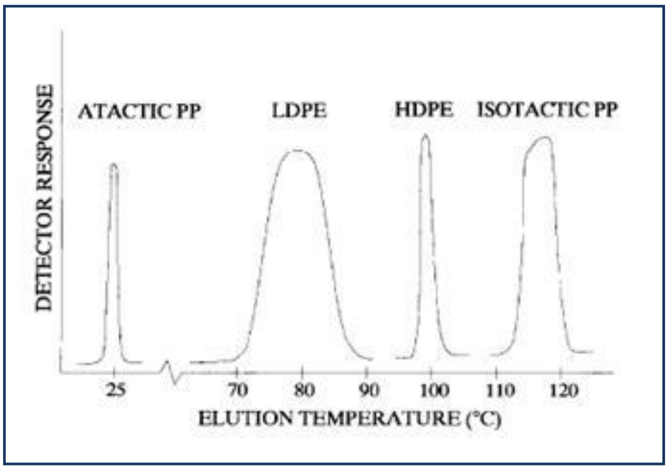

Figure 2.5 TREF chromatogram of some polyolefin.

Besides, polymerization of ethylene and propylene made $MgCl_2$-supported $TiCl_4$ catalysts, this product was fractionated by TREF and a variety of fractions were obtained, the fractions were characterized by DSC. The results found that product made from polymerization of ethylene and propylene has four types of components: random copolymer, multiple-block copolymer, ethylene-predominant copolymer and ethylene –propylene block copolymer. TREF can separate copolymer by its different crystallinity. Thermal analysis can be used to determine the microstructure of different components.

In 2002, L. Hongbin *et al.* [16] studied isotacticity distribution on crystallization of polypropylene by using TREF and SEG analysis. This research used polypropylene with molecular weight of 380000 and 403000. The results found molecular weight containing crystallizable units was increased. Isothermal crystallization rate of iPP (isotactic propylene) is dependent on higher temperature. Although low molecular weights and different isotacticity dritribution can induce variation in the nucleation mechanism of iPP.

In 2005, N. Samoth *et al.* [10] found that separation the polymers by TREF took a long time especially in the crystallization step. This problem was solved by using mixed solvent and various solvents, for example 1-butene, 1-hexene and 1-octene. The results found that time of analysis depended on the strength of solvent in the crystallization step and polarity of solvent in elution step. Using mixed solvent can reduce time consuming of the technique. It was found that the DSC thermogram of each fraction with mixed solvent, the rate of crystallization increased, elution temperature increased, sharper the melting peak and the temperature of melting curve shifted toward higher.

In 2007, M. Zhang *et al.* [17] used TREF and DSC in order to study distribution of molecular structure affect crystallization behavior and melting. The copolymers, made by using Ziegler-Natta catalyst and metallocene copolymer, have different structure as short chain branching (SCB), and methylene sequence length (MSL). The dominant factors of TREF analysis are physical properties of copolymers, which depend on the amount and distribution of the SCB. The results found that the copolymer made from Ziegler-Natta catalyst showed a broad of bimodal SCB distribution indicated by TREF. The copolymer from metallocene exhibited a narrow SCB distribution. The characterization by TREF depends on melting and crystallization from the difference of distributions in the SCB and MSL.

In 2011, R. Ramnath *et al.* [18] studied molecular weight distribution and branch length distribution of polyethylene effect from catalyst systems and catalyst activity fractionated by TREF and GPC. This paper studied the structure and branch content of a linear low-density polyethylene (LLDPE). The branch length distribution of this LLDPE was determined by using TREF

2.5.2 Separation component of waxes

Antonio de Lucas *et al.* [19] studied separation of long chain alcohols from sugar cane crude wax by supercritical CO_2 extraction.

Zaky *et al.* [20] reported the study of various grades of paraffin waxes. Comparison of two methods by using extraction technique and the crystallization technique in order to separated oil from the waxes. In the analysis of the paraffin, crystallization technique was found to be suitable for the separation.

A. Yasantha *et al.* [21] studied separation of wax from triticale straw by using supercritical carbon dioxide and hexane extraction. Extraction by SC-CO_2 condition at 70 ^{o}C and a flow rate of 50g/min at various pressures 250, 300, 350 and 400 bar for 90 min. Triticale straw wax was successfully extracted with SC-CO_2. The main components were fatty acids, fatty alcohols, sterols, hydroxylbeta-diketones, alkanes and beta-diketones.

Separation of waxes by extraction technique require high pressure and high cost. Waxes with similar molecular structures cannot be separated by this technique.

M. Chantdat *et al.* [22] studied separation of rice bran oil wax by fractional crystallization using hexane as the solvent. The wax was separated into 4 fractions at temperature of 20, 30, 40 and 50 ^{o}C, respectively. The major component was separated at 40 ^{o}C (29.64%).

Separation of natural waxes which are mixture of various components can only indicate the weight distribution in each fraction. It is difficult to separate and to identify pure component. The separated fractions are still mixtures of the components.

2.5.3 Crystallinezation of waxes

K. Rizwana *et al.* [23] studied separation of wax ester from seeds of A. pavonin using crystallization technique. In the first step, oil was separated from A. pavonin seed by extraction with hexane. The remaining waxes were crystallized by dissolving in hot acetone in order to crystallize the waxes. Finally, crystals were collected by filtration. The crystals were characterized by FT-IR and GC. It was found that the separated crystals were a mixture and the major components were wax esters from C_{34} to C_{48} and fatty alcohols from C_{14} to C_{28}.

In 2007, Mimma *et al.* [7-8, 24] studied the structures of edible oils. The structures of the various types of fat in food products were studied by crystallization. The structure of triacylglycerol (TAG) consisting of saturated fatty acids was found to contain three types of network structures, suspensions and emulsions depending on the ratio of the concentration of fats. Emulsions and suspensions are distribution of small inert particles dispersed in a continuous phase.

In 2007, Schaink and van Malssen. [8] studied differences in shape and size crystal of waxes which depend on ratios of the fatty acid and fatty alcohol. This research used stearic acid and stearyl alcohol in various ratios. The crystal structure of stearic acid is found to be plate-like (Fig. 2.6a). When the fatty acid: alcohol ratio was 1:1 (Fig 2.6b), the crystals were found to be more like small needles and the crystals of stearyl alcohol were found to form mica-like crystal (Fig. 2.6c). The shapes of the crystals can be changed depending on the compositions of the structurants.

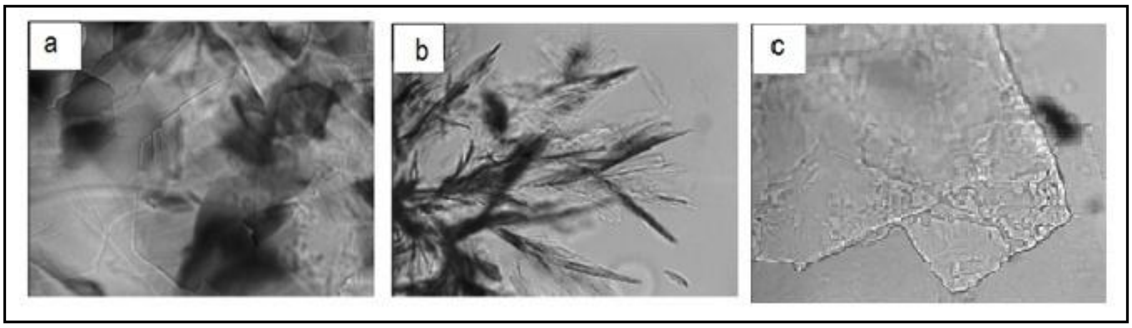

Figure 2.6 Microscopy images of the crystals for different components between fatty acid (C18:0): fatty alcohol (C18:0) ratios: 1:0 (a), 1:1 (b) and 0:1(c).

In 2003, S. Martini and M.C. Anon. [25] studied crystallization of sunflower oil waxes and melting behavior of purified waxes and solutions of waxes prepared at various concentrations. The melting diagrams of the purified waxes crystallized at 35°C were compared between slow (1°C/min) and fast (20°C/min) cooling rates. The melting thermograms of sample at slow cooling was a broader peak and a fast cooling rate compounds is a sharp peak exhibited. The samples crystallized at low temperatures have low-melting points. This indicates that cooling rate effects crystallization behavior. The important factors of wax crystallization are various concentrations (supersaturation) and T_c which affects crystallization at different temperatures and melting behavior. Activation free energies of nucleation (ΔG_c) values were studied in order to understand the nucleation process and measurements of induction times in crystallization of sunflower oil waxes.

Chapter III

Experimental Section

3.1 Apparatus

1. Water bath
2. Rotary evaporators
3. Column: inner diameter 4.42 cm, outside diameter 5.50 cm and length 61.50 cm.
4. Nuclear magnetic resonance spectroscopy : Varian Mercury 400MHz
5. Fourier transform infrared spectroscopy : Model Impect 410; Nicolet
6. Differential scanning calorimetry (DSC) : Netzsch ; 204 F1 Phoenix
7. Optical microscope (OM) : Leitz Metallovert
8. Gas chromatography (GC) : Shimadzu; GC-2010

3.2 Chemicals

All the chemicals were purchased from the commercial suppliers with the specified grade. They were used without further purification.

1. Hexane : analytical grade ; Lab-Scan
2. Dichloromethane : analytical grade ; Lab-Scan
3. Acetone : analytical grade ; Lab-Scan

4. Cyclohexane : analytical grade ; Fluka
6. Ethanol : analytical grade ; Fluka
7. Potassium hydroxide : analytical grade ; Merck
8. Potassium bromide : analytical grade ; Merck
10. Potassium dichromate : analytical grade ; Merck
11. Potassium iodine : analytical grade ; Merck
12. Potassium permanganate : analytical grade ; Merck
13. Phenolphathalein indicator : analytical grade ; Merck
14. Iodine : analytical grade; Merck
15. Soduim thiosulfate : analytical grade ; Fluka
16. 96% Sulfuric Acid : analytical grade ; Lab-Scan
17. 37% Hydrochloric acid : analytical grade; Merck
18. Stearic acid : analytical grade; Merck
19. Oleic acid : analytical grade; Merck
20. Palmitic acid : analytical grade; Merck
21. 1-Octadecanol : analytical grade; Merck
22. Linoleic acid : analytical grade; Merck
23. Decanoic acid : analytical grade; Merck
24. Sand : 710 µm and 510 µm

3.3 Preparation of esters

The esters were prepared by following the literature procedure as follow [26].

The stearyl decanoate was prepared by heating 8.52 gram (MW 270 g/mole) of stearyl alcohol in 20 mL of hexane with stirring to solution. Six grams of decanoic acid (MW 200 g/mole) was slowly poured at temperature 60 ^{o}C for 30 min and 2-3 drops of sulfuric acid was added as catalyst. The mixture was stirred for 2 hrs until completion of the reaction. The reaction mixture was washed several times with water in order to eliminate the acid and the remaining alcohol. Solution was filtered to separate the ester, and then water was removed by rotary evaporator and followed by heating in an oven at 110 ^{o}C for 5 hours.

All other esters (stearyl linolate, stearyl oleate, stearyl palmitate and stearyl stearate) were synthesized in the same manner.

3.4 Properties of the synthetic esters

3.4.1 Thermal analysis

The melting point (T_m) and crystallization temperature (T_c) of each ester were determined by differential scanning calorimetry (DSC). The sample (10 to 15 mg) hermetically sealed in an aluminum pan was heated to 100 ^{o}C. It was then cooled to 0 ^{o}C and heated again to 100 ^{o}C. The rate of temperature variation was 10 ^{o}C/min for heating and cooling.

The peak Tm, Tc, melting enthalpy (ΔH_m) and crystallization (ΔH_c) were determined using the software provided with the instrument. The average endothermic peak value was taken as T_m [27].

3.4.2 Determination of Solubility

The synthetic ester (stearyl linolate, stearyl oleate, stearyl decanoate, stearyl palmitate and stearyl stearate) was dissolved in 20 mL of *n*-hexane and isopropanol at various temperatures (0 ^oC, 5 ^oC, 10 ^oC, 15 ^oC, 20 ^oC, 25 ^oC, 30 ^oC, 35 ^oC, 40 ^oC, 45 ^oC, 50 ^oC and 55 ^oC). The amount of synthetic ester dissolved in 20 mL of solvent was recorded.

3.4.3 Determination of Microscopy of the synthetic esters

The appearance of the ester crystals in this research was observed by optical microscope. Photomicrographs of the developing crystal were taken by using magnification 50x and 100x.

3.4.4 Determination of wax by GC

The components and purity of wax in the each fraction were analyzed by gas chromatography following ASTM D5442-93. The column temperature was linearly increased at temperature program rate until the sample was completely eluted from the column. The eluted components were detected by a flame ionization detector and recorded on the computer system. The peak areas were obtained by the computer program.

Operating condition of the standard test method for analysis of petroleum waxes by GC (ASTM D5442-93) were as follow.

1. Column	DB-5 high temp 5% -Phenyl-methylpolysiloxane
2. Column length	30 m
3. Column ID	0.32 mm
4. Film thickness	0.25 μm
5. Carrier gas helium	Helium
6. Pressure	96.2 kPa
7. Column temperature	Initial temperature: 190 ^{o}C, temperature program rate: 17 ^{o}C/min, final temperature: 220 ^{o}C, holding time: 7 min
8. Injector temperature	230 ^{o}C
9. Detector temperature	250 ^{o}C
10. Sample preparation	0.1 - 1.0 wt%

3.4.5 Determination of wax by FTIR

The FTIR spectrum of each synthetic ester was recorded on a Nicolet Fourier Transform Infrared Spectrophotometer: Impact 410 (Nicolet Instruments Technologies, INC. WI, USA). Infrared spectra were recorded between 400 cm^{-1} to 4000 cm^{-1} in transmittance mode.

3.4.6 Determination of wax by NMR

The final degree of olefin conversion of wax was calculated by NMR spectroscopic analysis. The samples were dissolved in $CDCl_3$ at 1% (w/v) at atmospheric pressure and room temperature. The ^{1}H-NMR spectra were obtained on Varian Mercury 400MHz spectrometer using tetramethyl silane (TMS) as the internal standard.

Integration of spectra was used to determine the amount of characteristic protons of each structure in the polymer. The integration peak area for the saturated protons ($-CH_2-$ and $–CH_3$) in the range of 0.8-2.3 ppm and the unsaturated protons peak area at 5.2 ppm were measured in order to calculate the degree of unsaturation by using Eq. (Hinchiranan, 2004).

$$\%\, unstaturated \quad = \frac{2B}{A} \times 100$$

Where A is the integration peak area of $–CH_3$ protons and B is the integration peak area of unsaturated protons. The example for calculation of hydrogenation level is presented in Appendix B [28].

3.5 Chemical properties of synthetic ester

The chemical properties of synthetic ester were determined by following the standard method as shown in Table 3.1.

Table 3.1 Standard method for the determination of wax properties.

Characterization Properties	Standard Method
Saponification Number	ASTM D 1387
Acid Number	ASTM D 1386
Iodine Value	ASTM D 5554
Drop Melting Point	ASTM D127

3.5.1 Determination of saponification number (ASTM D 1387)

One gram of wax was dissolved in 40 mL of hexane at 60 ^{o}C, the solution was removed hot plate and then 0.10 N ethanolic KOH solution in the burette was added 50 mL into the solution and reflux condenser for 3 hours with hot plate. After that the solution was removed from the condenser, this solution was added 5 drops of phenolphthalein solution and this solution was titrated with 0.5 N HCl until the pink color disappears. And then the solution was reheated to boiling point and titrated until the pink color dose not reappear.

Calculation the saponification number

Calculation the saponification number as follows :

$$Saponification\, number\ =\ \frac{(B-A)\,N\ \times\ 56.1}{C}$$

A= milliliters of HCl solution required for titration of the sample.

B= milliliters of HCl solution required for titration of the blank.

C=grams of sample used.

N=normality of HCl Solution.

3.5.2 Determination of the iodine value of the synthetic wax (ASTM D5554)

The iodine value is a measure of unsaturation and expressed as the number of gram of iodine absorbed, under the prescribed conditions, by 100g of the test substance.

Wax (1.5 grams) was dissolved in 6 mL of chloroform while warming on the hot plate. The solution was then removed from the hot plate, 25 mL of wijs solution was added in this solution and the solution was kept in the dark for 1 hr. Potassium iodide and DI water were added in this solution. The solution was titrated with 0.1N sodium thiosulphate solution until the solution turned to primrose. A few drops of starch solution were added in this solution to give a blue solution. The titration was continued until the blue color disappears.

Calculation the acid number as follows:

$$Iodine\ value\ = \frac{(B - S)N * (12.69)}{C}$$

B= milliliters of sodium thiosulphate solution required for titration of the blank.

S= milliliters of sodium thiosulphate solution required for titration of the sample.

C= gram of the sample used.

N= normality of sodium thiosulphate solution.

3.5.3 Determination of acid number (ASTM D1386)

The test method covers the determination of the acid number of synthetic wax and natural waxes. The acid number was obtained by direct titration of the material. It indicates the amount of free acid present.

Procedure

Synthetic wax (0.001-1 gram) was dissolved in 40 mL of hexane and 5 drops of phenolphthalein solution was added. The solution was titrated with 0.1N ethanolic potassium hydroxide until the hot solution turned to pink color and remains for at least 10 sec. which indicated the end point.

Calculation the acid number as follows:

$$Acid\ number = \frac{\mathrm{A\,N} \times 56.1}{B}$$

A= milliliters of alkali solution required for titration of the sample.

B= gram of the sample used.

N= normality of alkali solution.

3.6 Preparation of synthetic wax

The synthetic wax used for this study was prepared by mixing an equal weight amount of the appropriate esters.

3.7 Preparation of inert support

One thousand and five hundred grams of sand, 150 mL of conc. H_2SO_4 and 1,500 mL of DI water were added into a beaker. The mixture was stirred for 24 hours. It was decanted, and the remaining sand was washed several times with excessive amount of DI water. The sand was then dried in an oven at 110 ^{o}C for 10 hours. The resulting sand was sieved through 510 µm and 710 µm sieves.

3.8 General procedure for the separation by TREF technique.

The apparatus used for the separation is shown in Figure 3.1

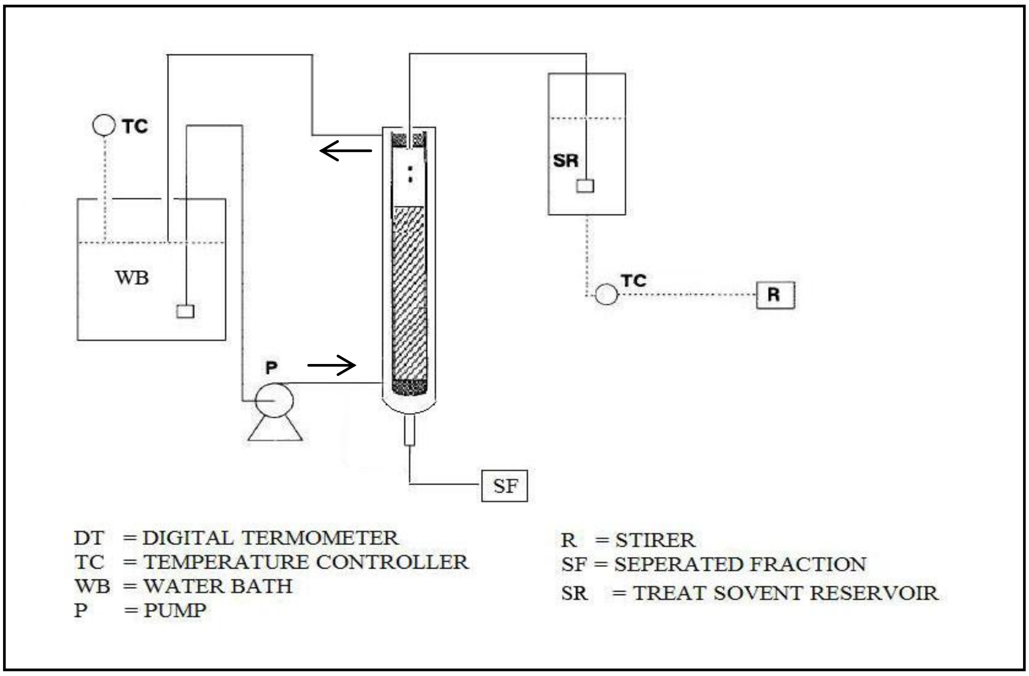

Figure 3.1 Schematic drawing of the apparatus for the separation.

The preparative TREF was performed by dissolving the wax (5 g) in 150 mL of a solvent at 60 ^{o}C. The solution was heated at above crystallizing temperature by stirring until the solution was homogeneous.

This solution is loaded into a preheated column containing an inert support. The column temperature was controlled by using water bath. The temperature was slowly decreased to 50, 40, 30, 20, 10 and 5 ^{o}C at a constant rate of 10 ^{o}C/h and was hold at each temperature for 1 hrs.

For the elution step, the column temperature was increased and hold at 10 ^{o}C for an hour. The column was then eluted with 50 mL of the pre-cooled solvent at 10 ^{o}C. The column temperature was slowly increased to 20 ^{o}C and hold at this temperature for an hour and was then eluted with 50 mL of the pre-cooled solvent at 20 ^{o}C. The same procedure was applied for the elution at 30, 40, 50 and 60 ^{o}C.

The composition for each eluated fraction at each temperature was analyzed by GC.

3.9 Factors affecting separation by TREF

3.9.1 Effect of solvent

The preparative TREF with 510 µm sand, various solvents (*n*-hexane, isopropanol and mixed solvent; hexane and isopropanol 1:1)

The study was performed by dissolving the wax (5 g) with 75 mL of a solvent (*n*-hexane, isopropanol or a mixed solvent of *n*-hexane and isopropanol) at 60 ^{o}C. The solution was loaded into the preheated column with sand support at 60 ^{o}C. The temperature was decreased to 50^{o}C, 40 ^{o}C, 30 ^{o}C, 20 ^{o}C, 10 ^{o}C and 5 ^{o}C at a constant rate of 10 ^{o}C/h and hold at each temperature for 1 hrs.

In the elution step, the column was eluted with 150 mL of the solvent for each temperature and the procedure was similar to that of the general procedure described above. The composition for each fraction was also analyzed by GC.

3.9.2 Effect of inert support size

The general procedure was performed using 5 grams of the synthetic wax in 75 mL of isopropanol and two different sizes of sand support (710±5 µm and 510±5µm) and without sand support.

3.10 Fractionation Crystallization

Five grams of the synthetic wax was dissolved in 75 mL of isopropanol at 60 ^{o}C. The temperature was decreased slowly at the rate of 10 ^{o}C/min to 50 ^{o}C and hold at this temperature for an hour. The precipitate was collected by quick filtration at the same temperature. The mixture was subjected to the same operation at 40, 30, 20 and 10 ^{o}C. The precipitate at each temperature was analyzed for their composition by GC.

CHAPTER IV

RESULTS AND DISCUSSION

In this work, synthetic wax was prepared and was used for the separation by TREF. Five esters were prepared from five fatty acids (stearic acid, palmitic acid, decanoic acid, oleic acid and linoleic acid) and a fatty alcohol (stearyl alcohol). The synthetic waxes were then prepared by mixing an equal weight amount of the appropriate esters.

4.1 Appearance of the esters

Stearyl stearate, stearyl palmitate, stearyl decanoate, stearyl oleate and stearyl linoleate were prepared. The appearances are shown in Figure 4.1 and summarized in Table 4.1.

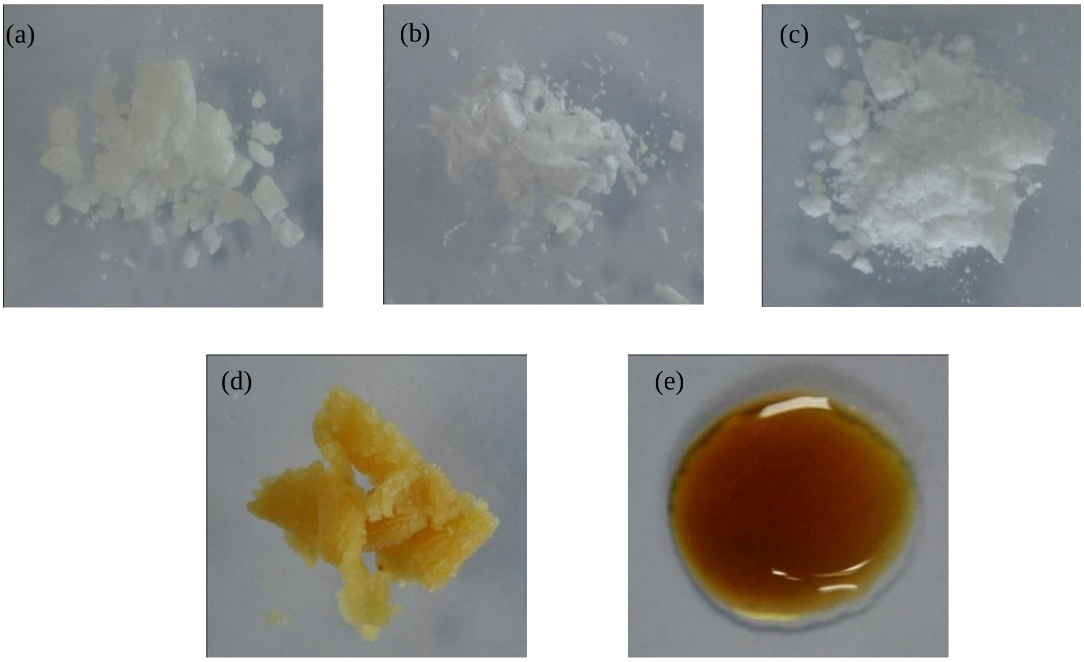

Figure 4.1 Physical appearances of synthetic esters, (a) SS is white solid waxy flakes, (b) SP is lutescent solid waxy flakes, (c) SD is white solid waxy flakes, (d) SO is pale yellow oily, (e) SL is pale ochre oily liquid.

Table 4.1 Appearance of the synthetic ester.

Sample	Formula Chemicals
stearyl linoleate	$C_{36}H_{68}O_2$
stearyl oleate	$C_{36}H_{70}O_2$
stearyl decanoate	$C_{28}H_{56}O_2$
stearyl stearate	$C_{36}H_{72}O_2$
stearyl palmitate	$C_{34}H_{18}O_2$

4.2 Crystallinity of the synthetic esters

Crystallinity and other properties of the esters were determined by DSC. The obtained DSC thermograms of the esters are shown in Figure 4.2. The thermograms indicate that esters are crystallizable at different temperature. Heats of fusion, melting temperature and crystallization temperature were also obtained and the results are given in Table 4.2.

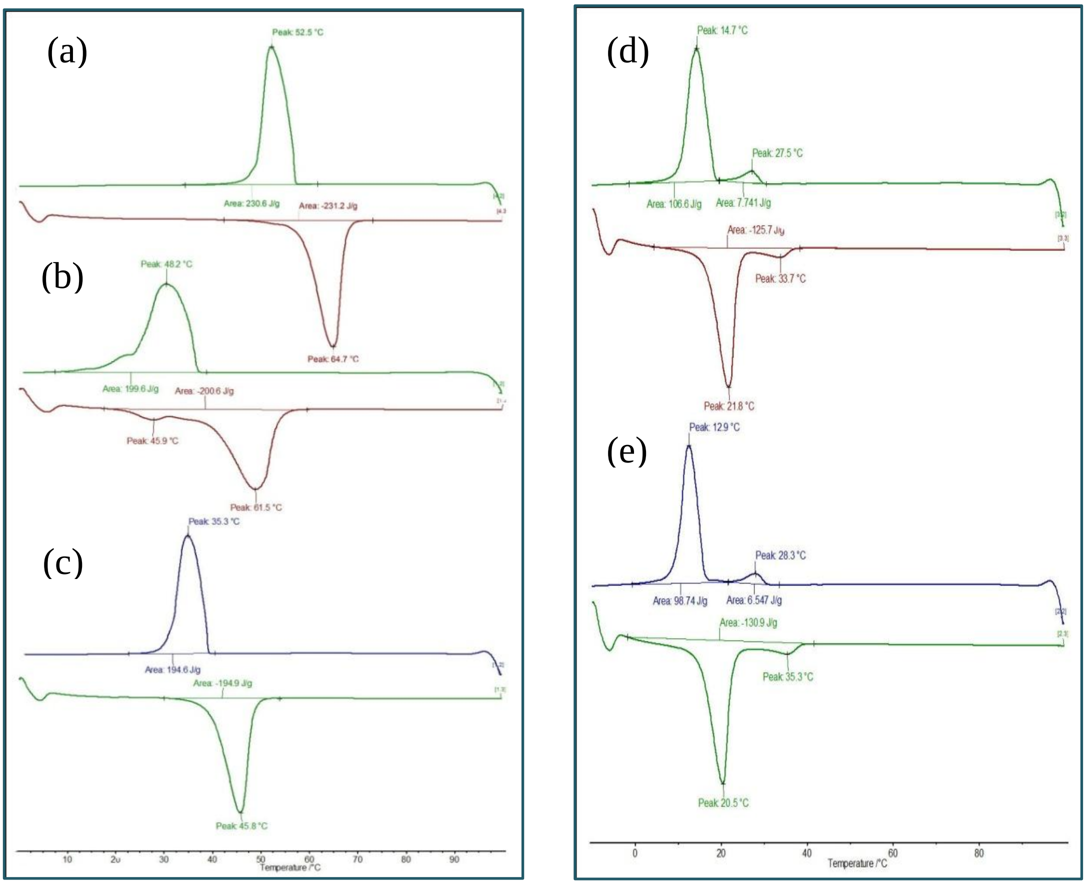

Figure 4.2 DSC thermograms of the synthetic esters: (a) stearyl stearate (SS), (b) stearyl palmitate (SP), (c) stearyl decanoate (SD), (d) stearyl oleate (SO), (e) stearyl linolate (SL).

Table 4.2 Heats of fusion (ΔH_m), melting temperature (T_m) and crystalline temperature (T_c) of synthetic esters.

Waxes	ΔH_m (Jg^{-1})	T_m (^oC)	T_C (^oC)
stearyl stearate	231.2	64.7	52.5
stearyl palmitate	200.6	61.5	48.2
stearyl decanoate	194.9	45.8	35.3
stearyl oleate	125.7	21.8	14.7
stearyl linoleate	130.9	20.5	12.9

Figure 4.2 and Table 4.2 show thermal properties of synthetic ester. They are compared on these properties. For the saturated esters (stearyl stearate C18:0, stearyl palmitate C16:0 and stearyl decanoate C10:0), T_m increases with increasing molecular weight. Therefore, stearyl decanoate has the lowest T_m and stearyl stearate has the highest T_m. The presence of a cis double bond in stearyl oleate lowers the melting point due to the unsaturated ester's "kink" at the position of the double bond. Kinked molecules cannot pack as tightly together in a solid as the uniform zigzag chains of a saturated ester. Stearyl oleate and stearyl linoleate have much lower T_m than stearyl stearate. ΔH_m and T_c of stearyl stearate, stearyl palmitate, stearyl decanoate and stearyl oleate are obviously different which indicates difference of degree of crystallization. However, stearyl linoleate has quite similar properties to those of stearyl oleate. In addition, stearyl stearate and stearyl palmitate are quite similar in their properties. This suggests that the two esters in each pair may not be separated from each other by this technique.

From the results of the properties of the esters discussed above, stearyl palmitate, stearyl decanoate and stearyl oleate were selected to make the synthetic wax for this study.

4.3 The crystal morphology of synthetic esters

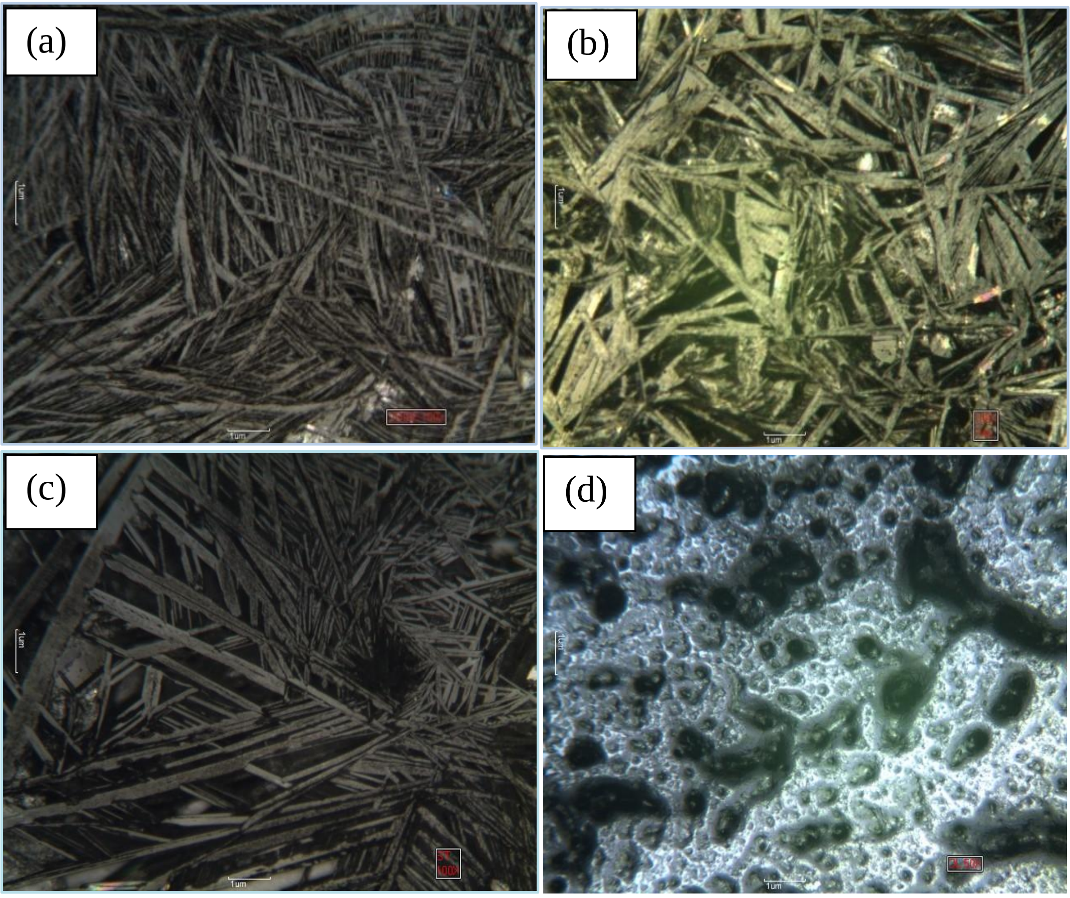

Figure 4.3 Photographs of the fatty esters: (a) SD, (b) SP, (c) SS and (d) SO (low melting point (20.5 ^{o}C).

Morphology of stearyl stearate, stearyl palmitate, stearyl decanoate and stearyl oleate at room temperature was obtained see in figture 4.3. These fatty esters have straight-chain hydrocarbons in their structures causing needle-type crystals and crystal size increases depending on their higher contents of long chain esters. This is evident from the result of morphology of stearyl stearate has needle crystal size larger than that of stearyl decanoate.

4.4 Chemical properties of the synthetic esters

Table 4.3 Chemical properties of synthetic ester

Sample	Saponification number	Acid number	Iodine number
stearyl linoleate	92.9623	1.9856	80.95
stearyl oleate	93.5638	2.0716	65.78
stearyl decanoate	106.1077	2.8882	1.75
stearyl stearate	92.7648	1.7656	1.03
stearyl palmitate	104.5431	1.8809	0.97

The saponification number is the number of milligrams of potassium hydroxide required to neutralize the fatty acids resulting from the complete hydrolysis of 1 g of ester. It indicates the fatty acids of the ester, the longer the carbon chain, the less acid is liberated per gram of fat hydrolyzed. It is also considered as a measure of the average molecular weight or chain length of all the fatty acids present. The long chain fatty acids found in esters have low saponification number because they have a relatively fewer number of carboxylic functional groups per unit mass of the ester and therefore high molecular weight [6]. Table 4.3 shows that stearyl linoleate, stearyl oleate and stearyl stearate have higher molecular weight than stearyl palmitate and stearyl decanoate, respectively.

The acid number indicates quality of oil and fat. This number indicates the amount of the free acid. For cosmetic and pharmaceutical standards, the acid number should be lower than 3. When it is stored for a long time, it can react with oxygen (lipid oxidation reaction) causing fatty ester to degenerate. The free acid increases viscosity and decreases solubility of the fatty ester. Therefore, the acid number of the ester should be determined before being used.

Iodine number indicates unsaturation in fat and oil. Therefore, stearyl linoleate and stearyl oleate have high Iodine number indicating the unsaturation of the components.

4.5 Solubility of synthetic esters

This experiment determines solubility of the fatty esters. Solubility is an important factor for the success of the separation of components. The solubility data obtained was used for finding the most suitable solvent for the separation. The solubility of the esters (stearyl stearate, stearyl palmitate, stearyl decanoate, stearyl oleate and stearyl linoleate) in *n*-hexane and isopropanol was determined at various temperatures (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 ^{o}C) and the results are summarized in Table 4.4. The plot between the solubility and temperature is shown in Figures 4.4 and 4.5.

Table 4.4 Solubility of the fatty esters in *n*-hexane at various temperatures (unit: grams of fatty esters in 20 mL of the solvent)

Ester	Temperature (^{o}C)										
	0	5	10	15	20	25	30	35	40	45	50
SL	0.22	0.32	0.40	1.30	1.91	5.20	10.53	16.39	10.53	G	G
SO	0.23	0.42	0.30	1.00	1.71	4.79	8.53	15.39	G	G	G
SD	0.30	0.32	1.24	1.51	3.75	4.28	4.61	5.17	6.68	6.73	6.97
SS	I	0.02	0.10	0.31	0.35	0.78	1.10	1.49	2.51	4.49	5.05
SP	0.02	0.03	0.49	0.53	0.77	1.20	1.45	1.92	2.91	4.88	5.25

Notes : I = Insoluble, G = Good solubility

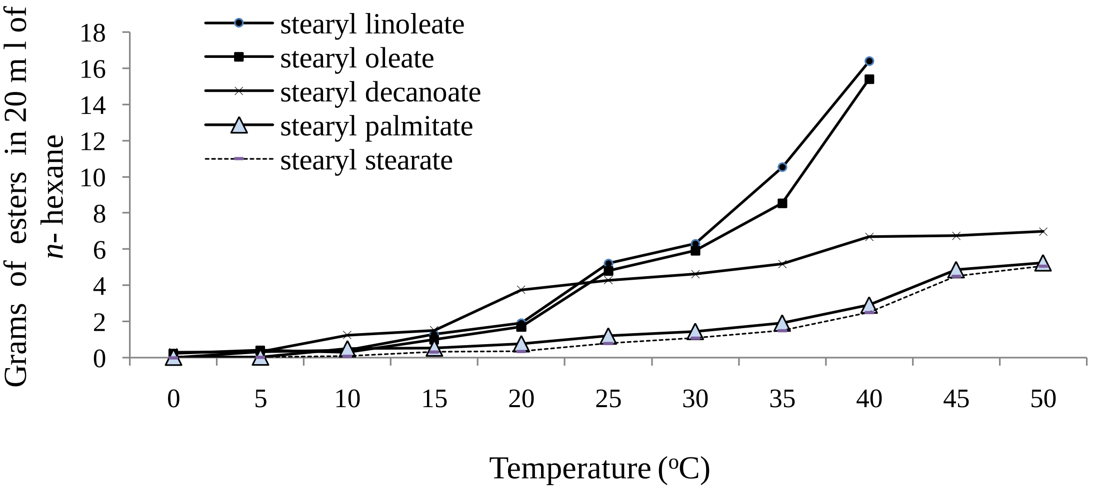

Figure 4.4 Temperature-solubility cures of fatty esters in *n*-hexane

In Figure 4.4, all the esters sparingly soluble in *n*-hexane at low temperature and more soluble with increasing temperature. The solubility of stearyl linoleate and stearyl oleate drastically increases at 25 ^{o}C and above.

Table 4.5 Solubility of fatty esters in isopropanol at various temperatures (unit: grams of fatty esters in 20 mL of the solvent)

Ester	Temperature (^{o}C)										
	0	5	10	15	20	25	30	35	40	45	50
SL	0.21	0.35	0.62	1.24	2.55	3.14	4.23	6.34	9.09	12.75	16.5
SO	0.25	0.40	0.57	1.04	2.55	3.00	4.03	5.95	8.09	12.43	15.87
SD	I	I	0.13	0.25	0.30	0.48	1.62	2.76	5.17	7.03	8.90
SS	I	I	0.02	0.06	0.06	0.06	0.12	0.19	0.23	0.45	0.65
SP	I	I	0.10	0.10	0.13	0.13	0.19	0.24	0.35	0.55	0.83

Notes: I = Insoluble, G = Good solubility

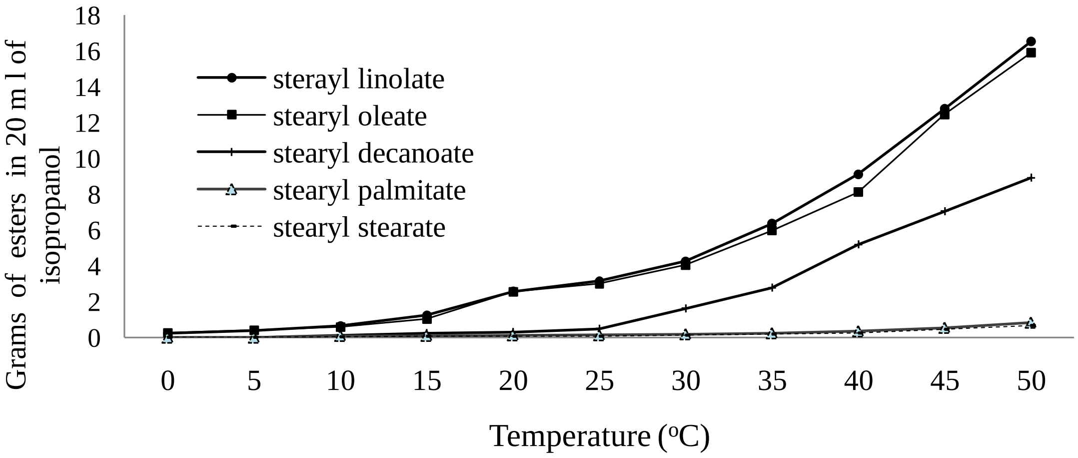

Figure 4.5 Temperature-solubility curves of fatty esters in isopropanol

Figure 4.5 shows that stearyl palmitate and stearyl stearate dissolved poorly at all temperatures in isopropanol. Stearyl decanoate starts to dissolve at temperature above 25 ^{o}C. On the other hand, solubility of stearyl linoleate and stearyl oleate increase rapidly above 15 ^{o}C.

Comparing between the two solvents, the esters are more soluble in *n*-hexane than in isopropanol at the same temperature.

Table 4.5.3 Solubility of fatty esters in *n*-hexane and isopropanol at various temperatures (unit: grams of fatty esters in 20 mL of solvent)

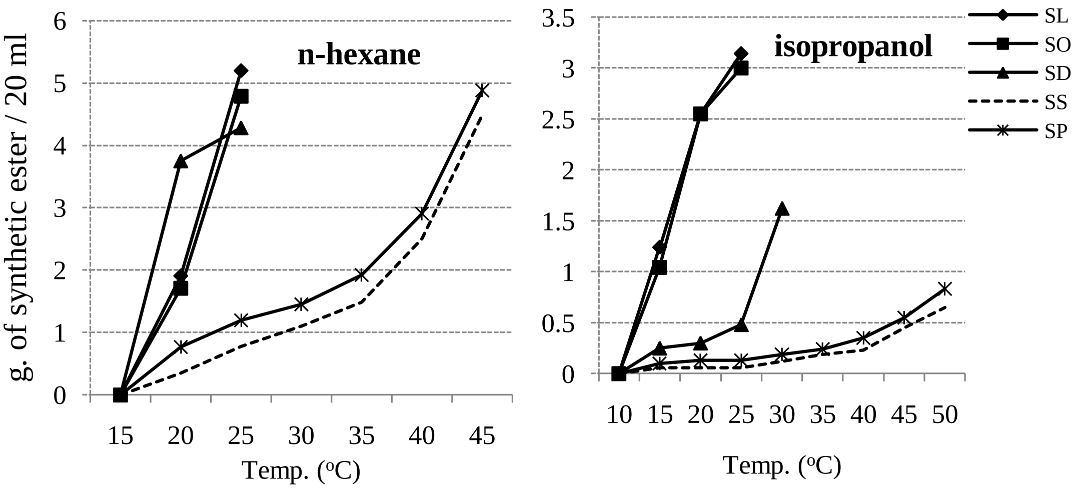

Figure 4.6 Comparing between the two solvents, temperature-solubility cures of fatty esters in *n*-hexane and isopropanol.

Table 4.5 and figures 4.6 show that solubilities of the esters are more different in isopropanol than in *n*-hexane. In isopropanol, stearyl linoleate and stearyl oleate have quite similar solubilities. Their solubilities increase rapidly above 15 ^{o}C. Stearyl decanoate is not quite soluble at low temperature but much more soluble above 25 ^{o}C. Stearyl palmitate and stearyl stearate have low solubilities at room temperature and their solubilities slowly increase upon increasing temperature. In *n*-hexane, on the other hand, stearyl linoleate, stearyl oleate and stearyl decanoate have similar hight solubilities. Stearyl stearate and stearyl palmitate also have similar solubilities but lower than the other three esters. In order to be successful in separating by TREF, solubilities of the ester components should be different. Comparing between these two solvents, isopropanol is more suitable than *n*-hexane. Therefore, isopropanol was used as the solvent for this study. In order to make synthetic wax for this work, all five esters were considered. Stearyl linoleate and stearyl oleate have similar solubilities, these two esters may not be able to be separated by this technique. Stearyl stearate and stearyl palmitate may also not be to be separated. For this reason, only one for each pair is chosen as the components for the wax. Therefore, the synthetic wax was made by mixing equal amounts of stearyl oleate, stearyl palmitate and stearyl decanoate. This synthetic wax was used for this work.

4.6 Separation of the components of the synthetic wax by TREF

The components of the synthetic wax were separated by TREF. The fractions were obtained at 10 ^{o}C, 20 ^{o}C, 30 ^{o}C, 40 ^{o}C and 50 ^{o}C and were analyzed by GC and NMR.

4.7 Factors affecting separation by TREF

4.7.1 Effect of solvent

4.7.1.1 Hexane as solvent, 510 µm sand support

The separation of the wax components by TREF using *n*-hexane as the solvent with 510 µm sand support was performed and the compositions of each fraction are summarized in Table 4.6 The plot of % area of wax components for each fraction at each temperature is as shown in Figure 4.7

Table 4.6 The distribution of wax components and GC results in each fraction

Elution temperature	Weight(g)	% area P1	% area P2	% area P3	% wt. of ester		
					SD	SO	SP
10	2.11	29.89	70.10	-	14.29	33.51	0
20	1.19	47.65	16.92	35.41	12.88	4.57	9.57
30	0.59	-	-	100	0	0	13.56
40	0.51	-	-	100	0	0	11.59

*P1= peak at retention time 24.18 min, P2= peak at retention time 27.18 min and P3= peak at retention time 26.12 min.

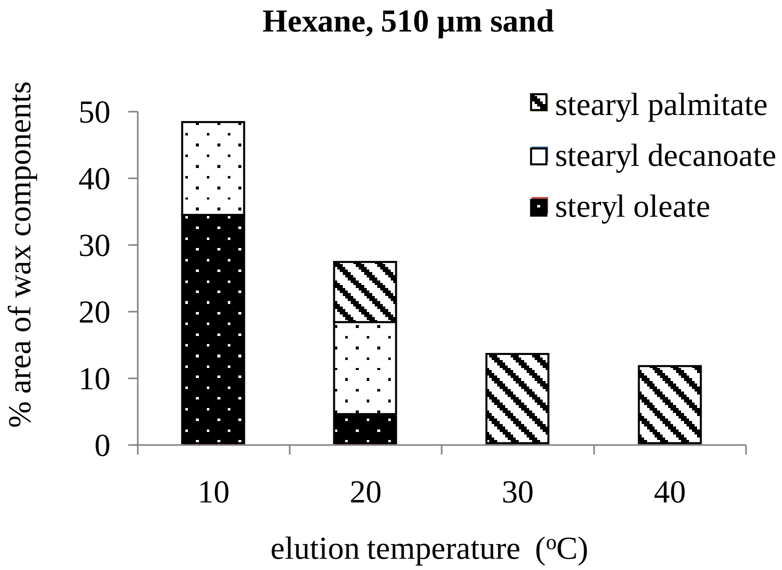

Figure 4.7 The plot of % area of wax components with elution temperature by TREF, Hexane as solvent, 510 µm sand as an inert support.

The GC result of the fraction eluted at 10 °C was a mixture of stearyl oleate as the major component and stearyl decanoate as the minor component. The fraction at 20 °C was a mixture of stearyl oleate, stearyl decanoate and stearyl palmitate. The fraction at 30 °C was a pure stearyl palmitate.

4.7.1.2 Isopropanol as solvent, 510 µm sand support

The separation of the wax components by TREF using isopropanol as the solvent with 510 µm sand support was performed. The area of GC chromatograms in each fraction is shown in Figure 4.8 and the compositions of each fraction are summarized in Table 4.7.

Table 4.7 The distribution of wax components and GC results in each fraction

Elution temperature (°C)	Weight (g)	% area P1	% area P2	% area P3	% wt. of ester		
					SD	SO	SP
10	0.88	3.18	96.82	-	0.62	19.02	0
20	0.72	57.82	42.18	-	9.29	6.78	0
30	1.13	89.99	10.01	-	22.69	2.52	0
40	0.56	-	-	100	0	0	12.50
50	0.84	-	-	100	0	0	18.75

*P1= peak at retention time 24.18 min, P2= peak at retention time 27.18 min and P3= peak at retention time 26.12 min.

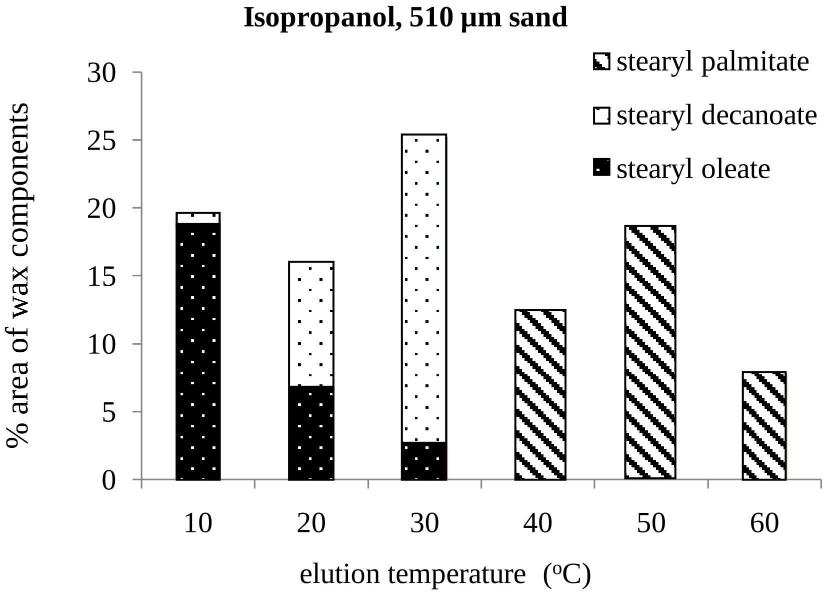

Figure 4.8 The plot of % area of wax components with elution temperature by TREF, isopropanol as solvent, 510 µm sand as an inert support.

The GC result of fraction eluted at 10 °C was mainly the stearyl oleate with a little amount of stearyl decanoate. The fraction at 20 °C was a mixture of the two components with stearyl oleate as the major component and stearyl decanoate as the minor component. The fraction at 30 °C was mainly the stearyl decanoate with a little amount of stearyl oleate. The fractions at 40 °C and above were pure stearyl palmitate. The fraction obtained at 30 °C was further separated by repeating the same procedure. The results are summarized in Table 4.8 and Figure 4.9

4.7.1.2.1 Isopropanol as solvent, 510 µm sand support at 30 °C and repeated.

Table 4.8 The distribution of the components in each fraction

Elution temperature (°C)	Weight (g)	% area P1	% area P2	% wt. of ester	
				SD	SO
10	0.05	-	100	0	5.32
15	0.07	-	100	0	7.45
20	0.04	14.36	85.64	0.61	3.64
25	0.12	55.95	44.05	7.14	5.62
30	0.27	85.54	14.46	24.57	4.15
35	0.39	100.00	-	41.49	0

*P1= peak at retention time 24.18 min, P2= peak at retention time 27.18 min and P3= peak at retention time 26.12 min.

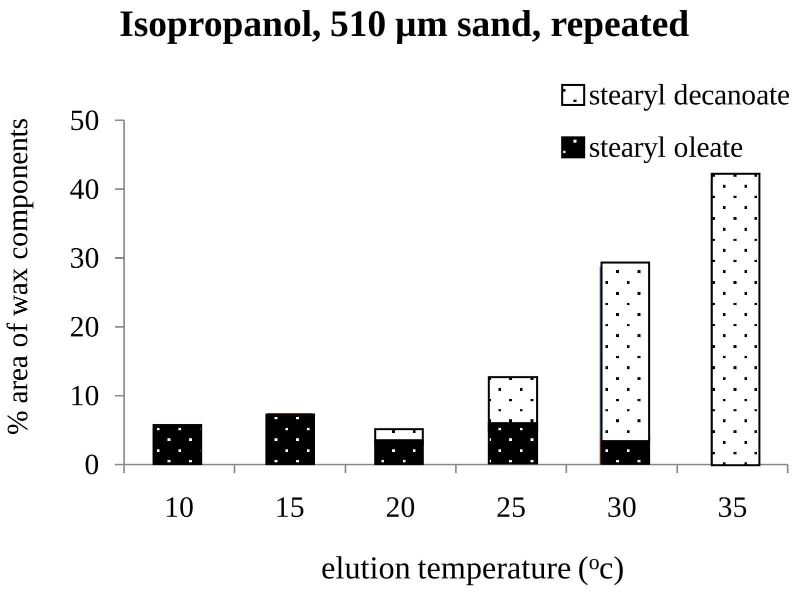

Figure 4.9 The plot of % area of wax components with elution temperature by TREF, isopropanol as solvent, 510 µm sand as an inert support.

The results indicate that the two components were separated as pure stearyl oleate at 10 and 15 ^{o}C and as pure stearyl decanoate at 35 ^{o}C. The fractions at 20, 25 and 30 ^{o}C were mainly stearyl decanoate with a minor amount of stearyl oleate. This suggests that the two components can be separated by repeating the same procedure.

4.7.1.3 Mixed solvent (hexane: isopropanol) as solvent, 510 µm sand support

The separation of the wax components by TREF using a mixture of hexane and isopropanol (1:1 ratio) as the solvent with 510 µm sand support was performed. The compositions of each fraction are summarized in Table 4.8 and the plot of % area of wax components for each fraction at each temperature is as shown in Figure 4.10

Table 4.8 The distribution of wax in each fraction and GC results

Elution temperature (^{o}C)	Weight (g)	% area P1	% area P2	% area P3	% wt. of ester		
					SD	SO	SP
10	2.13	43.51	56.49	-	21.55	27.98	0
20	0.62	55.83	44.17	-	8.05	6.37	0
30	0.84	-	-	100	0	0	19.53
40	0.46	-	-	100	0	0	10.70
50	0.25	-	-	100	0	0	5.81

*P1= peak at retention time 24.18 min, P2= peak at retention time 27.18 min and P3= peak at retention time 26.12 min.

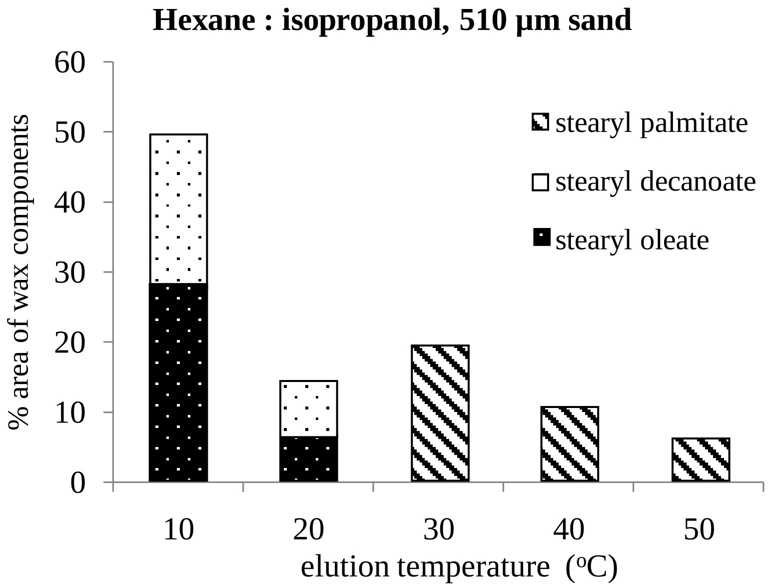

Figure 4.10 The plot of % area of wax components with elution temperature by TREF, mixed solvent (hexane: isopropanol) as solvent, 510 µm sand as an inert support.

As shown in Figure 4.10, The GC results of fractions eluted at 10 and 20 °C were mixtures of stearyl decanoate and stearyl oleate. The fractions eluted at 30 °C and above were pure stearyl palmitate. This results show these conditions can separate pure stearyl palmitate but cannot separate stearyl decanoate and stearyl oleate.

4.7.2 Effect of the support size

4.7.2.1 Isopropanol as solvent, 710 µm sand support

Table 4.9 The distribution of wax in each fraction and GC results

Elution temperature (°C)	Weight (g)	% area P1	% area P2	% area P3	% wt. of ester		
					SD	SO	SP
10	0.86	9.41	90.59	-	1.86	17.87	0
20	0.73	24.33	75.67	-	4.07	12.67	0
30	1.14	32.89	49.66	17.45	8.60	12.98	4.56
40	1.07	24.88	-	75.12	6.11	0	18.44
50	0.56	-	-	100	0	0	12.84

*P1= peak at retention time 24.18 min, P2= peak at retention time 27.18 min and P3= peak at retention time 26.12 min.

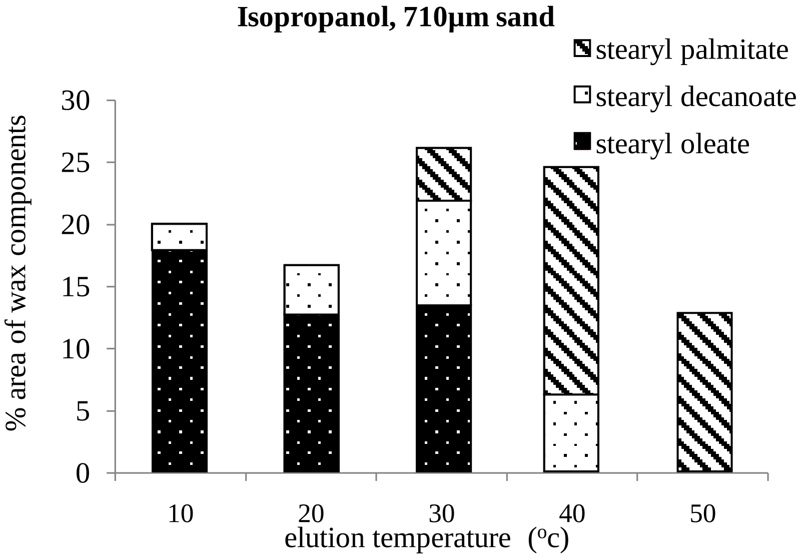

Figure 4.11 The plot of % area of wax components with elution temperature by TREF, isopropanol as the solvent, 710 µm sand as the inert support.

As shown in Figure 4.11, the fraction eluted at 10 ^{o}C was pure stearyl oleate. The fractions at 20 and 30 ^{o}C were mixtures of stearyl oleate and stearyl and the fraction eluted at 50 ^{o}C was pure stearyl palmitate. It can be seen that, the smaller the size of the sand support, the better the separation. This is due to the higher surface of the sand support.

4.7.2.2 Isopropanol as sovent, without inert support

The separation was performed with isopropanol as the solvent but without sand support. The results are summarized in Table 4.10 and Figure 4.12.

Table 4.10 The distribution of wax in each fraction and GC results

Elution temperature (^{o}C)	Weight (g)	% area P1	% area P2	% area P3	% wt. of ester		
					SD	SP	SO
10	0.45	17.539	82.461	-	2.22	10.42	0.00
20	0.78	42.54	54.01	3.456	9.32	11.83	0.76
30	0.66	28.45	60.43	11.12	5.27	11.20	2.06
40	0.72	30.52	-	69.48	6.17	0	14.05
50	0.95	42.57	-	57.43	11.36	0	15.33

*P1= peak at retention time 24.18 min, P2= peak at retention time 27.18 min and P3= peak at retention time 26.12 min.

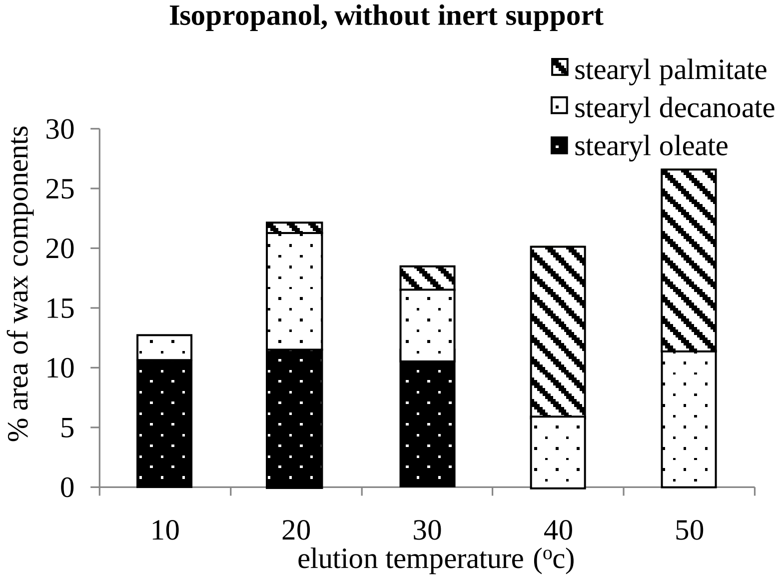

Figure 4.12 The plot of % area of wax components with elution temperature by TREF, isopropanol as the solvent, without inert support.

As shown in Figure 4.12, the fraction separated at 10 ^{o}C was pure stearyl oleate. Other fractions were mixtures of the components. This technique fails to separate the components without sand support. This indicates the importance of the support to the success of this technique. This may also support the principle of the TREF technique.

4.7.3. Fractionation Crystallization using isopropanol as the solvent and without sand support.

The Fractionation Crystallization was perform in order to compare with TREF. Results of the separation are summarized in Table 4.11

Table 4.11 The distribution of wax in each fraction and GC results

Elution temperature (°C)	Weight (g)	% area P1	% area P2	% area P3	% wt. of wax		
					SD	SO	SP
10	0.75	23.55	71.72	4.73	7.99	24.34	1.61
20	0.44	43.46	19.11	37.43	8.65	3.80	7.45
30	0.64	38.54	16.23	45.23	11.16	4.70	13.10
40	0.38	42.85	0	57.15	7.37	0	9.83

*P1= peak at retention time 24.18 min, P2= peak at retention time 27.18 min and P3= peak at retention time 26.12 min.

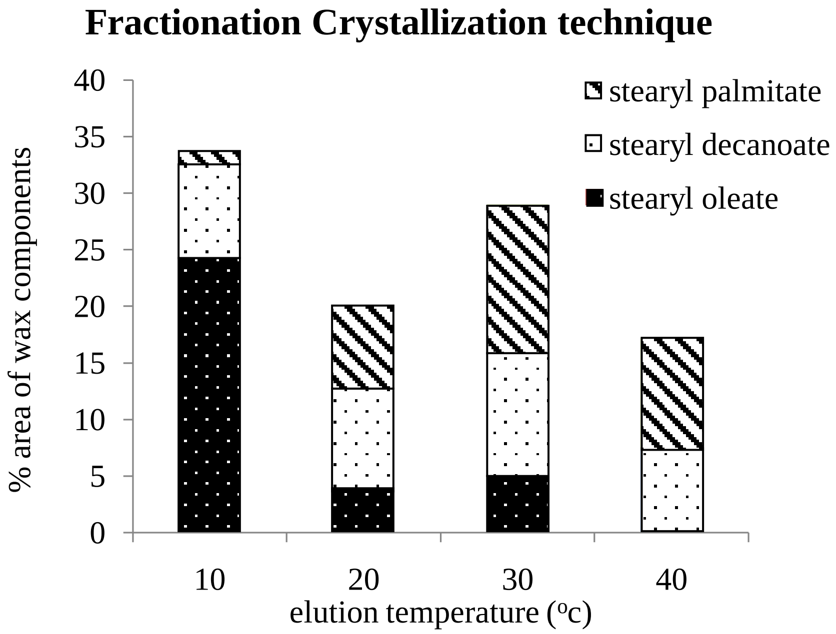

Figure 4.13 The plot of % area of wax components separated by Fractionation Crystallization process.

As shown in Figure 4.13, the components were poorly separated. The fractions separated at each temperature are mixtures of the components. The fraction at 10 ^{o}C was a mixture of stearyl oleate as the major component, stearyl decanoate and stearyl palmitate as the minor components. The fraction at 20 ^{o}C, 30 ^{o}C was a mixture of stearyl oleate, stearyl decanoate and stearyl palmitate. The fraction at 40 ^{o}C was a mixture of stearyl decanoate and stearyl palmitate. This Fractionation Crystallization fails to separate the components of the synthetic wax.

This work suggests that TREF can separate components of the synthetic wax. The success of this technique depends on the crystallinities of the components. The components to be separated must be able to crystallize for the effective separation by this technique. Many factors affecting the separation have been studied. It is found that isopropanol is a better choice than hexane as a solvent as it can give a better separation of the components. Inert support is the crucial factor for this technique. Smaller size of the support gives more effective of the separation. This supports the principle of TREF. The support induces crystallization of the components [5].

The support acts as a nucleating agent in polymer crystallization. It induces crystallization (heterogeneous nucleation) as a result, the crystallization occurs faster. The support produces crystal with smaller size and uniformity and controls the growth rate of nucleus (see Figure 4.14). The small size and uniformity will result in a better layer formation of the crystal on the support. This will increase the efficiency of the separation by TREF technique.

Crystallization without a support (homogeneous nucleation) resulted in crystals with a wide range of size distribution.

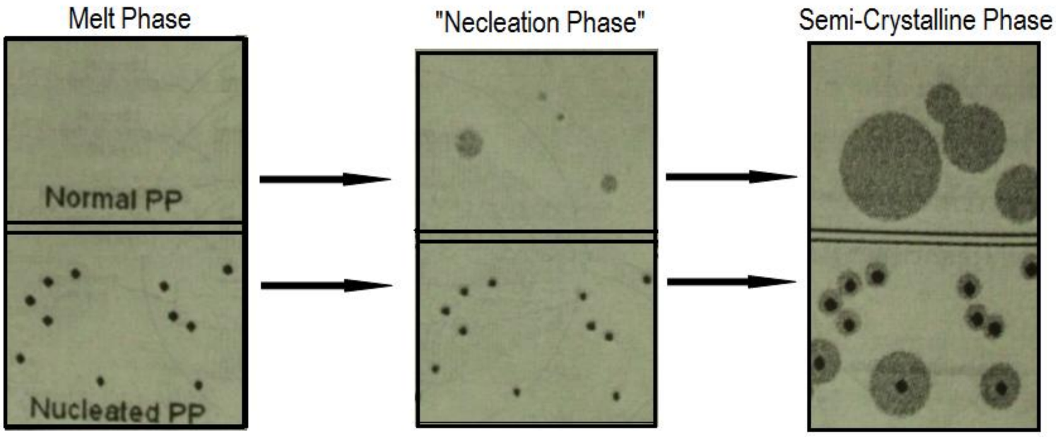

Figure 4.14 Crystallization in polymer; Top: general crystallization in polymer, bottom: crystallization using support.

In this study, the inert support help increase efficiency in the separation of components of waxes due to induce crystallization. Therefore, it is easy to induce adhesion layer and lamination on the support. On the reverse step, the layers of the components can be selectively eluted with increasing temperature. This results in more efficiency in separation of the wax components. The study also demonstrates the higher efficiency of TREF technique compared to the Fractionation Crystallization technique.

Chapter V

Conclusion and suggestion

5.1 Conclusion

Stearyl stearate, stearyl palmitate, stearyl decanoate, stearyl oleate and stearyl linoleate were synthesized from the reactions of stearyl alcohol and the respective fatty acids. FTIR spectra showed the important bands of esters at 1730-1743 cm^{-1} (C=O stretching) and 1158-117 cm^{-1} (C-O stretching). Melting points of these five esters were determined by DSC and found to be 64.7 ^{o}C, 61.5 ^{o}C, 45.8 ^{o}C, 21.8 ^{o}C and 20.5 ^{o}C, respectively. Study of the morphology by optical microscopy found the crystal structures of stearyl stearate, stearyl palmitate, stearyl decanoate to be needle shaped crystals. The solubility of these esters depends on types of solvent and temperature. Melting point, solubility and crystallinity are important factors for the separation by TREF. Depend on these factors, stearyl palmitate, steayl decanoate and stearyl oleate were selected as components for the synthetic ester for the separation by TREF in this work.

In this research, TREF could be applied for the separation of the components of the synthetic wax. The suitable conditions are with 510 µm sand as an inert support and isopropanol as the solvent. Under these conditions, steryl palimtate, steayl decanoate and stearyl oleate were successfully separated at temperature of 10 ^{o}C, 30 ^{o}C and 50 ^{o}C, respectively. Size of the inert support is also an important factor for the success of the separation. Smaller inert support was found to be more effective than larger size. Comparison between TREF technique and

Fractionation Crystallization technique, TREF is a much better technique for the separation of these components of the synthetic ester.

5.2 Suggestion

1. Further studies should be performed in applying TREF for the separation of components of natural waxes.
2. Further studies should be performed on types and size of inert support and solvent to get the optimum conditions to purify waxes.
3. Further work should be done on the apparatus, such as the size and the shape design of the column, in order to improve the separation.

REFERENCES

[1] Ophardt, C.E. Wax [online]. 2010. Available from : http://www.elmhcx9.elmhurst.edu/chm/vchembook/554wax.html [2012,April 11]

[2] Xu, J., Fu, Z., Fan, Z., and Feng, L. Temperature rising elution fractionation of PP/PE alloy and thermal behavior of the fractions. European Polymer Journal 38 (2002) : 1739–1743.

[3] Bennett, H. Commercial Waxes - Natural and Synthetic. Brooklyn, N.Y.; Chemical Pub, 2008.

[4] C.N., A., *et al.* Characterization of oil bodies in jelly fig achenes. Plant Physiology and Biochemistry 46 (2008) : 525-532.

[5] Saravari, A.P.O. polymer additive. Bangkok,Thailand : chulalongkorn university, 2003.

[6] Saengarun, C. Separation of microcrystalline wax, paraffin wax and oil fractions from intermediate waxes by solvent fractionation extraction and fractionation crystallization. Mester's Thesis, Faculty of Science, Chulalongkorn University, 1999.

[7] Schaink, H.M., Van Malssen, K.F., Morgado-Alves, S., Kalnin, D., and Van der Linden, E. Crystal network for edible oil organogels: Possibilities and limitations of the fatty acid and fatty alcohol systems. Food Research International 40 (2007) : 1185–1193.

[8] Pernetti, M., Van Malssen, K.F., Flöter, E., and Bot, A. Structuring of edible oils by alternatives to crystalline fat. Current Opinion in Colloid & Interface Science 12(4-5) (2007) : 221-231.

[9] Müller, A., and Arnal, M. Thermal fractionation of polymers. Progress in Polymer Science 30(5) (2005) : 559-603.

[10] Samoth, N., Tantayanon, S.,Tangpasuthadol, V., and Phalakornkul, K. Polyethylene Fractionation by Modified Temperature Rising Elution Fractionation Technique. Advanced Technologies International Symposium (2005) : 1-3.

[11] Kulinl, L.I., Meijerink, N.L., and Starck, P. Long and short chain branching frequency in low density polyethylene (LDPE). Pure&Appl. Cherm. 60 (1988) : 1403-1415.

[12] Monrabal, B. Microstructure Characterization of Polyolefins. Polymer char (2006) : 35-42.

[13] Gabriela, C., and Lilgeb, D. Comparison of different methods for the investigation of the short-chain branching distribution of LLDPE. Polymer 42 (2000) : 297–303.

[14] Vivillea, P., *et al.* Characterization of the molecular structure of two highly isotactic polypropylenes. Polymer 42 (2000) : 1953–1967.

[15] Soares, J.B.P., and Hamielec, A.E. Temperature rising elution fractionation of linear polyolefins. Polymer International 36 (1994) : 1639-1654.

[16] Lu, H., Qiao J., and Yang, Y. Effect of isotacticity distribution on crystallization kinetics of polypropylene. Polymer International 51 (2002) : 1304-1309.

[17] Zhang, M., Lynch, D.T., and Wanke, S.E. Effect of molecular structure distribution on melting and crystallization behavior of 1-butene/ethylene copolymers. Polymer 42 (2001) : 3067-3075.

[18] Ramachandran, R., *et al.* Branch length distribution in TREF fractionated polyethylene. Polymer 52(12) (2011) : 2661-2666.

[19] De Lucas, A., García, A., Alvarez, A., and Gracia, I. Supercritical extraction of long chain n-alcohols from sugar cane crude wax. The Journal of Supercritical Fluids 41(2) (2007) : 267-271.

[20] Zaky, M.T., Mohamed, N.H., and Farag, A.S. Separation of different paraffin wax grades using two comparative deoiling techniques. Fuel Processing Technology 88(9) (2007) : 913-920.

[21] Athukorala, Y., and Mazza, G. Optimization of Extraction of Wax from Flax Straw by Supercritical Carbon Dioxide. Separation Science and Technology 46(2) (2010) : 247-253.

[22] Khositcharoenkul, C. Fractional Crystallization and Characterization of Rice bran wax. Mester's Thesis, Faculty of Science, Chulalongkorn University, 2005.

[23] Soomro, R.K., and Sherazi, S.T.H. Spectroscopic and chromatographic evaluation of the wax ester fraction of Adenanthera pavonina oil. Industrial Crops and Products 36(1) (2012) : 294-298.

[24] François, *et al*. Structuring of Edible Oils by Long-Chain FA, Fatty Alcohols, and Their Mixtures. JAOCS 81 (2004) : 1–6.

[25] Martini, S. and Añón, M.C. Crystallization of Sunflower Oil Waxes. JAOCS 80 (2003) : 525–532.

[26] Aranda, D.A.G., *et al*. Acid-Catalyzed Homogeneous Esterification Reaction for Biodiesel Production from Palm Fatty Acids. Catalysis Letters 122(1-2) (2007) : 20-25.

[27] Dassanayake, L.S.K., *et al.* Physical Properties of Rice Bran Wax in Bulk and Organogels. J. Am. Oil Chem. Soc. 86 (2009): 1163–1173.

[28] Simma, K., Rempel, G.L., and Prasassarakich, P. Improving thermal and ozone stability of skim natural rubber by diimide reduction. Polymer Degradation and Stability 94(11) (2009): 1914-1923.

APPENDICES

Appendix A

Appendix A DSC thermogram of synthetic ester and FTIR

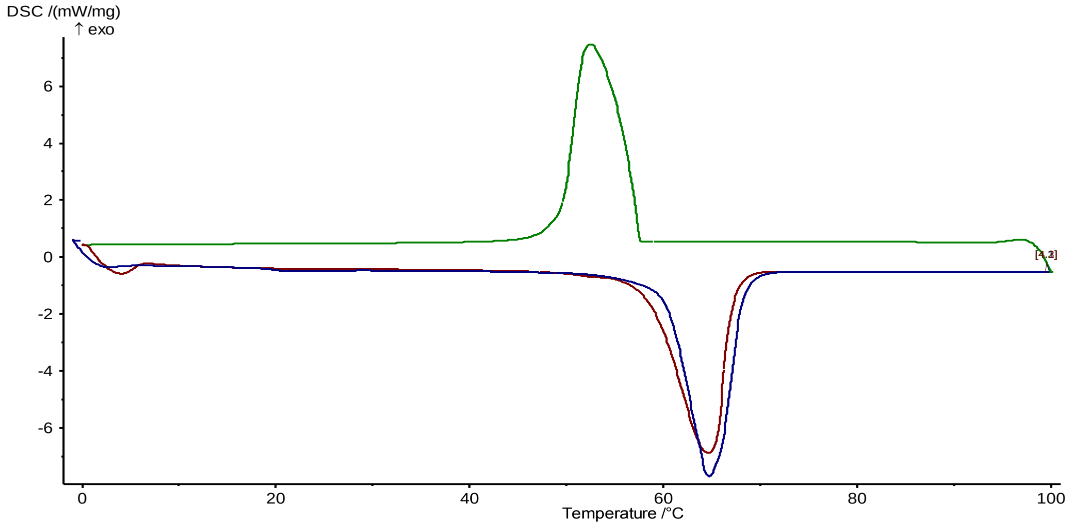

Figure 1A DSC thermogram of of stearyl stearate.

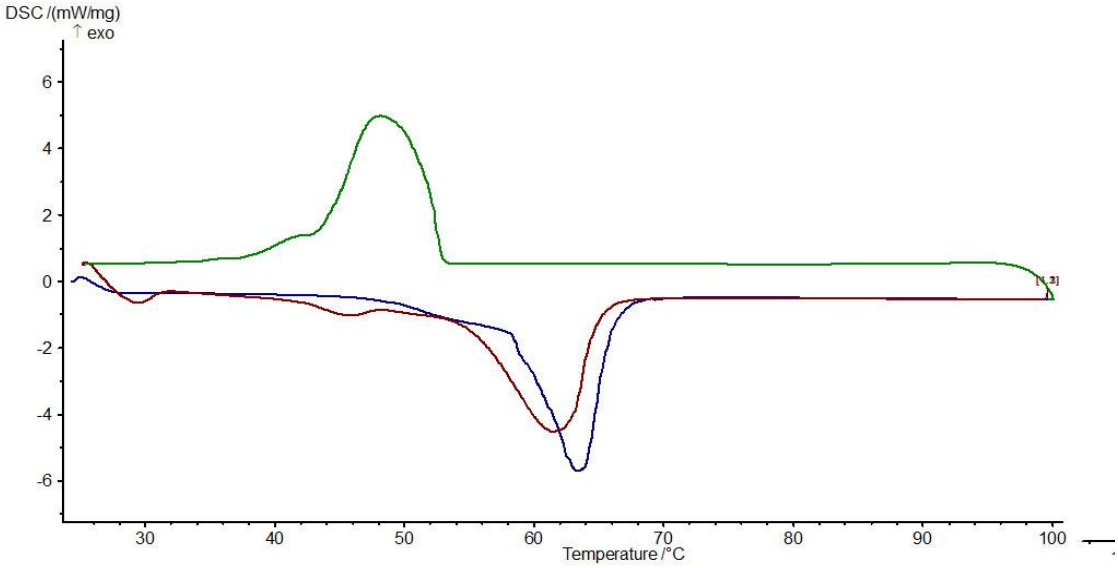

Figure 1B DSC thermogram of stearyl palmitate.

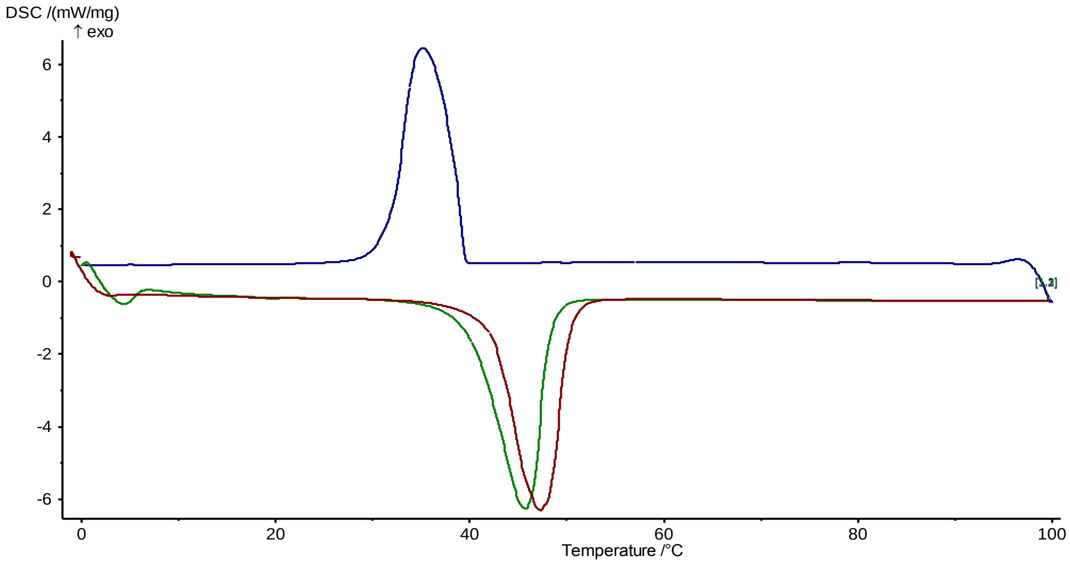

Figure 1C DSC thermogram of stearyl decanoate.

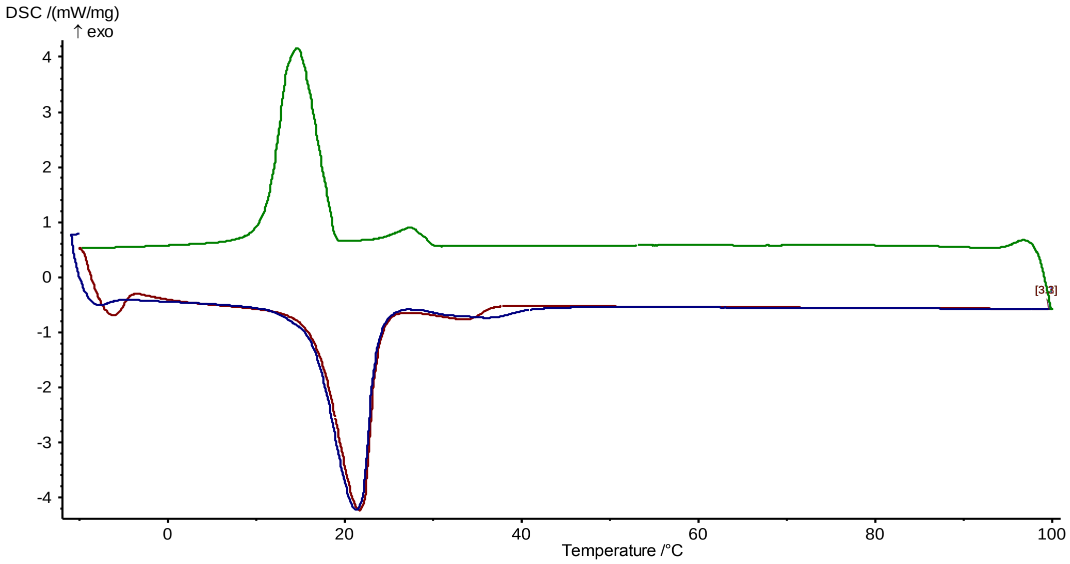

Figure 1D DSC thermogram of stearyl oleate.

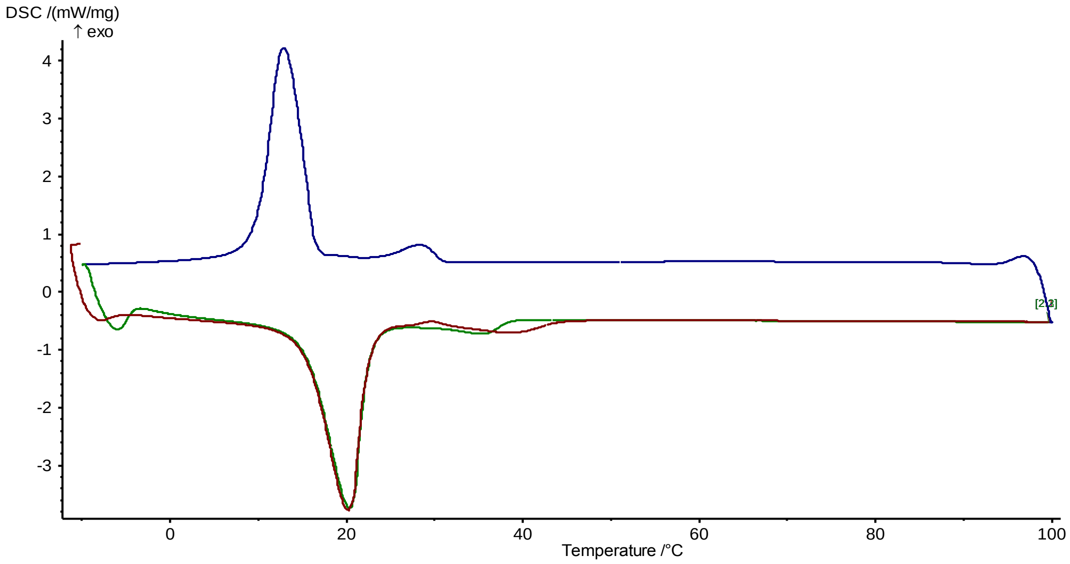

Figure 1F DSC thermogram of stearyl linoleate.

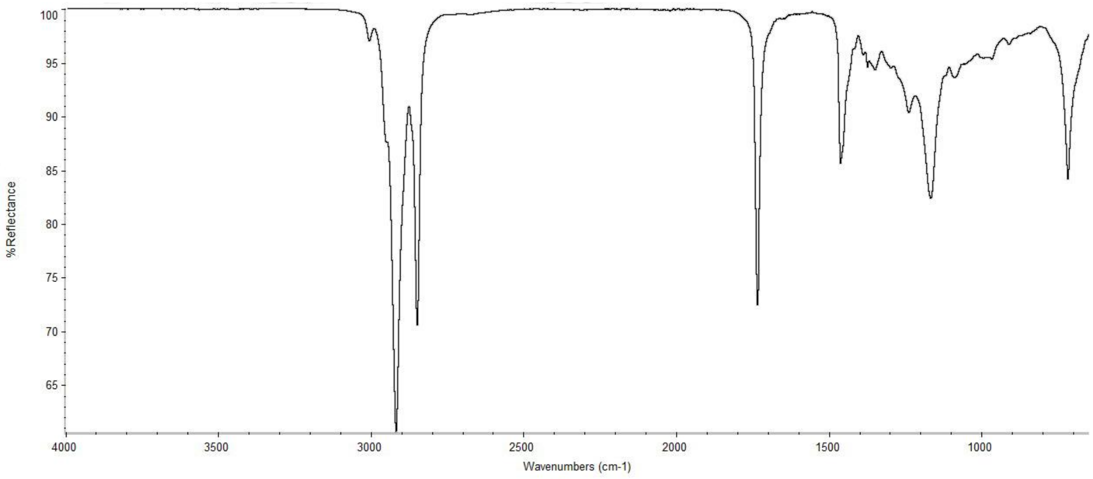

Figure 2A FTIR spectrum of stearyl linoleate.

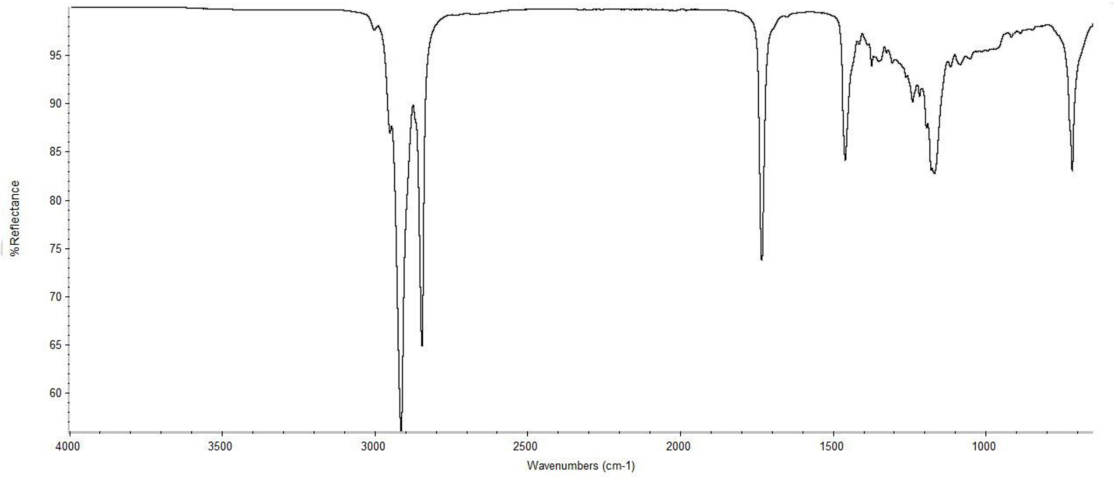

Figure 2B FTIR spectrum of stearyl oleate.

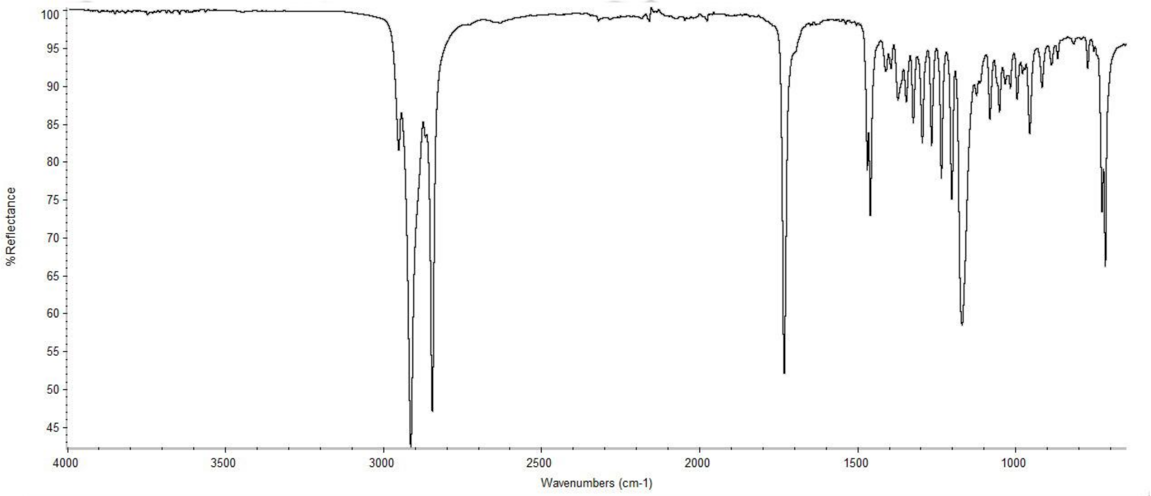

Figure 2C FTIR spectrum of stearyl decanoate.

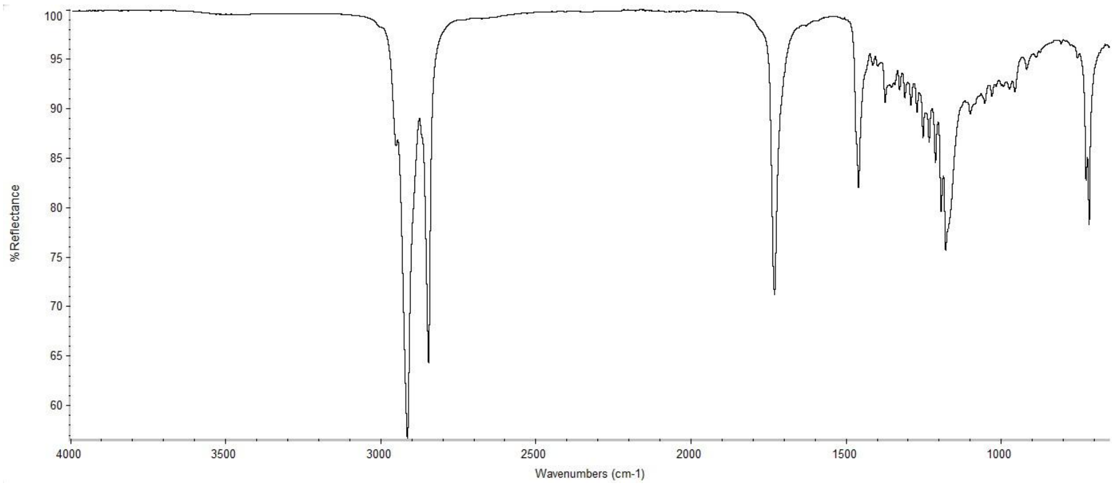

Figure 2D FTIR spectrum of stearyl palmitate.

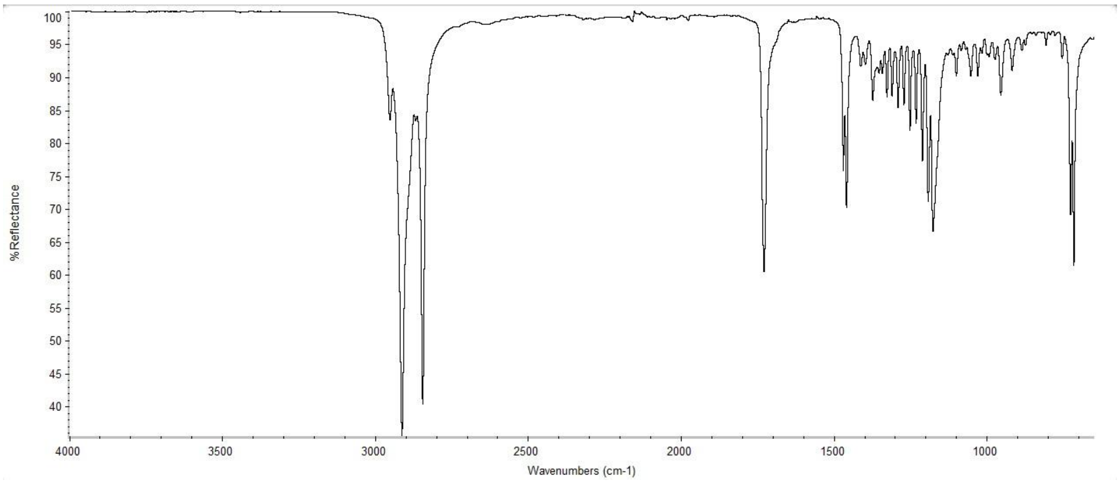

Figure 2E FTIR spectrum of stearyl stearate.

Appendix B

Appendix B: GC spectrum of the wax fraction eluted by TREF and crystalline technique.

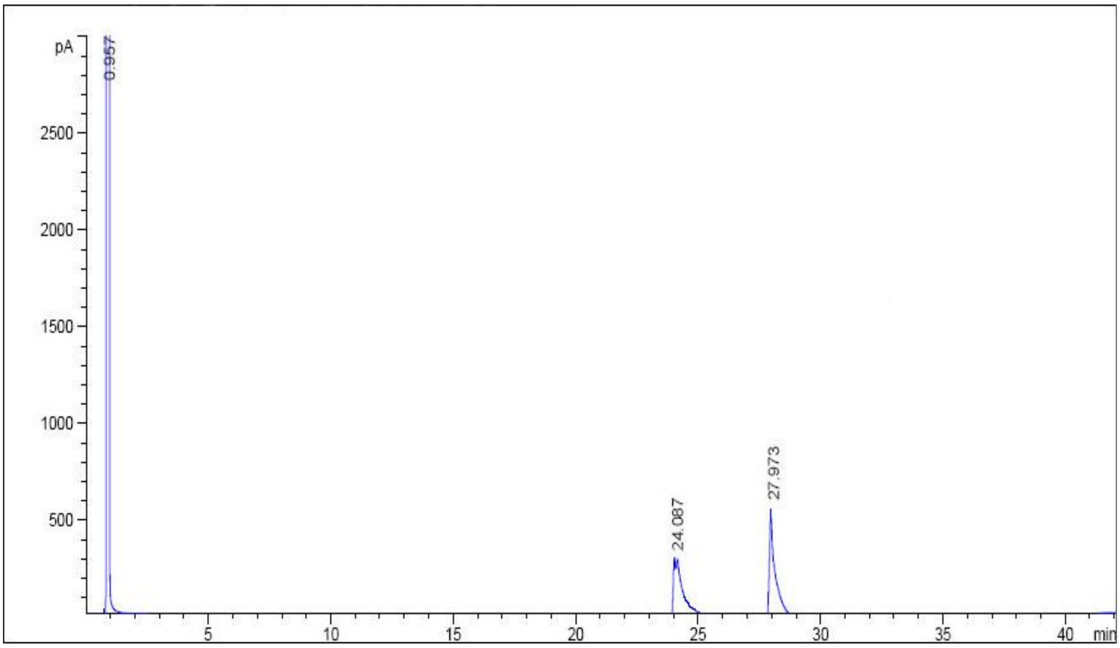

Figure 1A GC spectrum of the wax fraction eluted by using *n*-hexane as the solvent at 10 °C with 510 µm sand support.

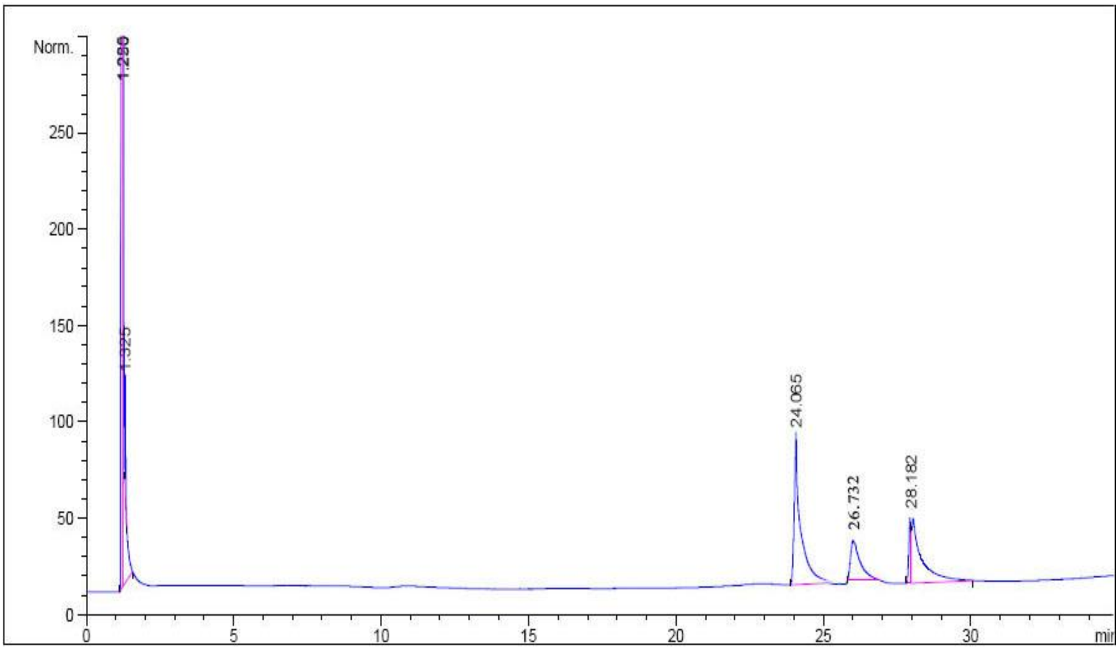

Figure 1B GC spectrum of the wax fraction eluted by using *n*-hexane as the solvent at 20 °C with 510 µm sand support.

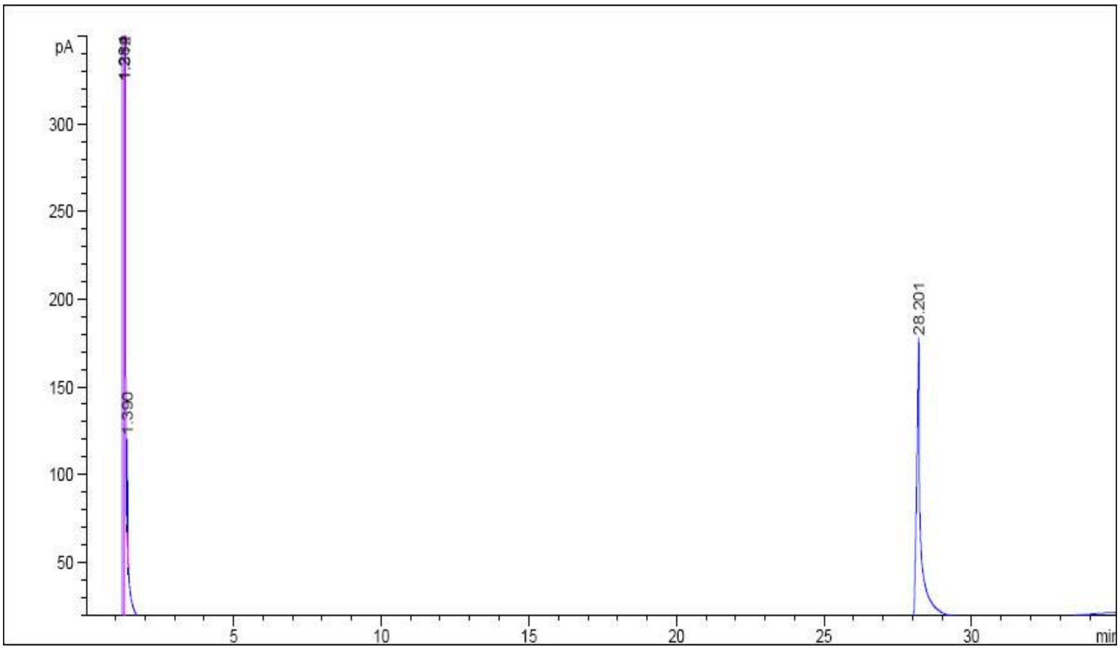

Figure 1C GC spectrum of the wax fraction eluted by using *n*-hexane as the solvent at 30 °C with 510 µm sand support.

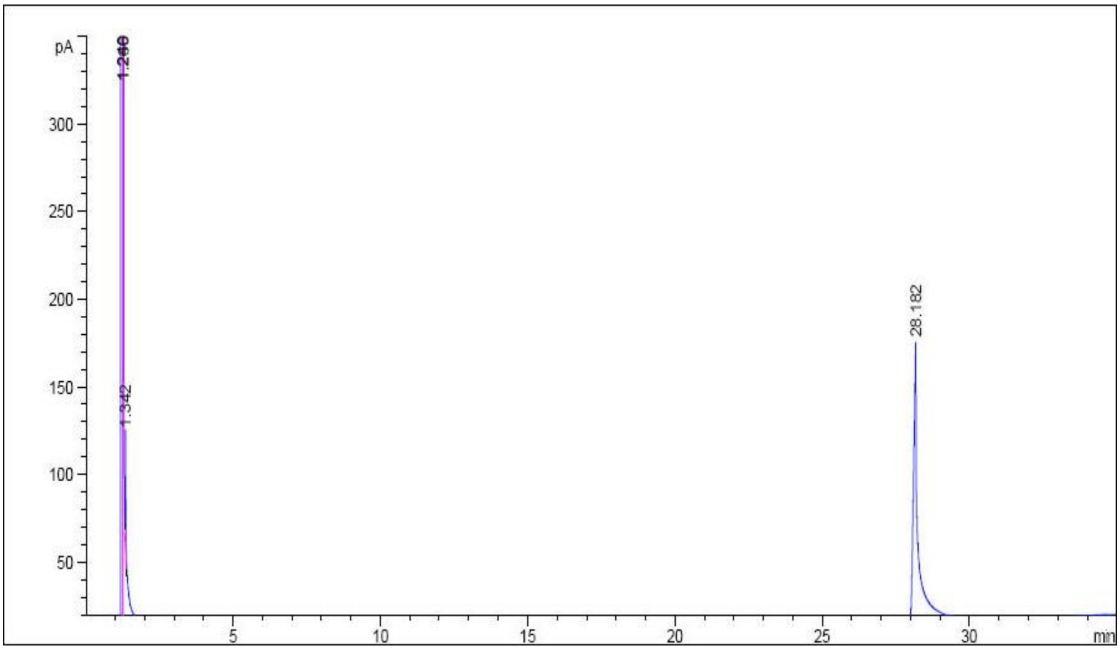

Figure 1D GC spectrum of the wax fraction eluted by using *n*-hexane as the solvent at 40 °C with 510 µm sand support.

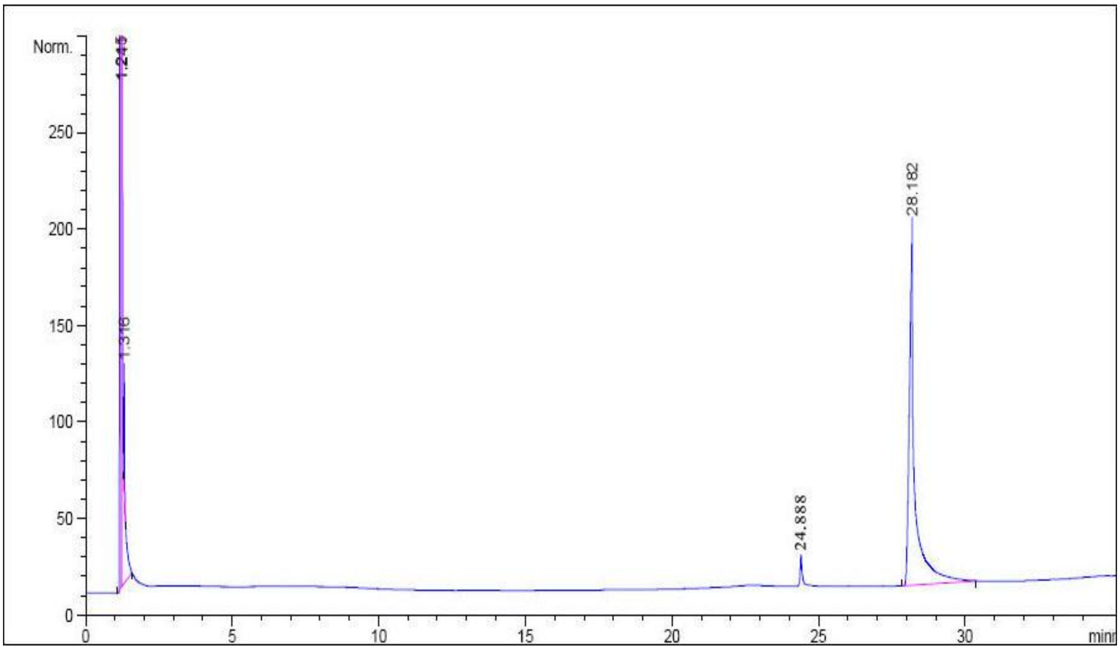

Figure 1.2A GC spectrum of the wax fraction eluted by using isopropanol as the solvent at 10 °C with 510 µm sand support.

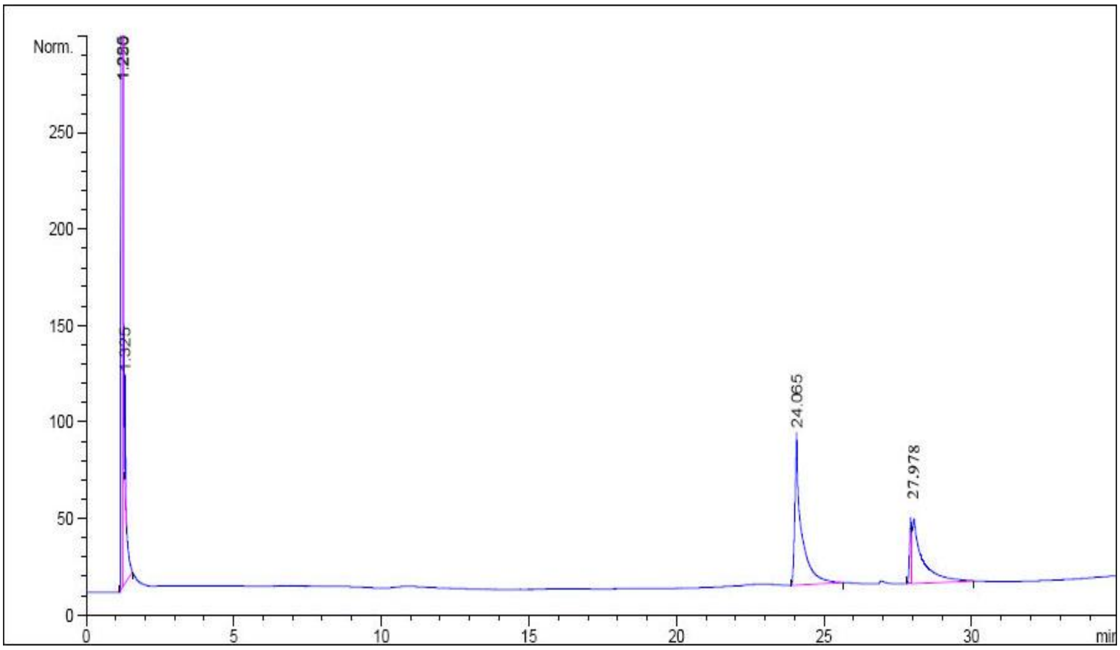

Figure 1.2B GC spectrum of the wax fraction eluted by using isopropanol as the solvent at 20 °C with 510 µm sand support.

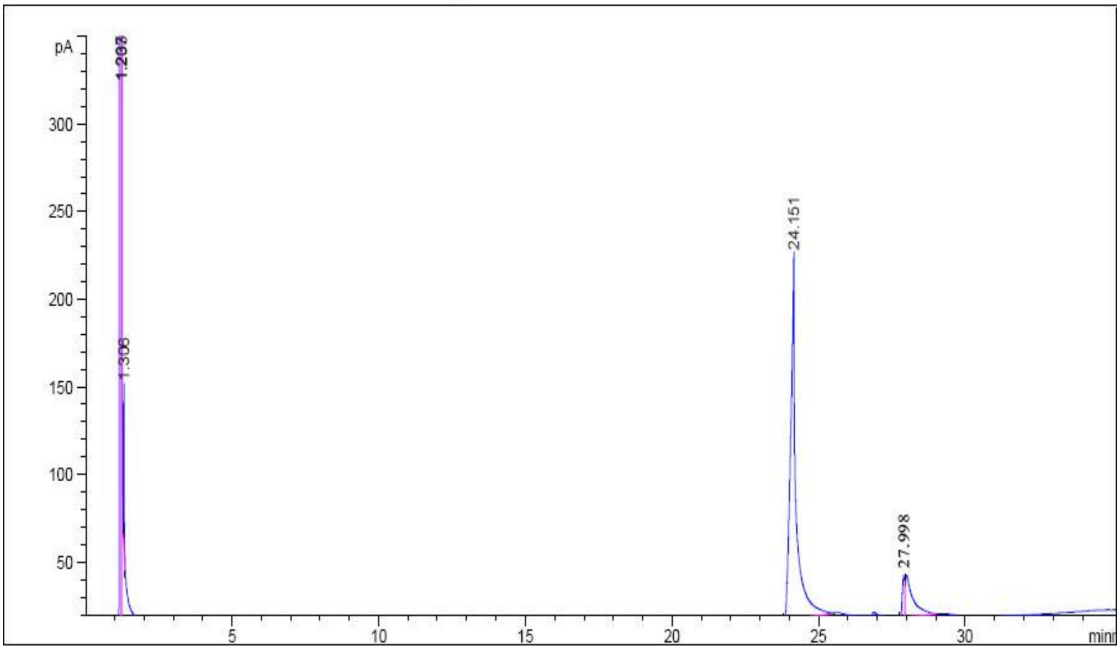

Figure 1.2C GC spectrum of the wax fraction eluted by using isopropanol as the solvent at 30 ^{o}C with 510 μm sand support.

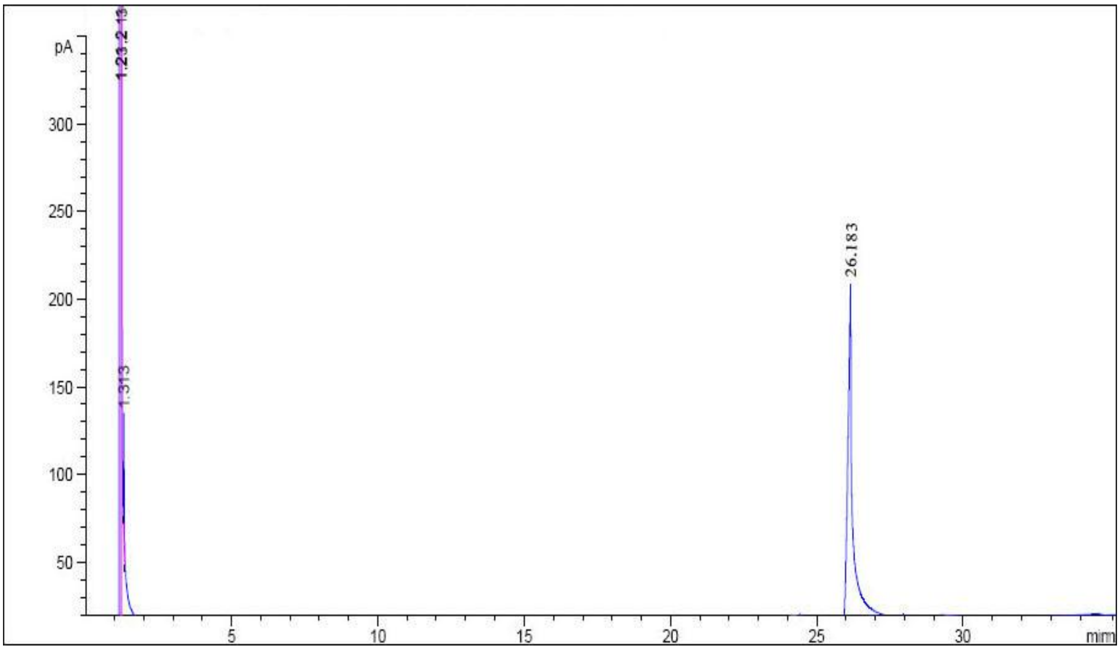

Figure 1.2D GC spectrum of the wax fraction eluted by using isopropanol as the solvent at 40 ^{o}C with 510 μm sand support.

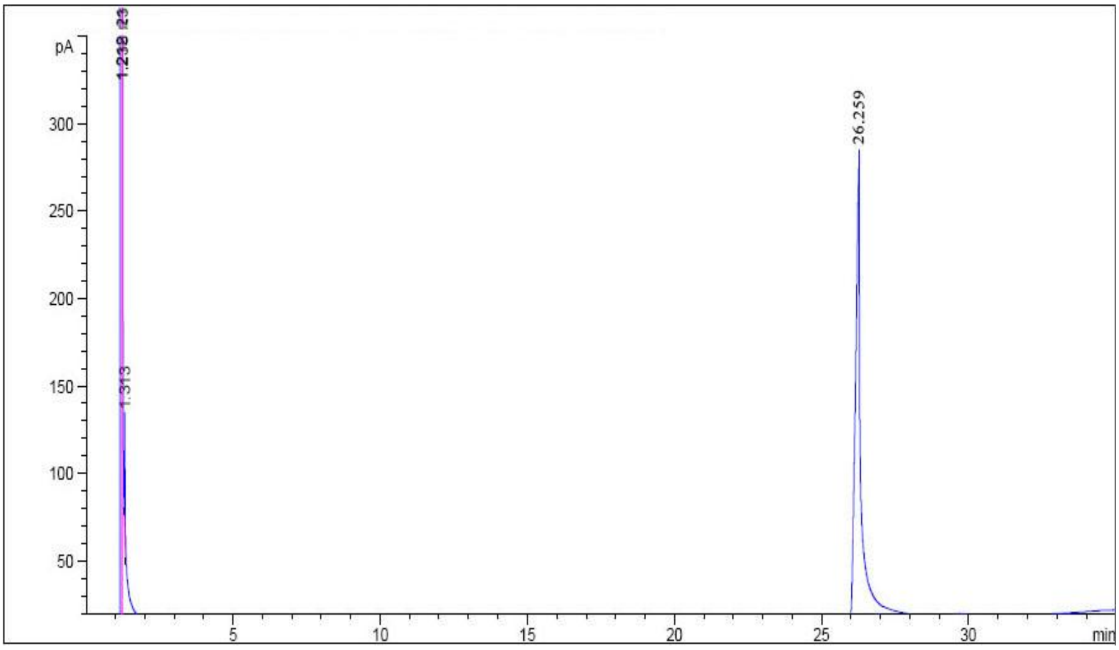

Figure 1.2E GC spectrum of the wax fraction eluted by using isopropanol as the solvent at 50 ^{o}C with 510 µm sand support.

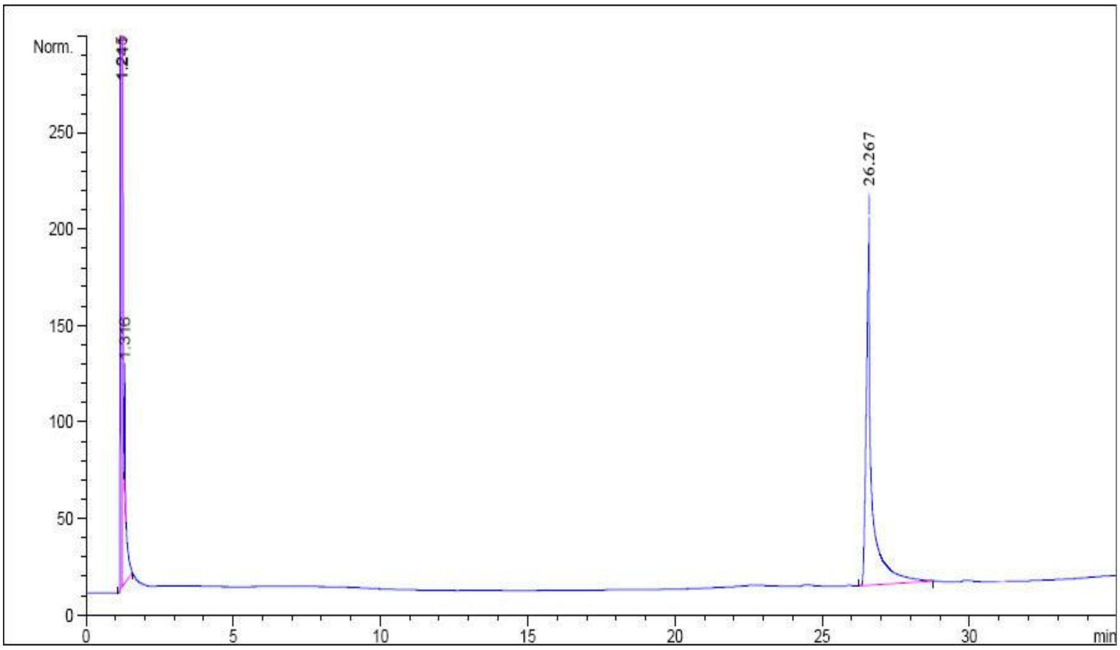

Figure 1.2F GC spectrum of the wax fraction eluted by using isopropanol as the solvent at 60 ^{o}C with 510 µm sand support.

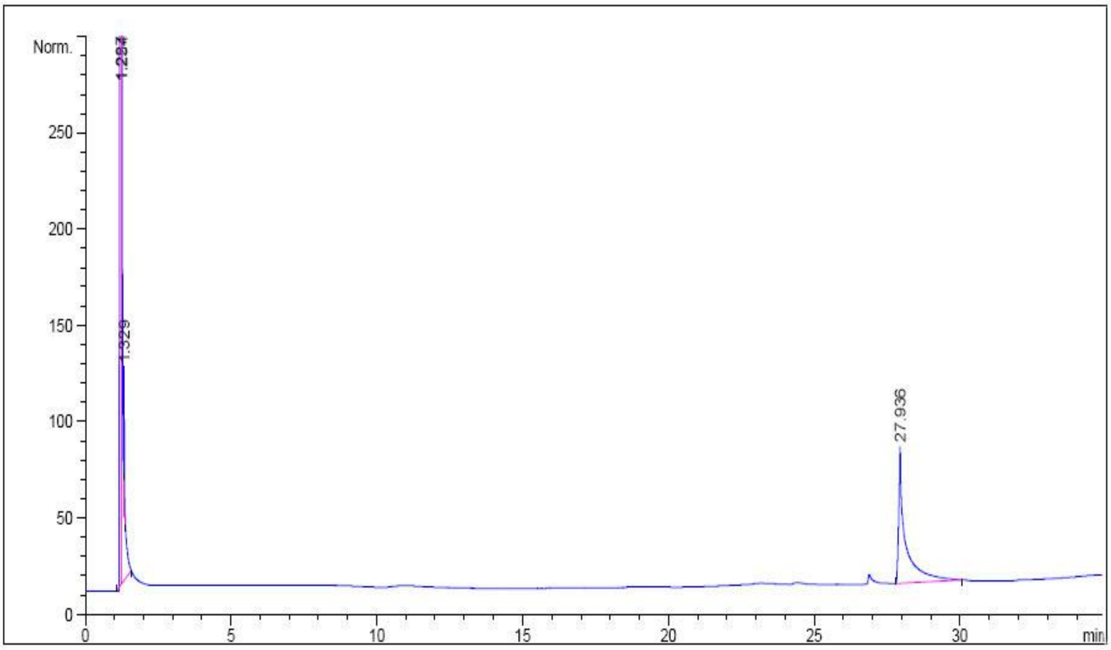

Figure 1.2.1A GC spectrum of the wax fraction eluted by using isopropanol as the solvent at 10 ^{o}C with 510 µm sand support, repeated.

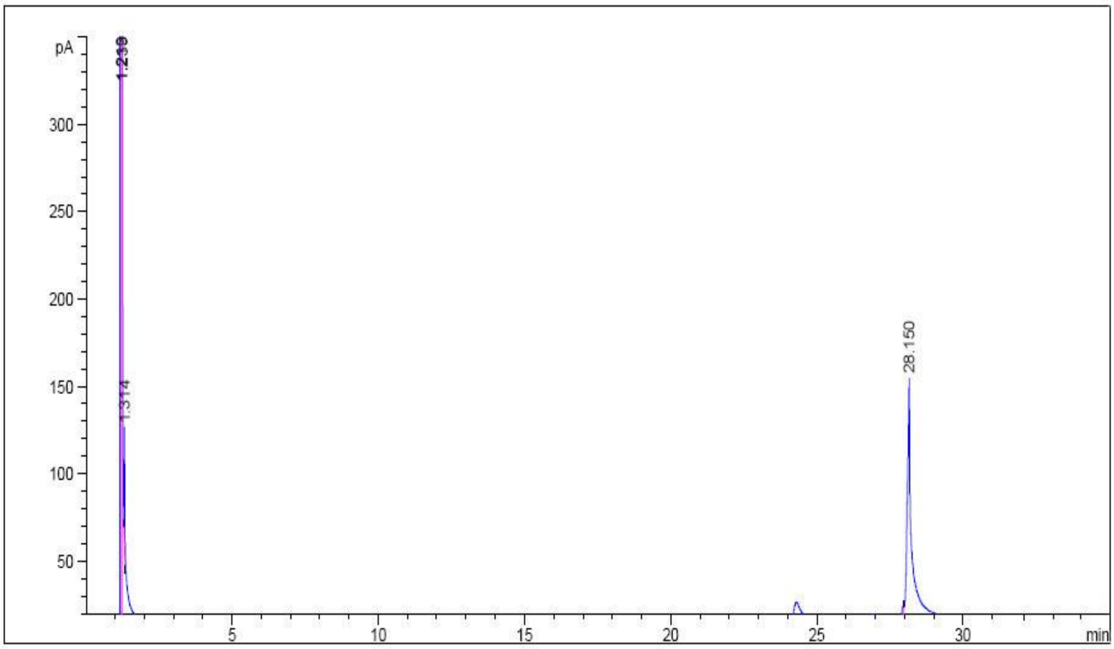

Figure 1.2.1B GC spectrum of the wax fraction eluted by using isopropanol as the solvent at 15 ^{o}C with 510 µm sand support, repeated.

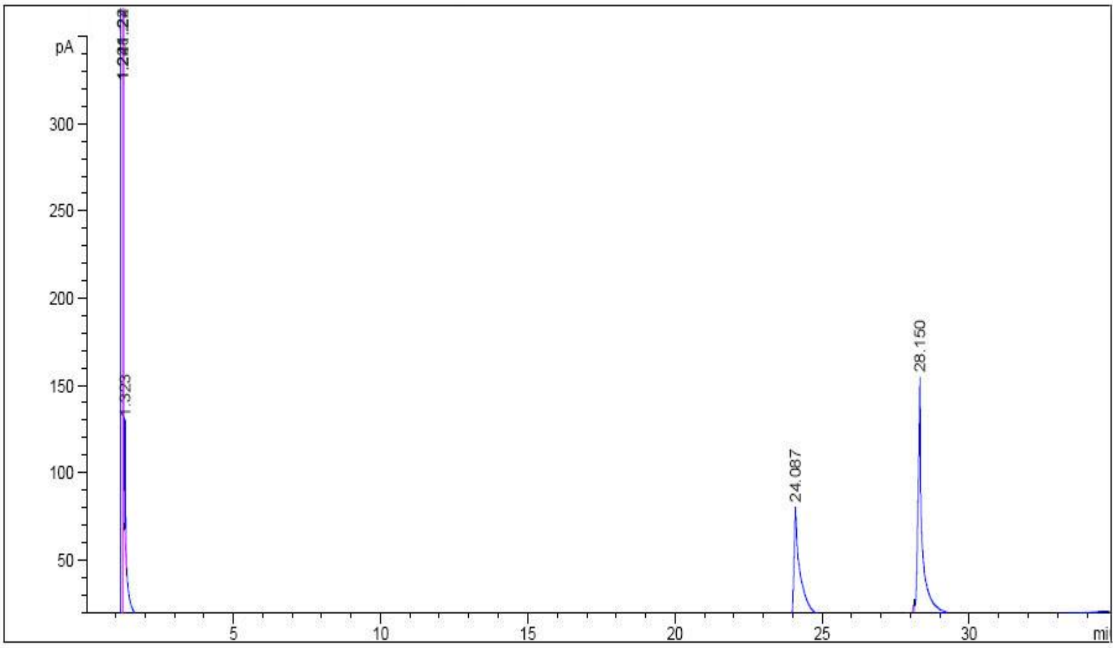

Figure 1.2.1C GC spectrum of the wax fraction eluted by using isopropanol as the solvent at 20 °C with 510 µm sand support, repeated.

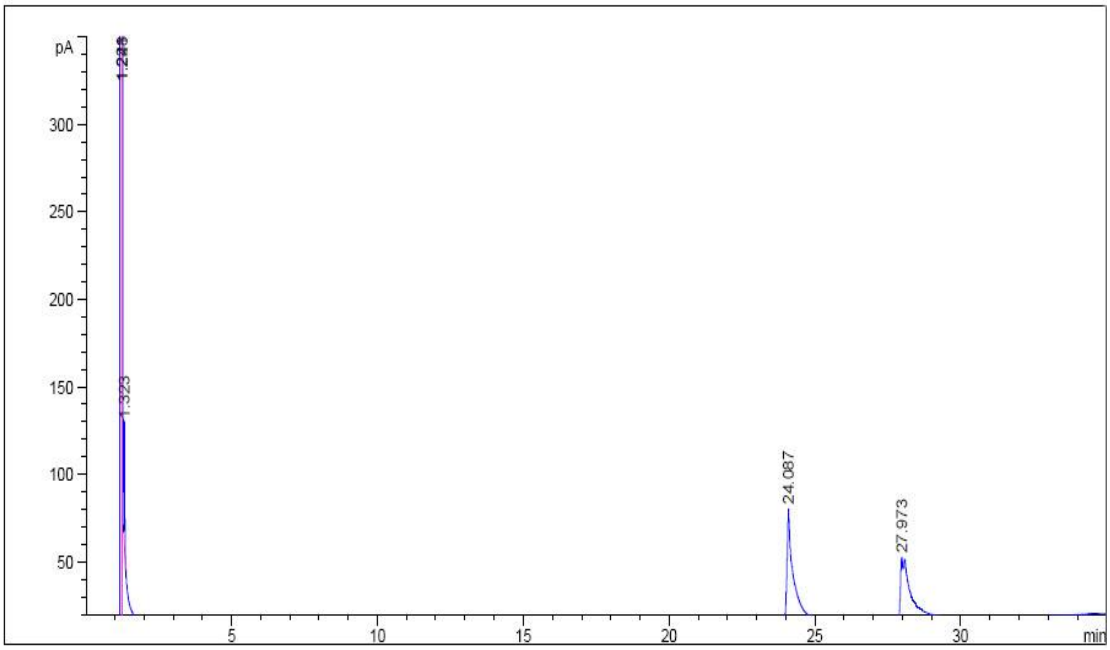

Figure 1.2.1D GC spectrum of the wax fraction eluted by using isopropanol as the solvent at 25 °C with 510 µm sand support, repeated.

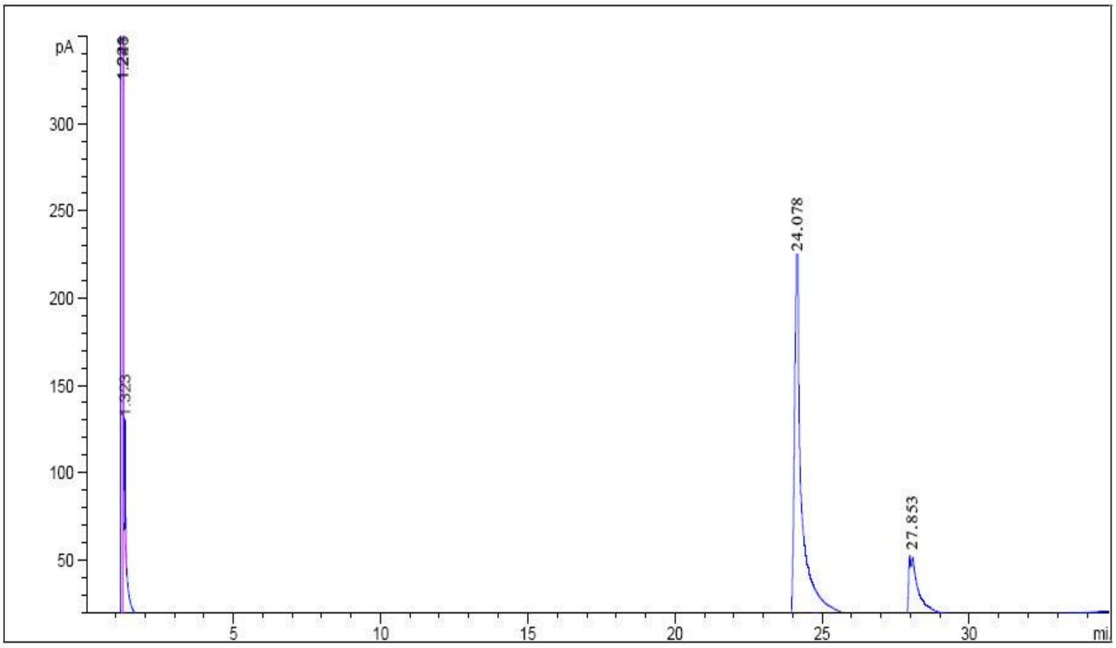

Figure 1.2.1E GC spectrum of the wax fraction eluted by using isopropanol as the solvent at 30 °C with 510 µm sand support, repeated.

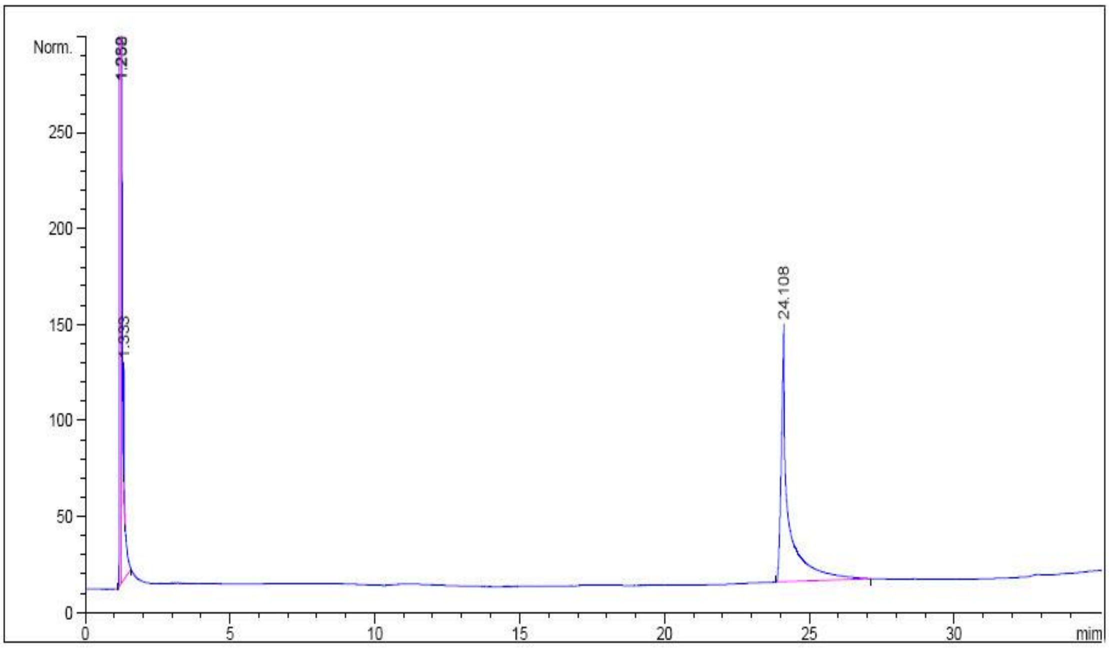

Figure 1.2.1F GC spectrum of the wax fraction eluted by using isopropanol as the solvent at 35 °C with 510 µm sand support, repeated.

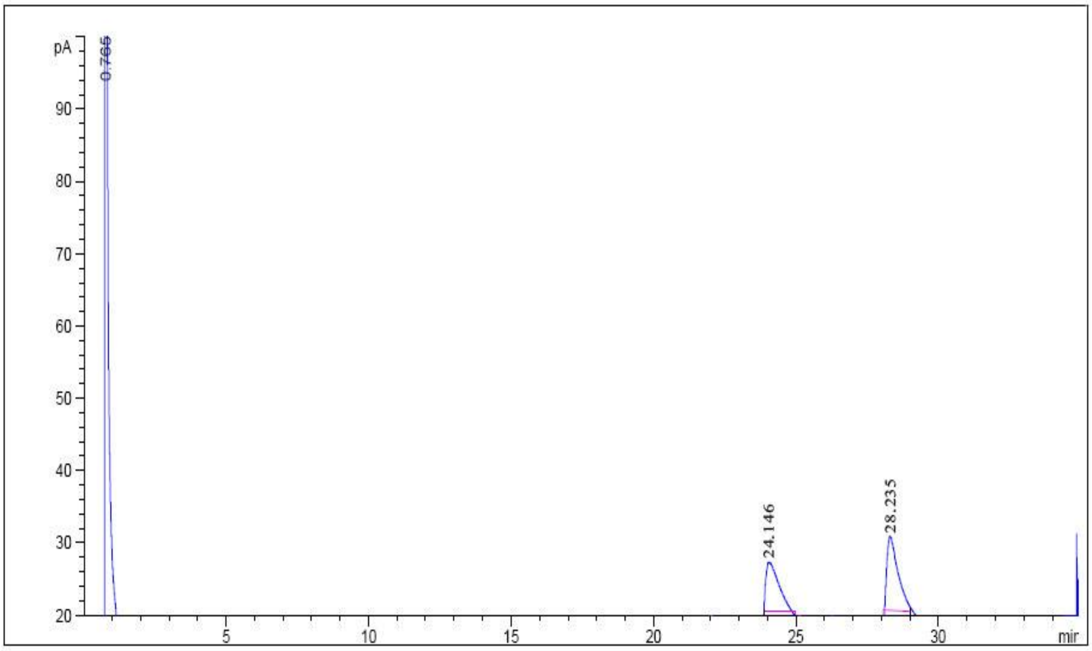

Figure 1.3A GC spectrum of the wax fraction eluted by using mixed solvent at 10 ^{o}C with 510 µm sand support.

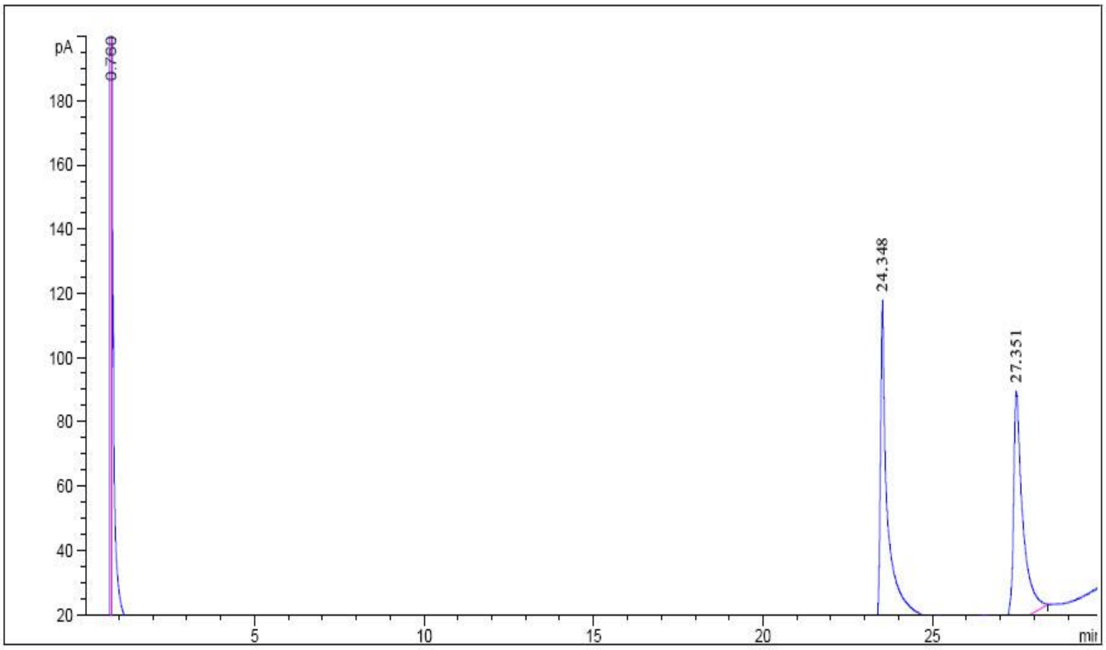

Figure 1.3B GC spectrum of the wax fraction eluted by using mixed solvent at 20 ^{o}C with 510 µm sand support.

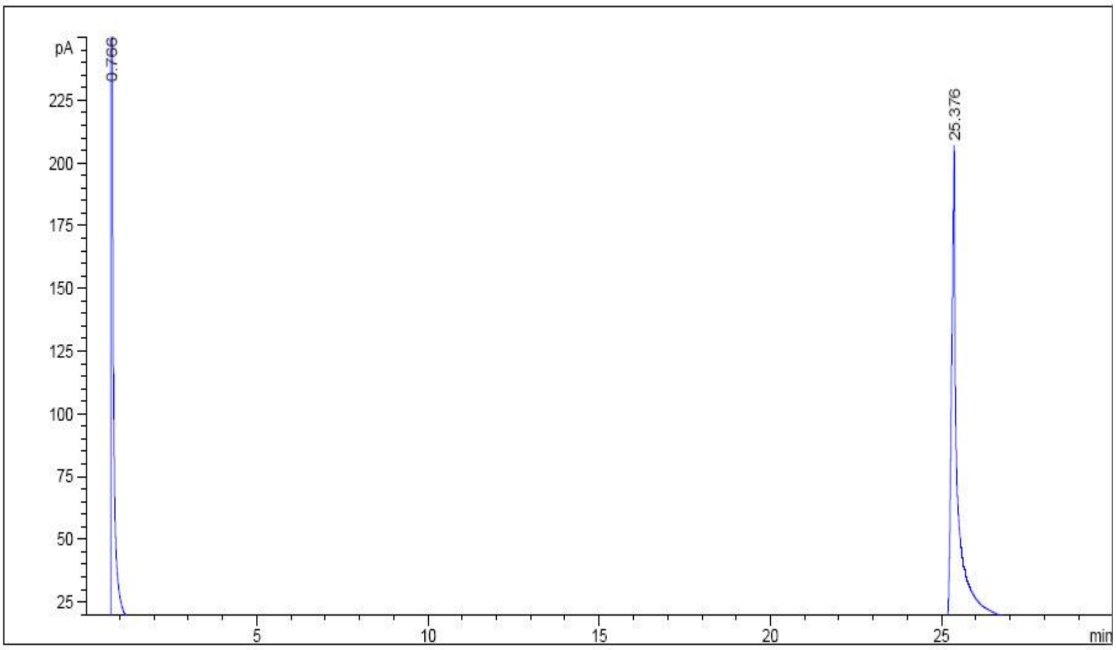

Figure 1.3C GC spectrum of the wax fraction eluted by using mixed solvent at 30 °C with 510 µm sand support.

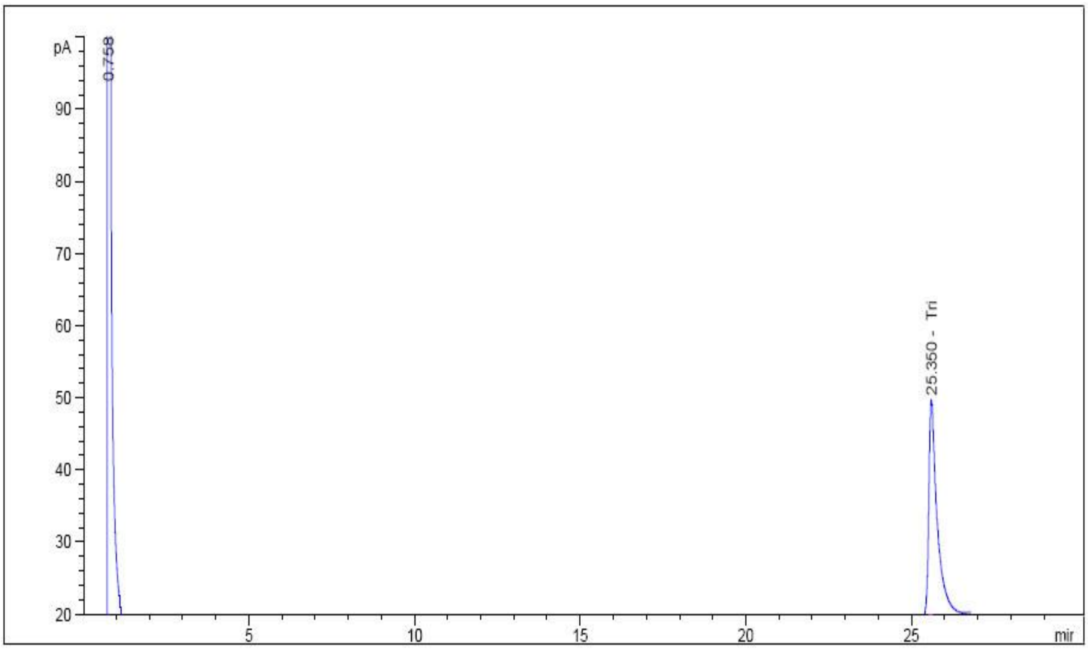

Figure 1.3D GC spectrum of the wax fraction eluted by using mixed solvent at 40 °C with 510 µm sand support.

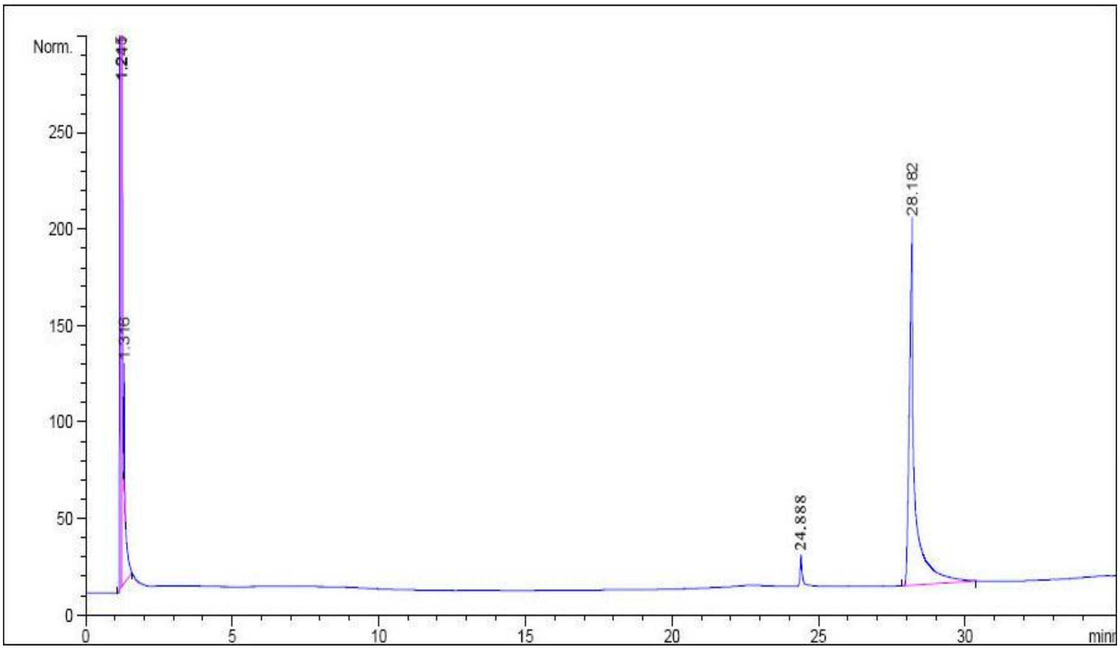

Figure 1.4A GC spectrum of the wax fraction eluted by using 710 µm sand support and isopropanol as the solvent at 10 ^{o}C.

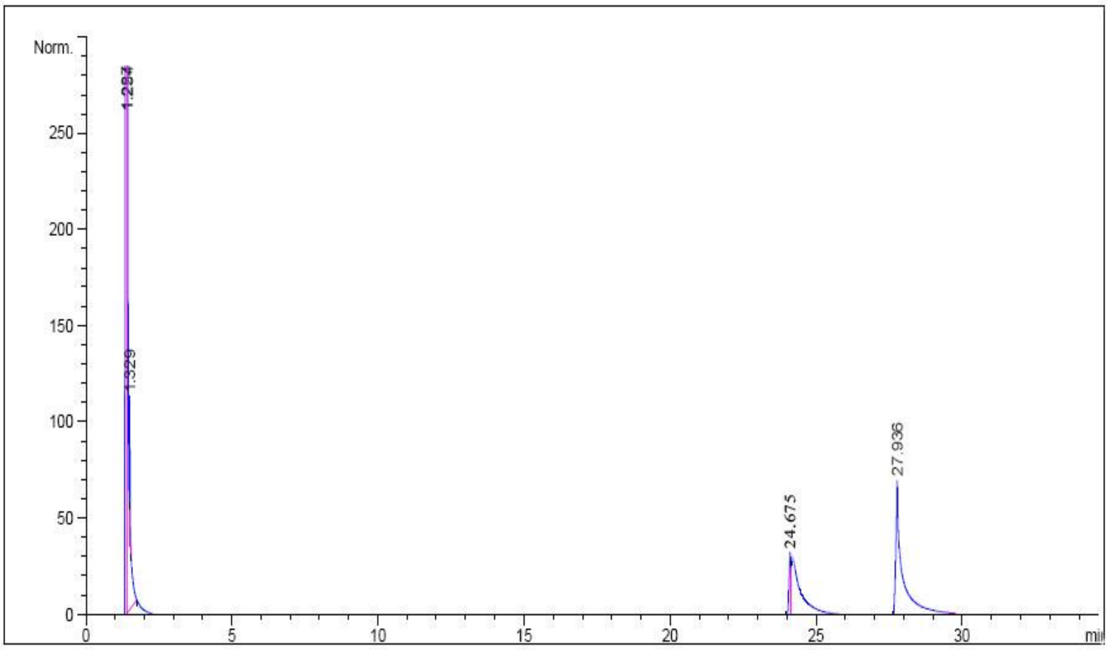

Figure 1.4B GC spectrum of the wax fraction eluted by using 710 µm sand support and isopropanol as the solvent at 20 ^{o}C.

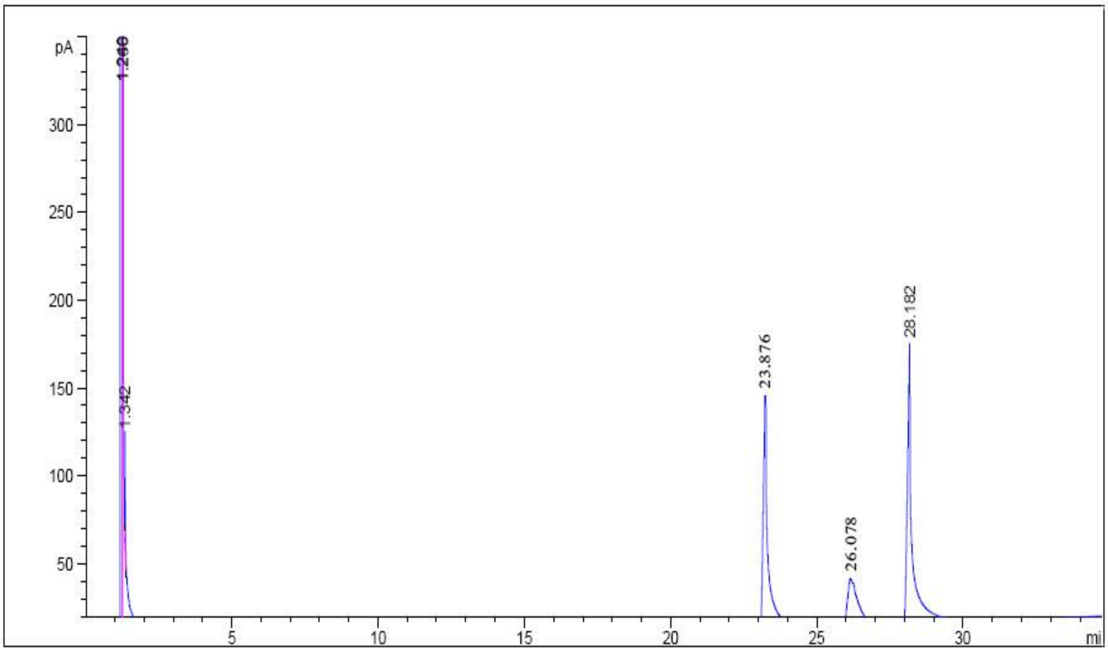

Figure 1.4C GC spectrum of the wax fraction eluted by using 710 µm sand support and isopropanol as the solvent at 30 °C.

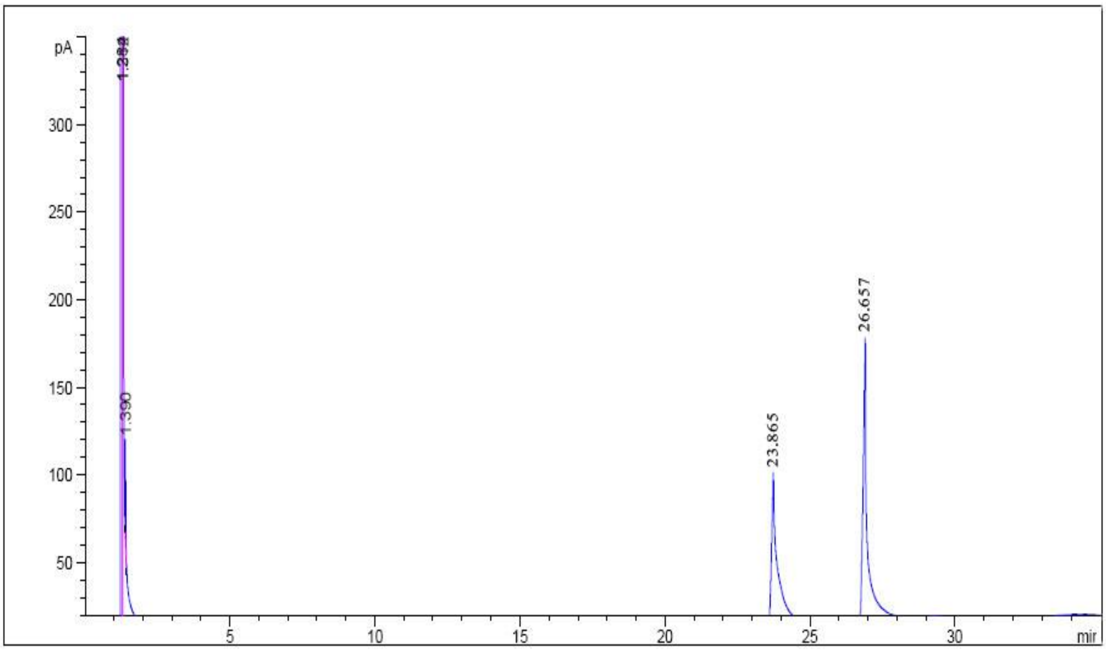

Figure 1.4D GC spectrum of the wax fraction eluted by using 710 µm sand support and isopropanol as the solvent at 40 °C.

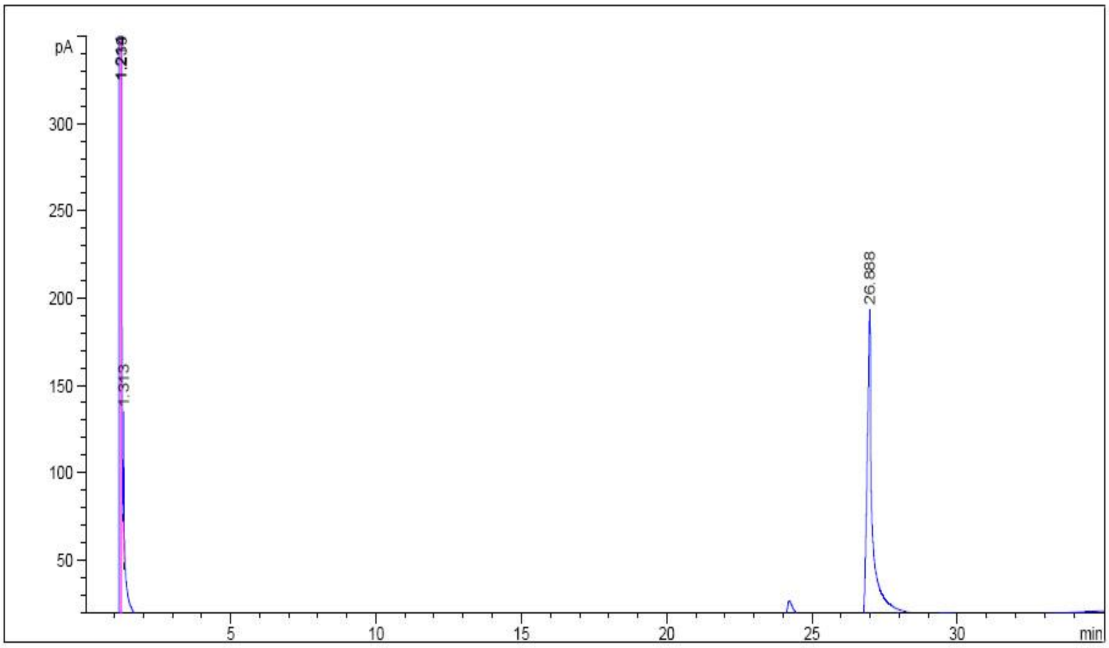

Figure 1.4E GC spectrum of the wax fraction eluted by using 710 µm sand support and isopropanol as the solvent at 50 ^{o}C.

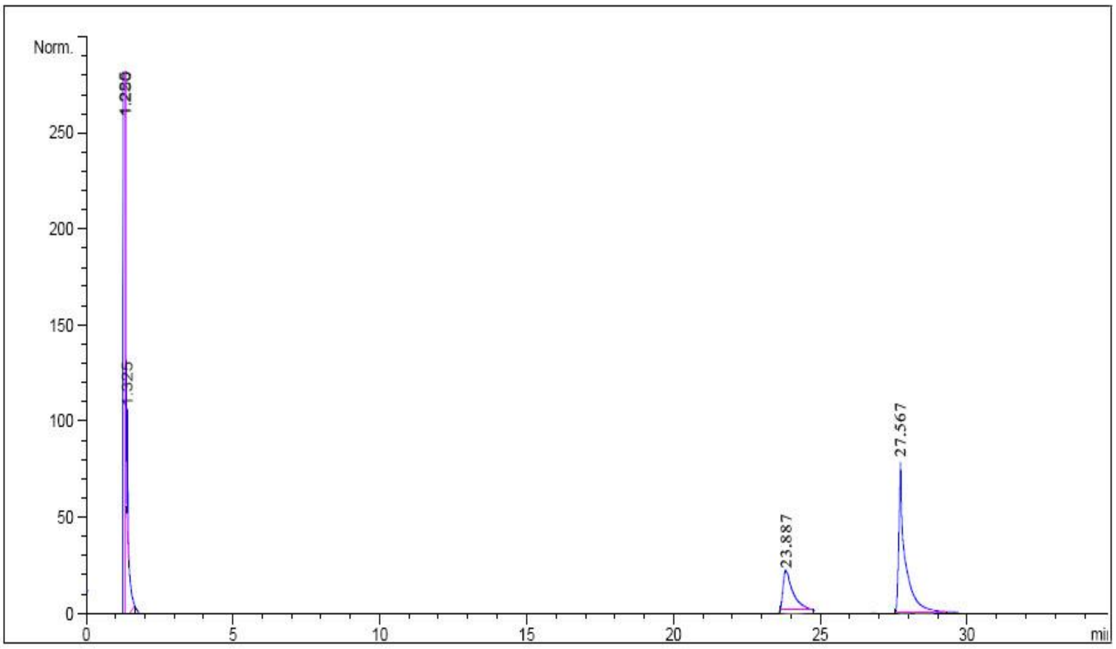

Figure 1.5A GC spectrum of the wax fraction eluted by using isopropanol as the solvent without an inert support at 10 ^{o}C.

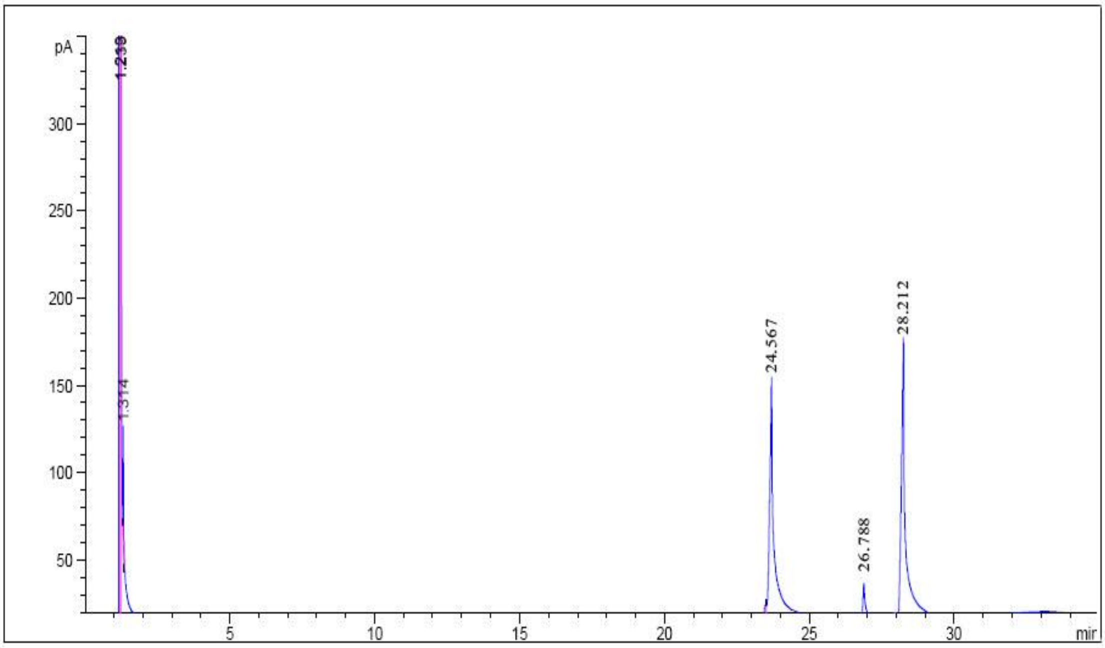

Figure 1.5B GC spectrum of the wax fraction eluted by using isopropanol as the solvent without an inert support at 20 ^{o}C.

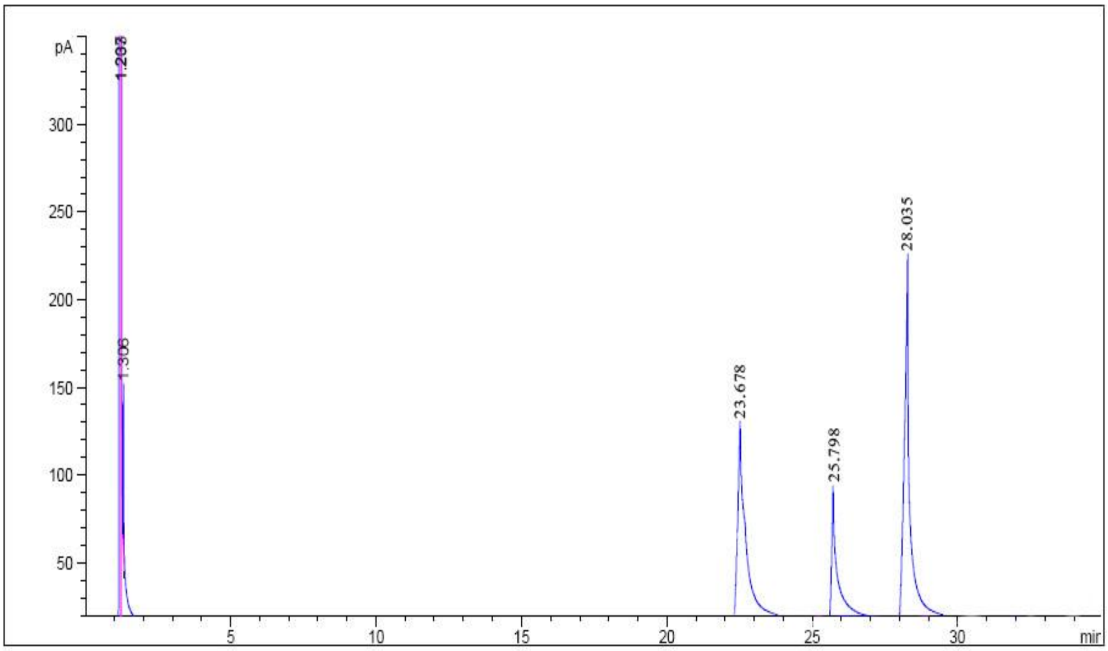

Figure 1.5C GC spectrum of the wax fraction eluted by using isopropanol as the solvent without an inert support at 30 ^{o}C.

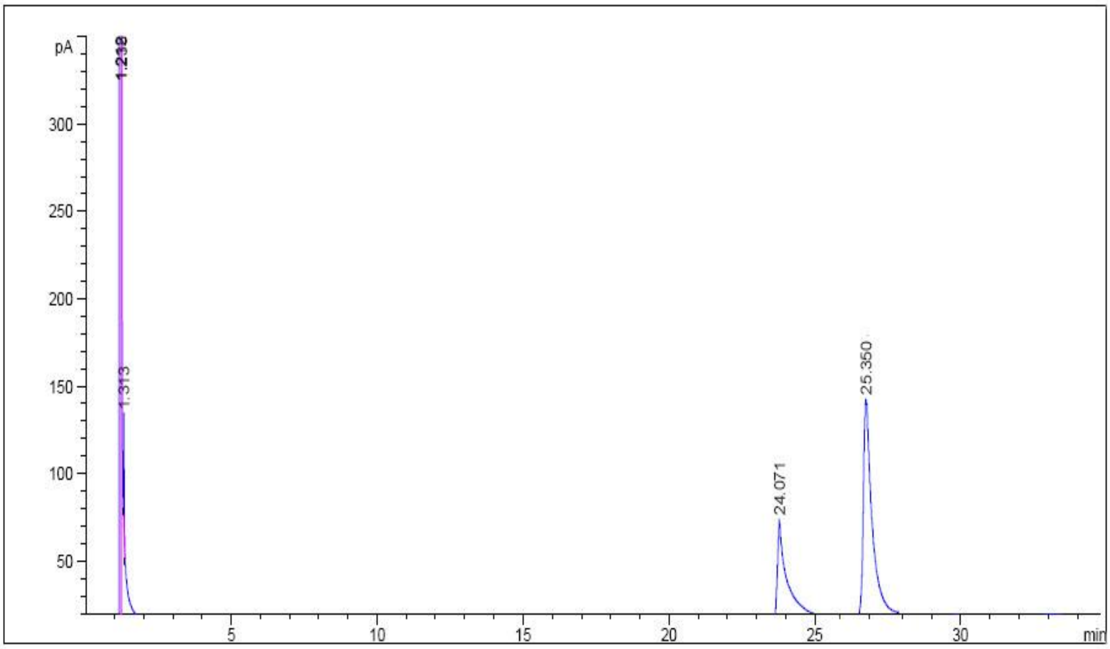

Figure 1.5D GC spectrum of the wax fraction eluted by using isopropanol as the solvent without an inert support at 40 °C.

Fractionation Crystallization technique condition: isopropanol as solvent.

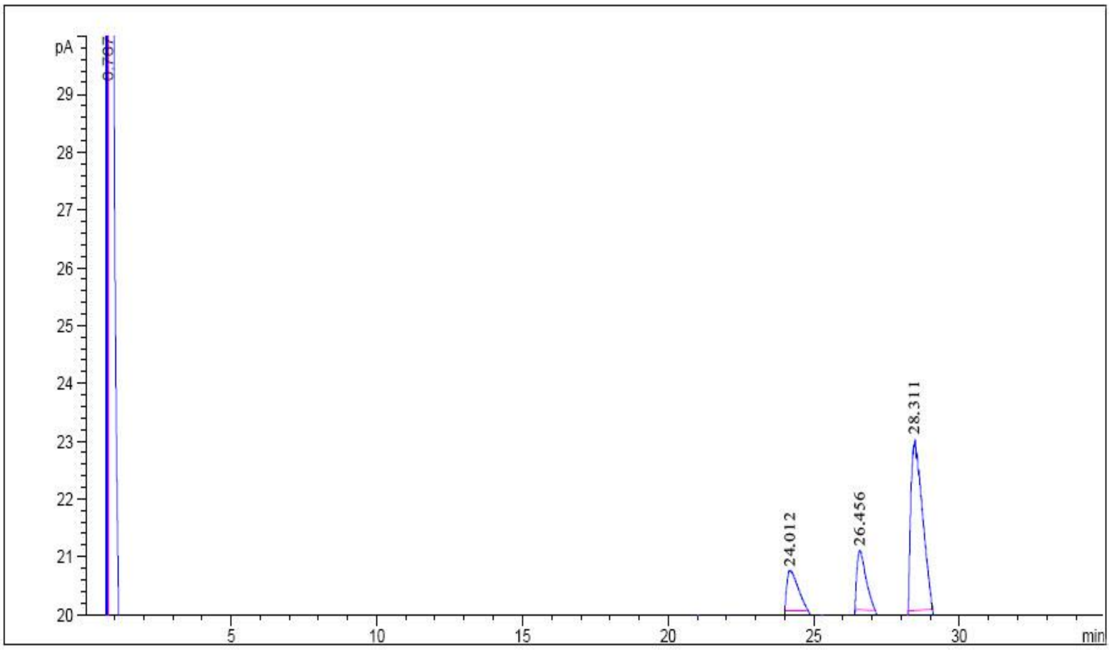

Figure 1.6A GC Spectrum of the wax fraction eluted at 10 °C.

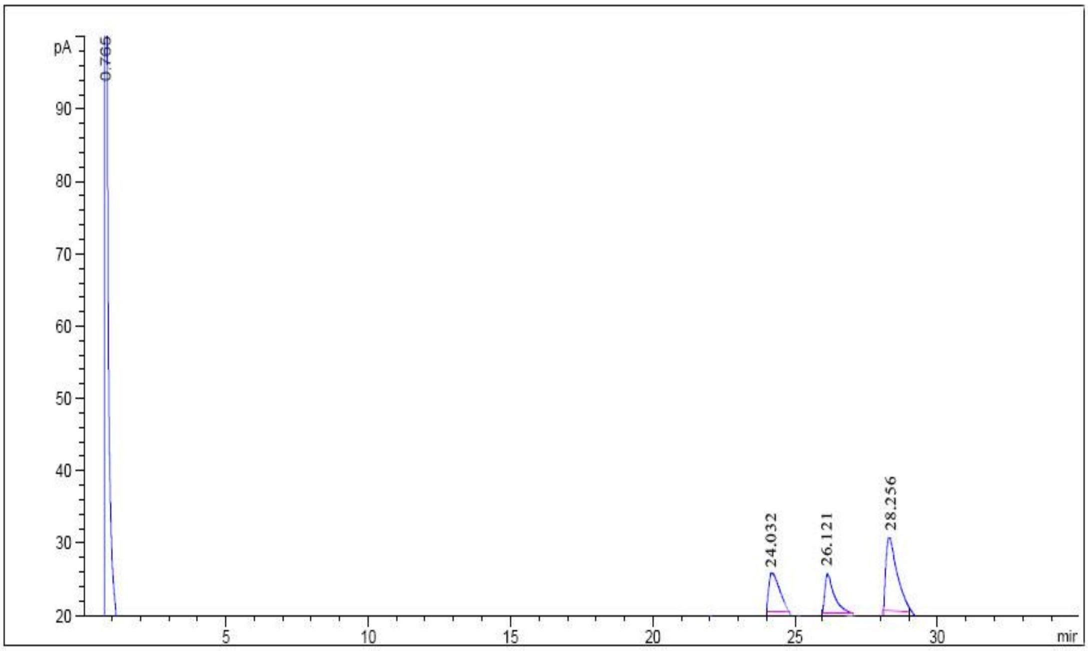

Figure 1.6B GC Spectrum of the wax fraction eluted at 20 ^oC.

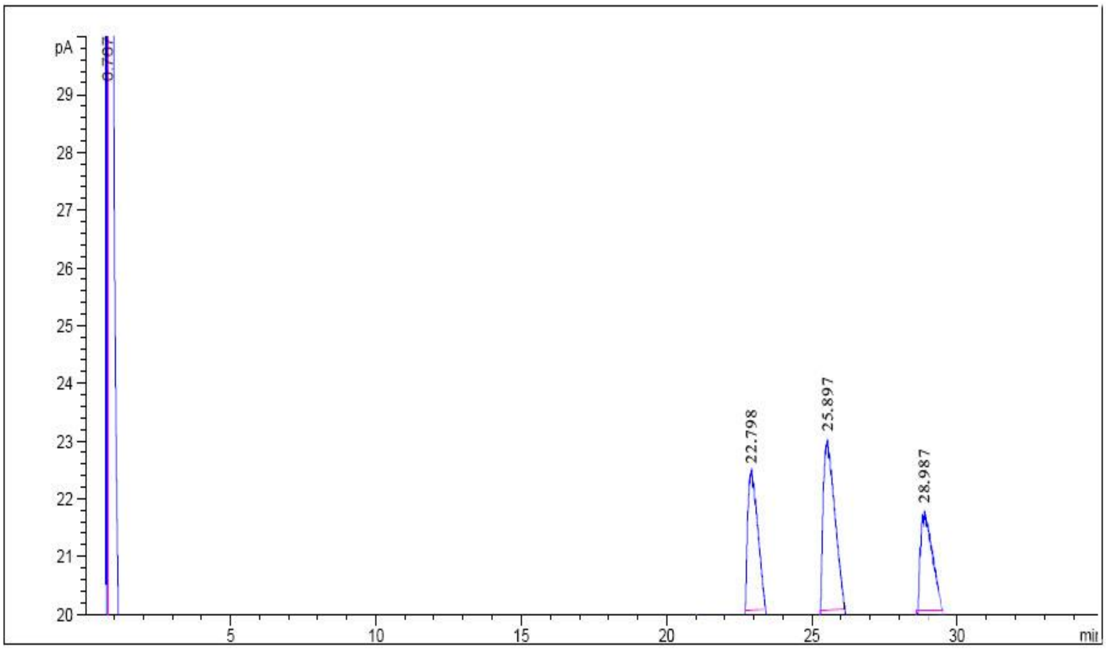

Figure 1.6C GC Spectrum of the wax fraction eluted at 30 ^oC.

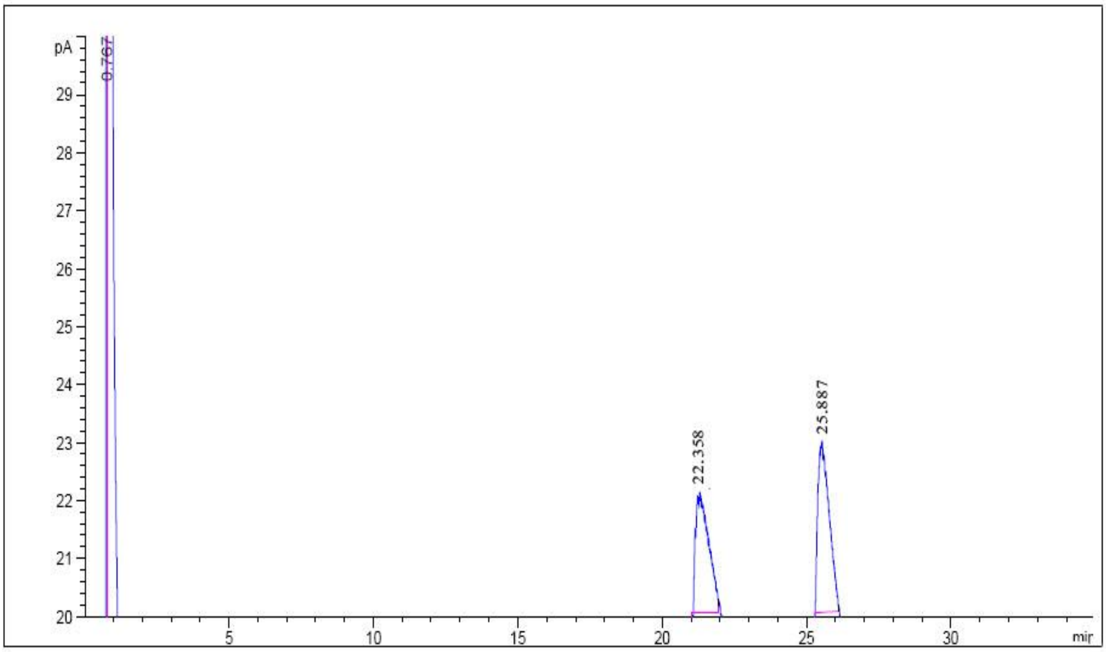

Figure 1.6D GC Spectrum of the wax fraction eluted at 40 °C.

Table 1. GC Spectrum of the wax fraction eluted by TREF technique each condition

Condition	Temperature eluted	peak	area
510 µm, *n*-hexane	10	24.067 27.973	434.4727 1018.7097
	20	24.065 26.732 28.182	242.7831 86.2530 180.4387
	30	28.201	1875.3416
	40	28.182	1734.9529
510 µm, isopropanol	10	24.888 28.1820	74.3941 2265.6618
	20	24.151 27.998	195.6785 142.7736
	30	24.065 27.978	2466.17212 274.3365
	40	26.183	2243.5463
	50	26.259	3654.2391
	60	26.267	3587.8442

Condition	Temperature eluted	peak	area
510 µm, isopropanol repeated	10	27.936	185.9753
	15	28.150	2014.0547
	20	24.067 28.150	247.3652 1475.1944
	25	24.067 27.973	174.8690 137.6773
	30	24.078 27.853	2587.9462 437.4769
	35	24.106	1429.6013
510 µm, mixed solvent	10	24.146 28.235	213.7716 277.5645
	20	24.348 27.351	766.3659 606.3118
	30	25.376	2594.64795
	40	25.350	506.2773
710µm, isopropanol	10	24.888 28.182	235.3961 2265.6661
	20	24.675 27.935	75.6453 235.2684
	30	23.876 26.078 28.182	663.6917 352.1259 1014.0962
	40	23.865 26.657	707.1129 2134.9807

Condition	Temperature eluted	peak	area
710µm, isopropanol	50	26.888	2724.2446
Without inert support, isopropanol	10	23.887 27.567	35.0447 164.7654
	20	24.567 26.788 28.212	2029.6058 164.8759 2576.2345
	30	23.678 25.798 28.035	1249.3263 488.3131 2653.6657
	40	24.072 25.35	154.9887 352.8379
Fractionation Crystallization, isopropanol	10	24.012 26.456 28.311	75.7716 277.5694 1245.8760
	20	24.012 26.121 28.256	65.3224 53.2102 159.9091
	30	22.798 25.897 28.987	27.5114 65.3291 76.6693
	40	22.338 25.887	74.0590 98.7742

Appendix C

Appendix C: ^{1}H NMR spectra of the wax fraction eluted by TREF technique and Fractionation Crystallization in different condition

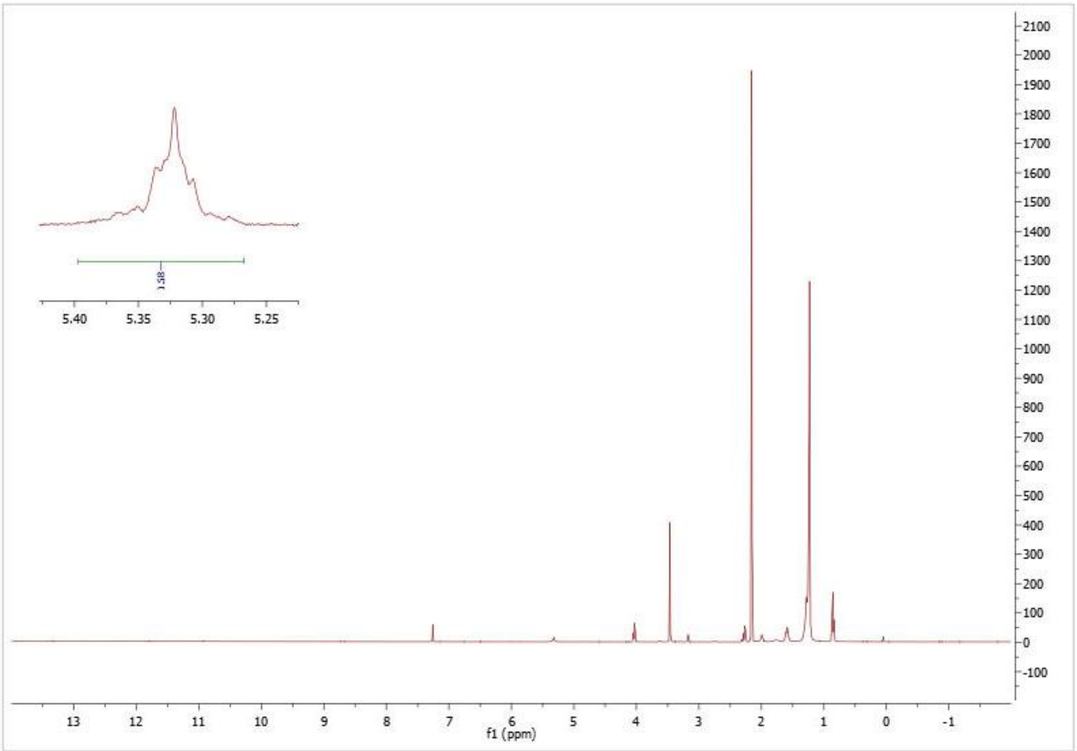

Figure 2.1A NMR Spectrum of the wax fraction eluted by using *n*-hexane as the solvent at 10 ^{o}C with 510 µm sand support.

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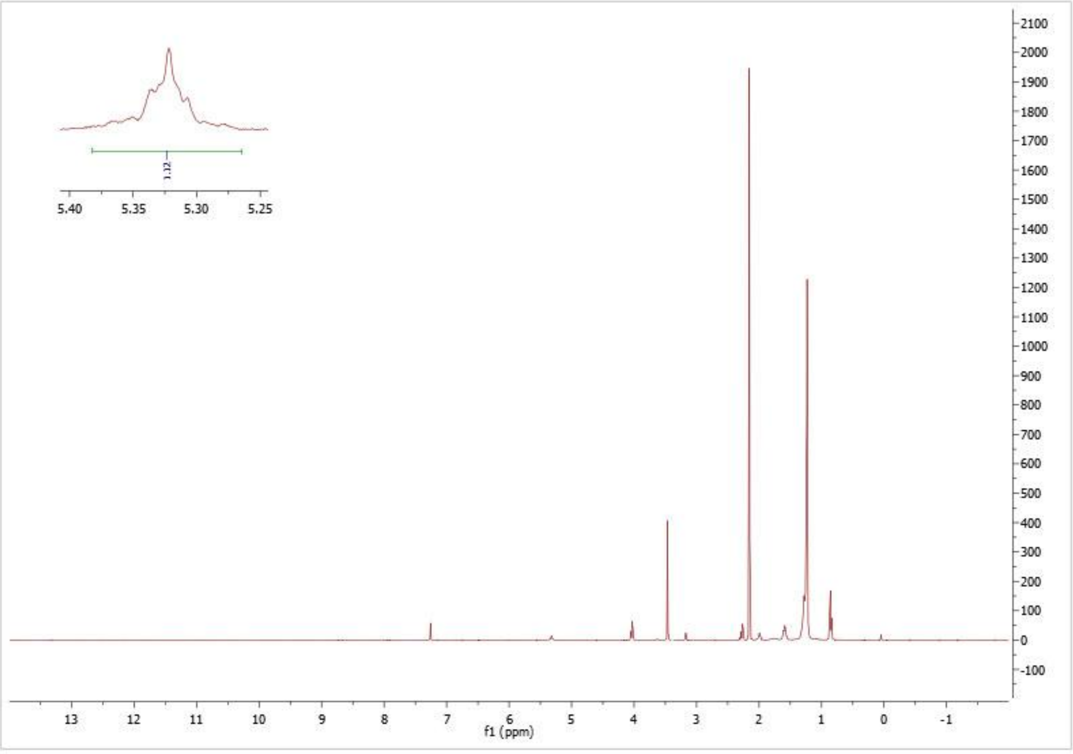

Figure 2.1B NMR Spectrum of the wax fraction eluted by using *n*-hexane as the solvent at 20 ^{o}C with 510 µm sand support.

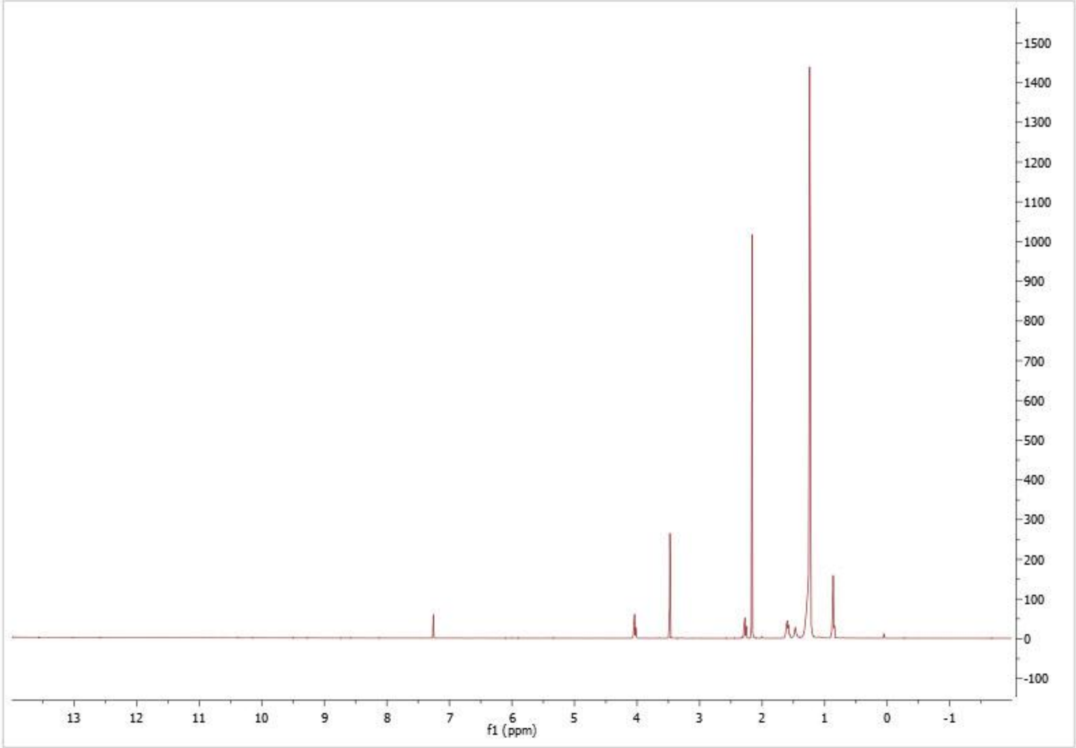

Figure 2.1C NMR Spectrum of the wax fraction eluted by using *n*-hexane as the solvent at 30 °C with 510 µm sand support.

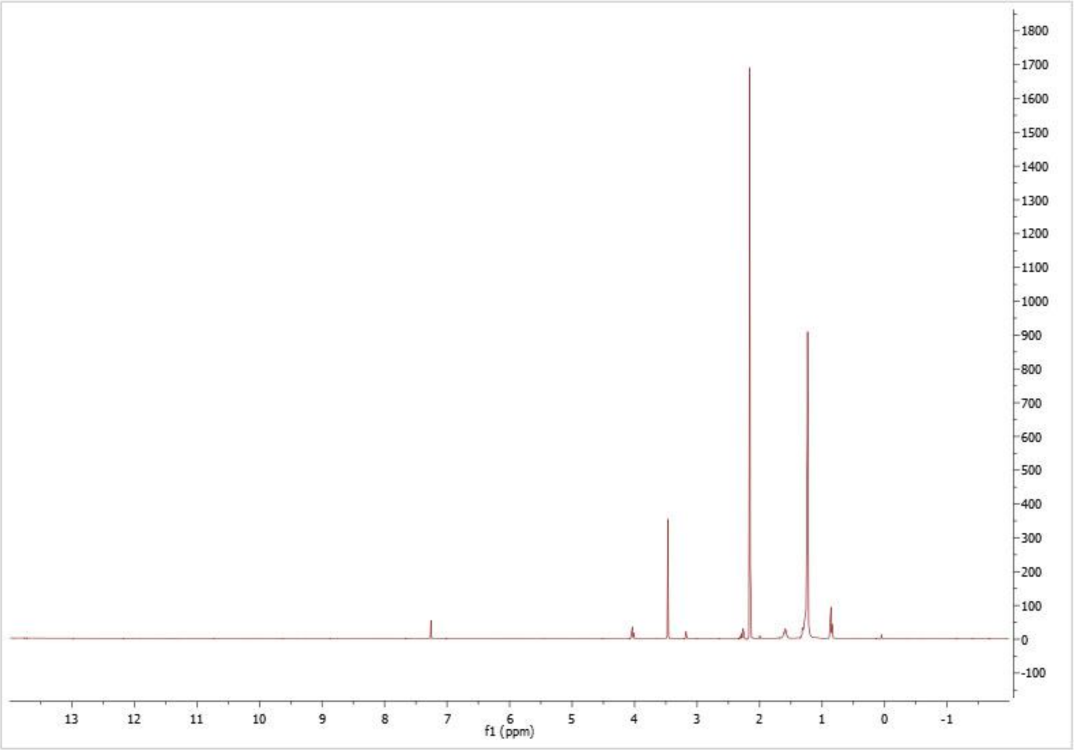

Figure 2.1D NMR Spectrum of the wax fraction eluted by using *n*-hexane as the solvent at 40 °C with 510 µm sand support.

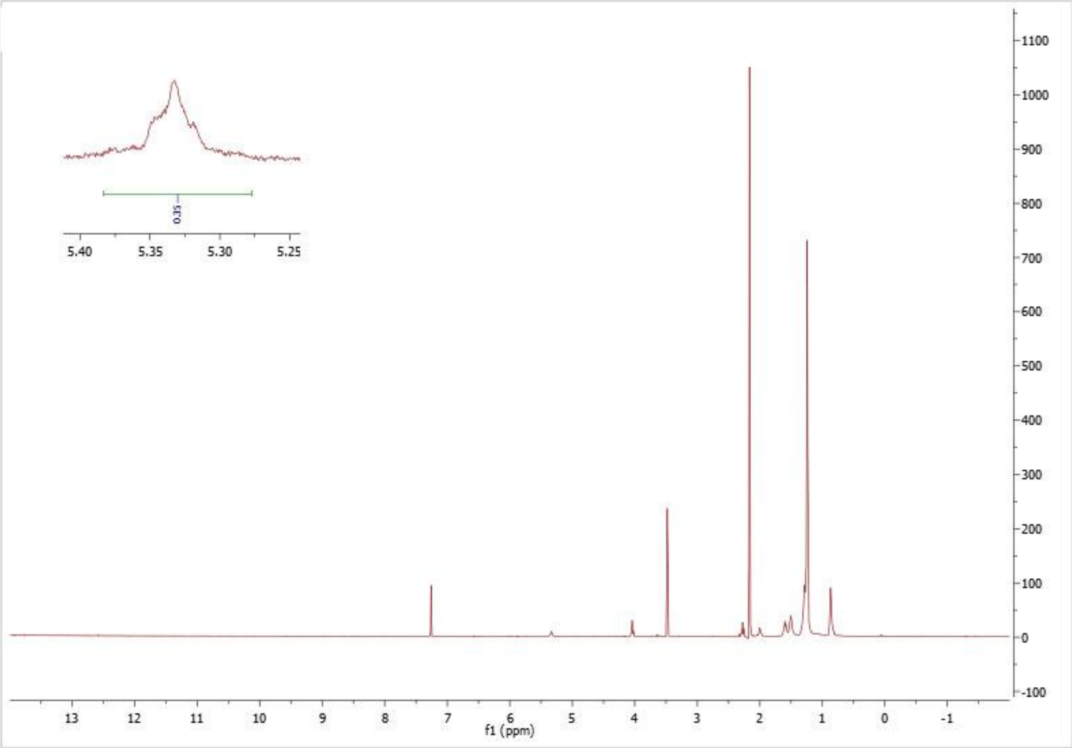

Figure 2.2A NMR Spectrum of the wax fraction eluted at 10 ^{o}C by using 510 µm sand support and isopropanol as the solvent.

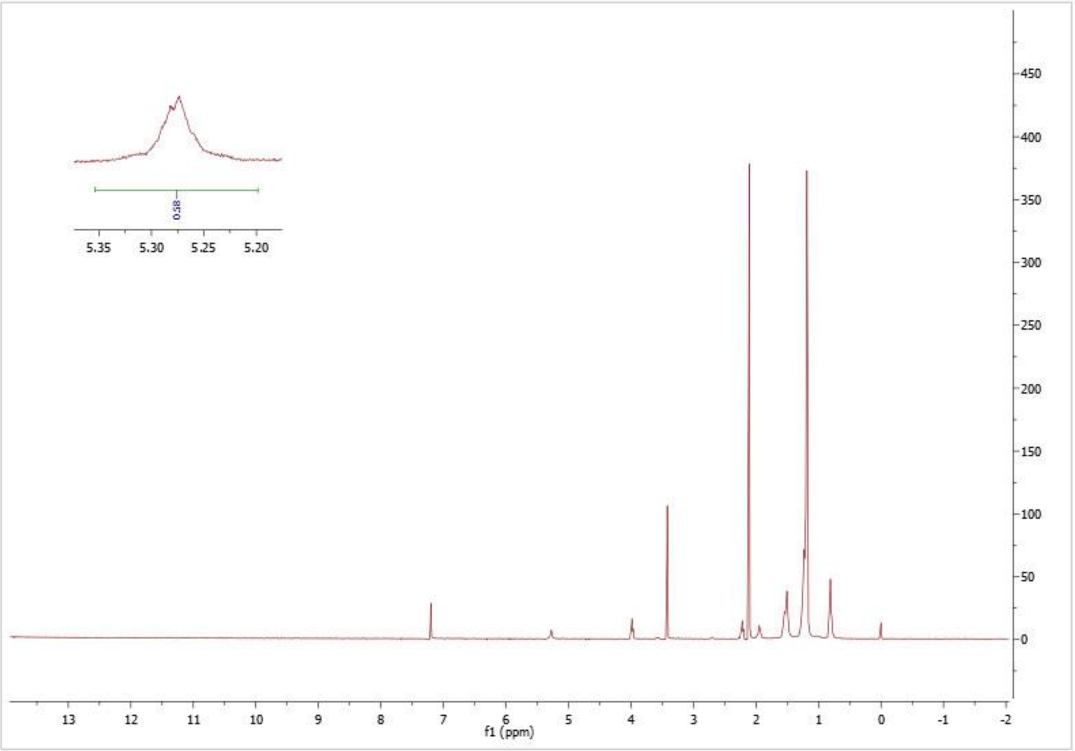

Figure 2.2B NMR Spectrum of the wax fraction eluted at 20 ^{o}C by using 510 µm sand support and isopropanol as the solvent.

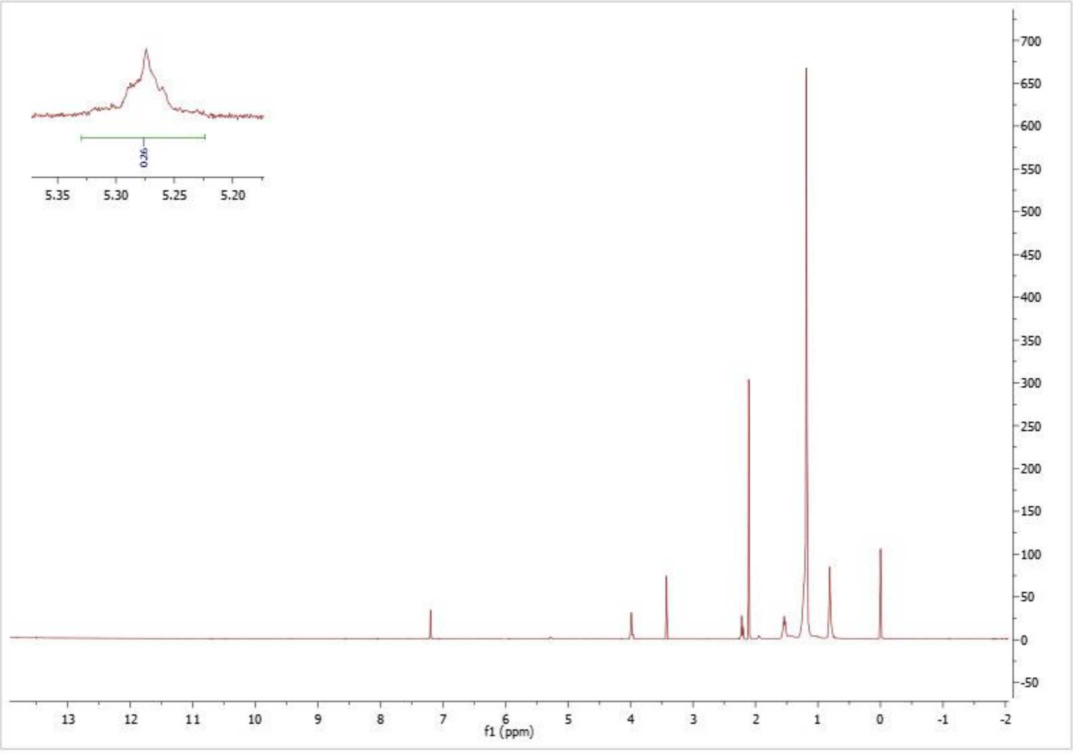

Figure 2.2C NMR Spectrum of the wax fraction eluted at 30 ^{o}C by using 510 µm sand support and isopropanol as the solvent.

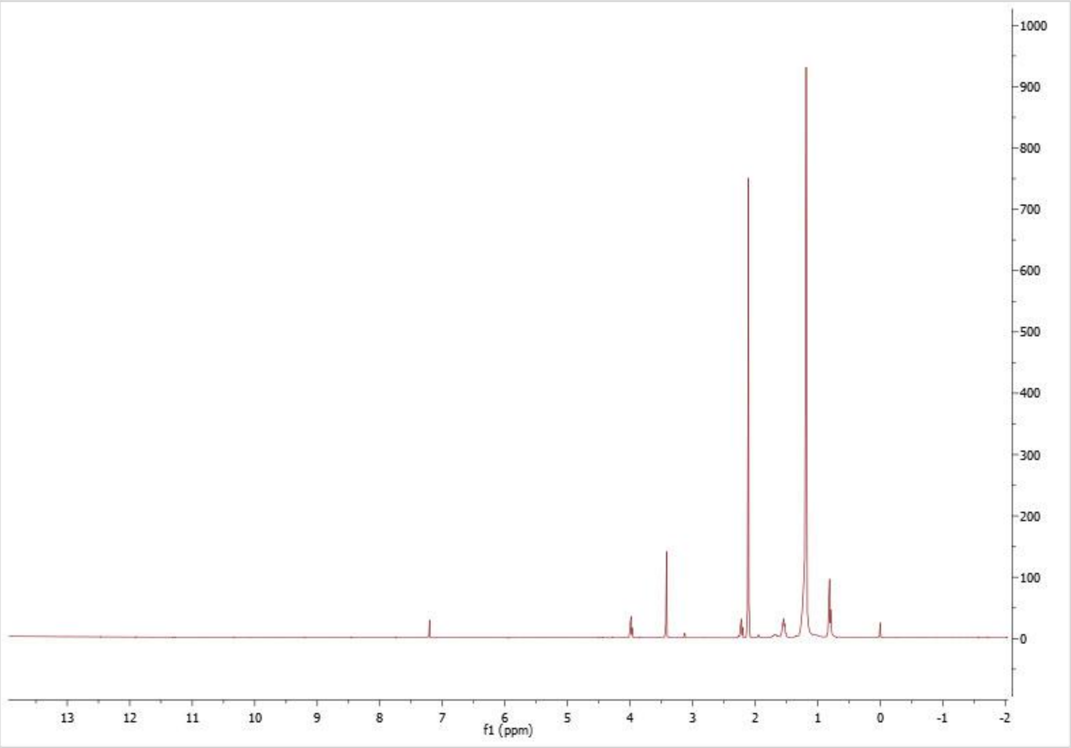

Figure 2.2D NMR Spectrum of the wax fraction eluted at 40 °C by using 510 µm sand support and isopropanol as the solvent.

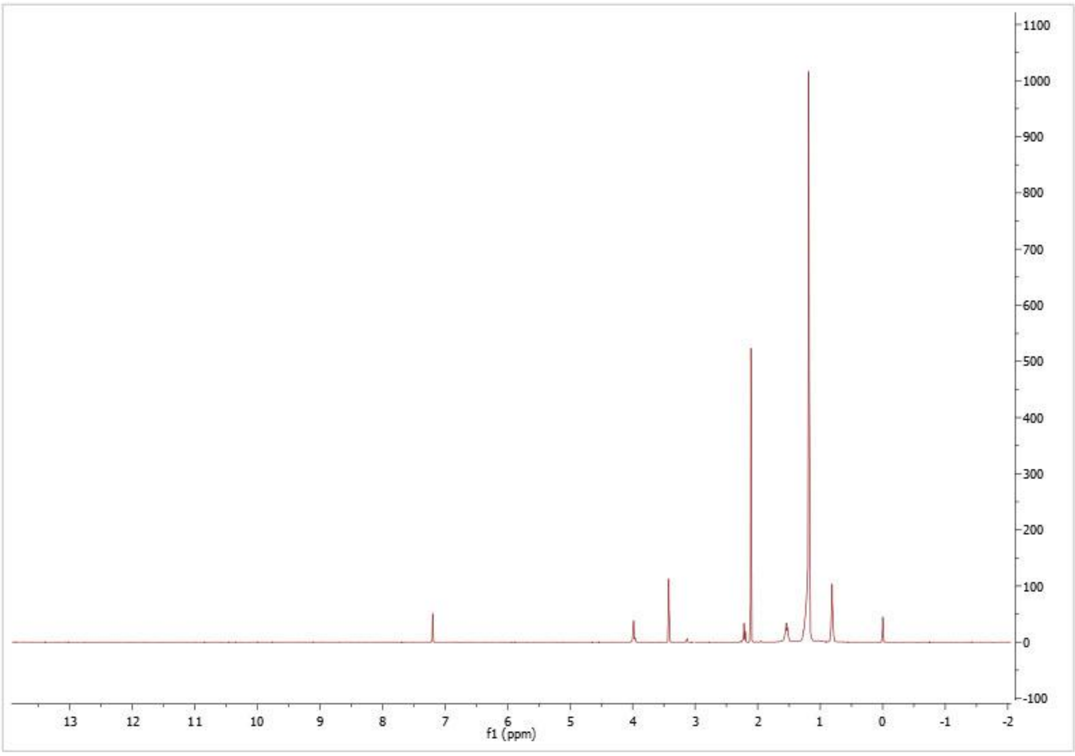

Figure 2.2E NMR Spectrum of the wax fraction eluted at 50 ^{o}C by using 510 µm sand support and isopropanol as the solvent.

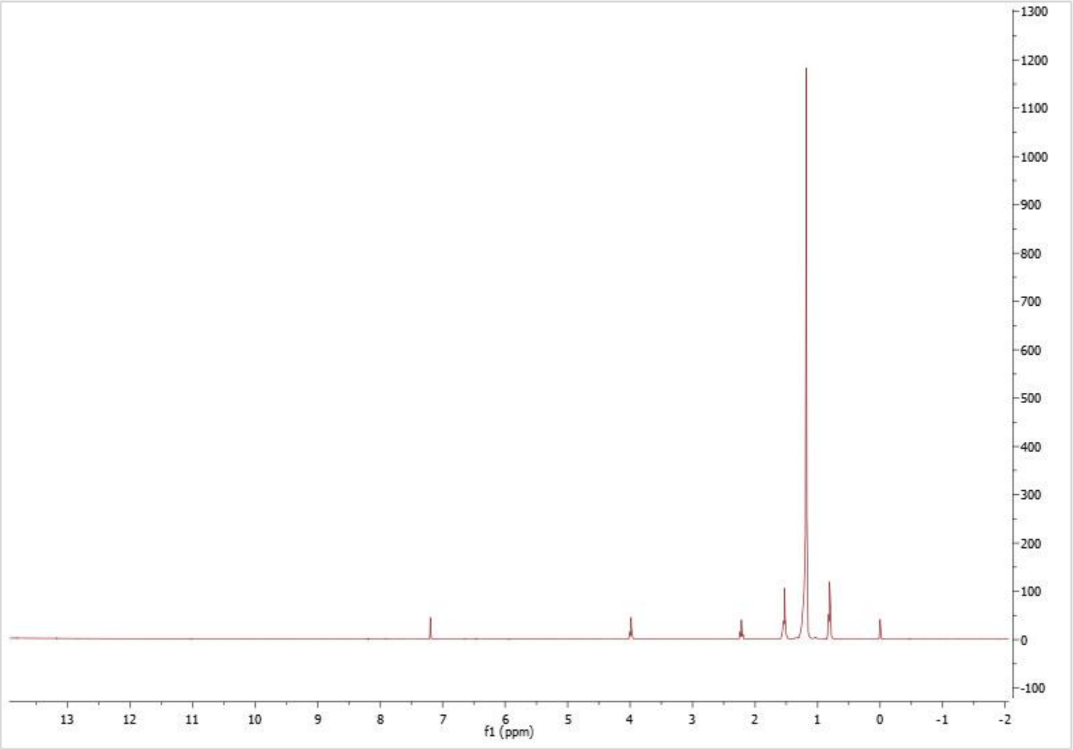

Figure 2.2F NMR Spectrum of the wax fraction eluted at 60 ^{o}C by using 510 µm sand support and isopropanol as the solvent.

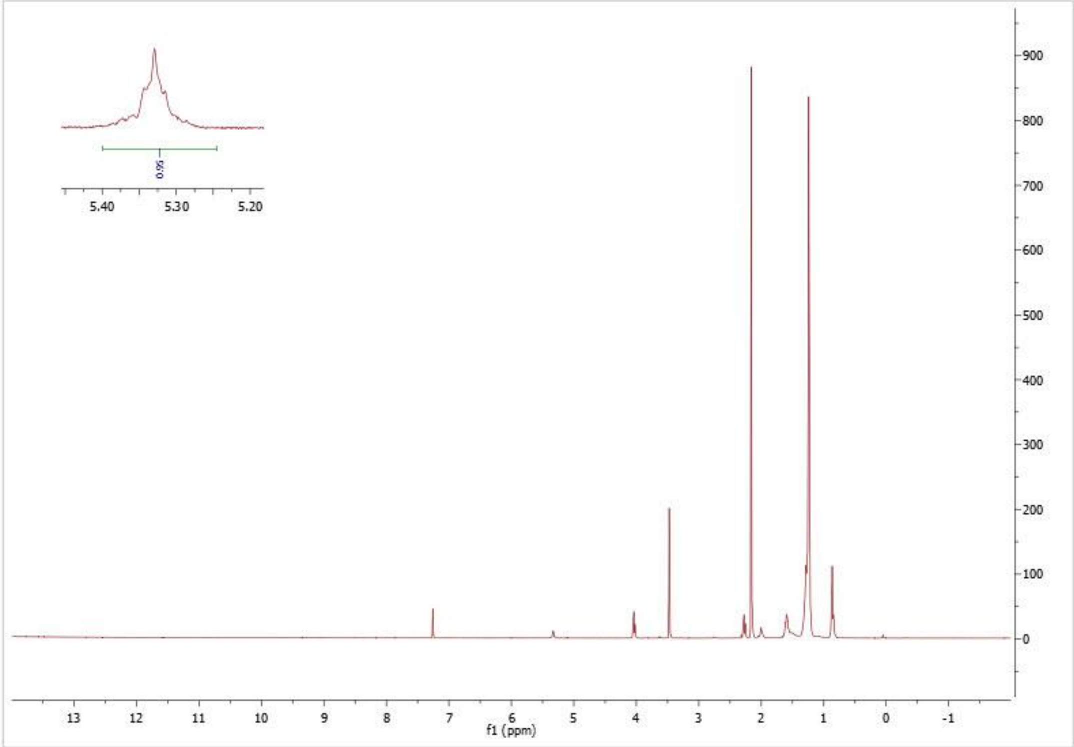

Figure 2.3A NMR Spectrum of the wax fraction eluted at 10 ^{o}C by using 510 µm sand support and mixed as the solvent (*n*-hexane: isopropanol).

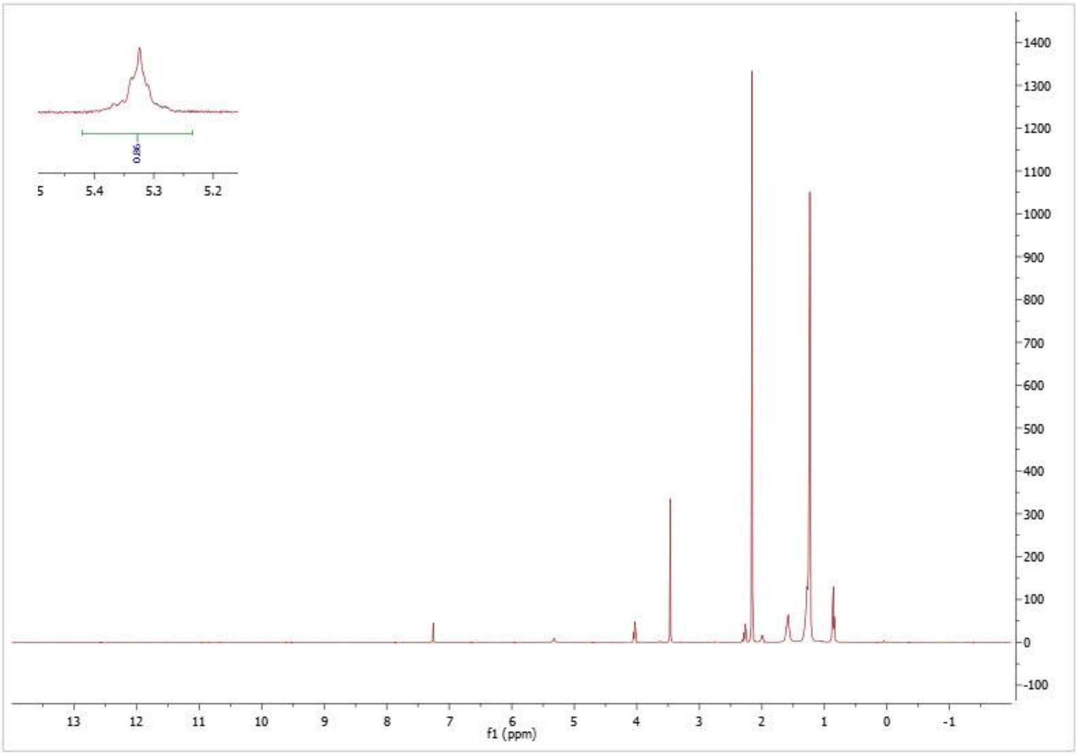

Figure 2.3B NMR Spectrum of the wax fraction eluted at 20 ^{o}C by using 510 µm sand support and mixed as the solvent (*n*-hexane: isopropanol).

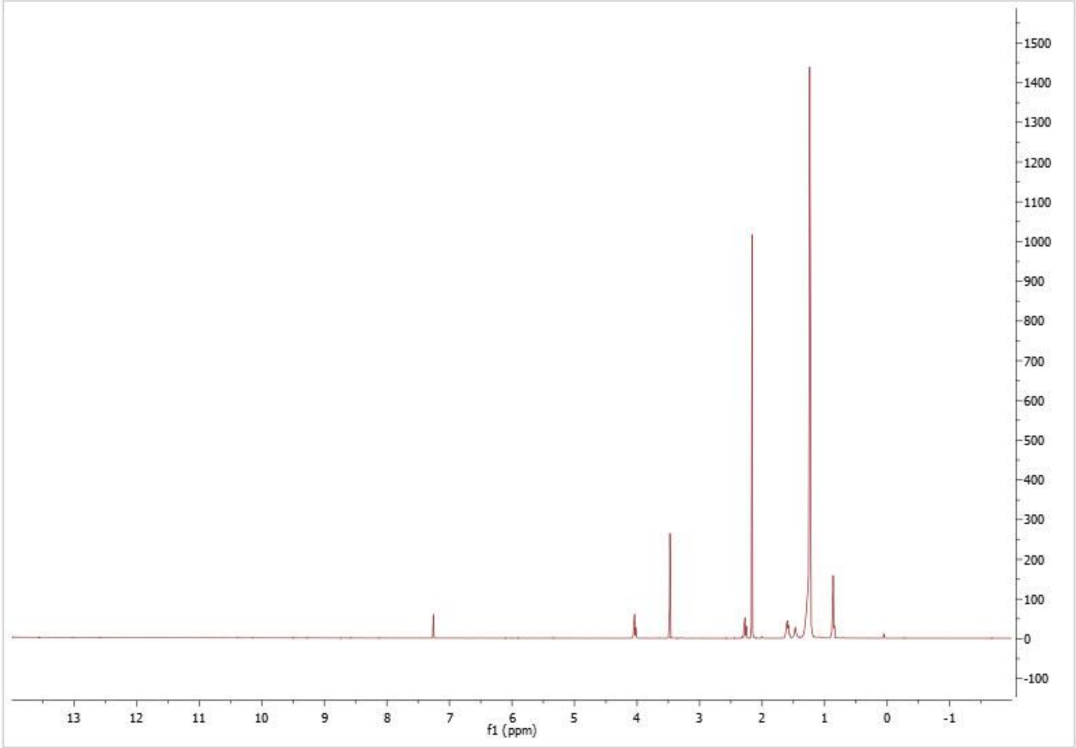

Figure 2.3C NMR Spectrum of the wax fraction eluted at 30 °C by using 510 µm sand support and mixed as the solvent (*n*-hexane:isopropanol).

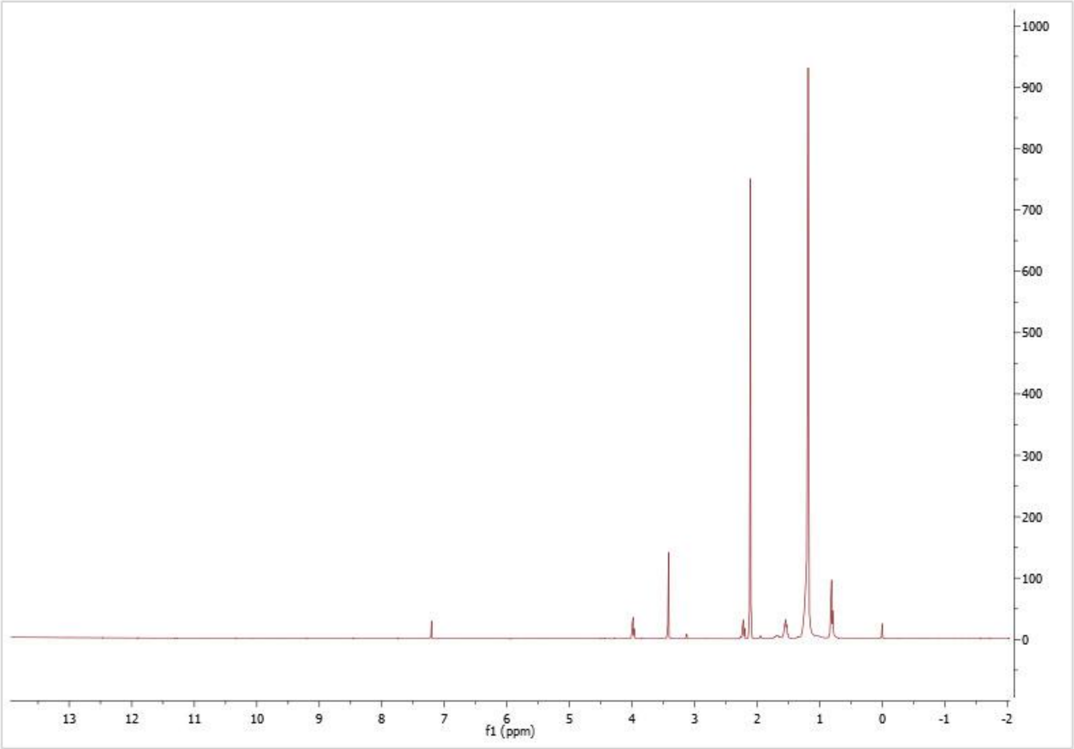

Figure 2.3D NMR Spectrum of the wax fraction eluted at 40 ^{o}C by using 510 µm sand support and mixed as the solvent (*n*-hexane: isopropanol).

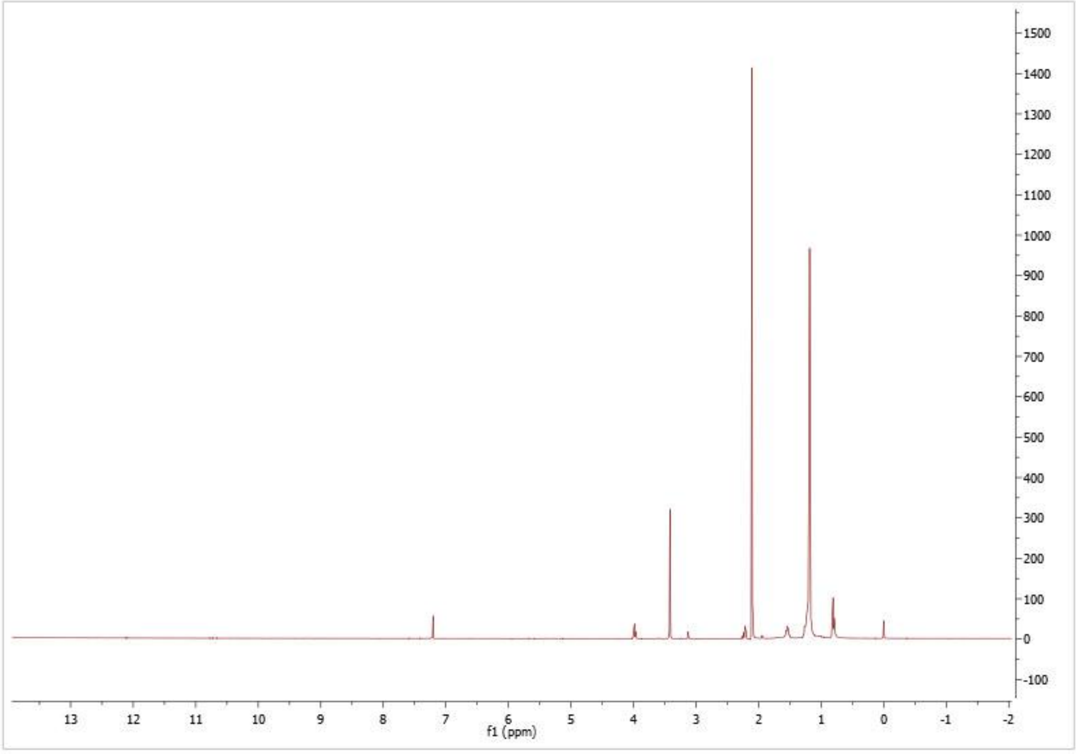

Figure 2.3E NMR Spectrum of the wax fraction eluted at 50 ^{o}C by using 510 µm sand support and mixed as the solvent (*n*-hexane: isopropanol).

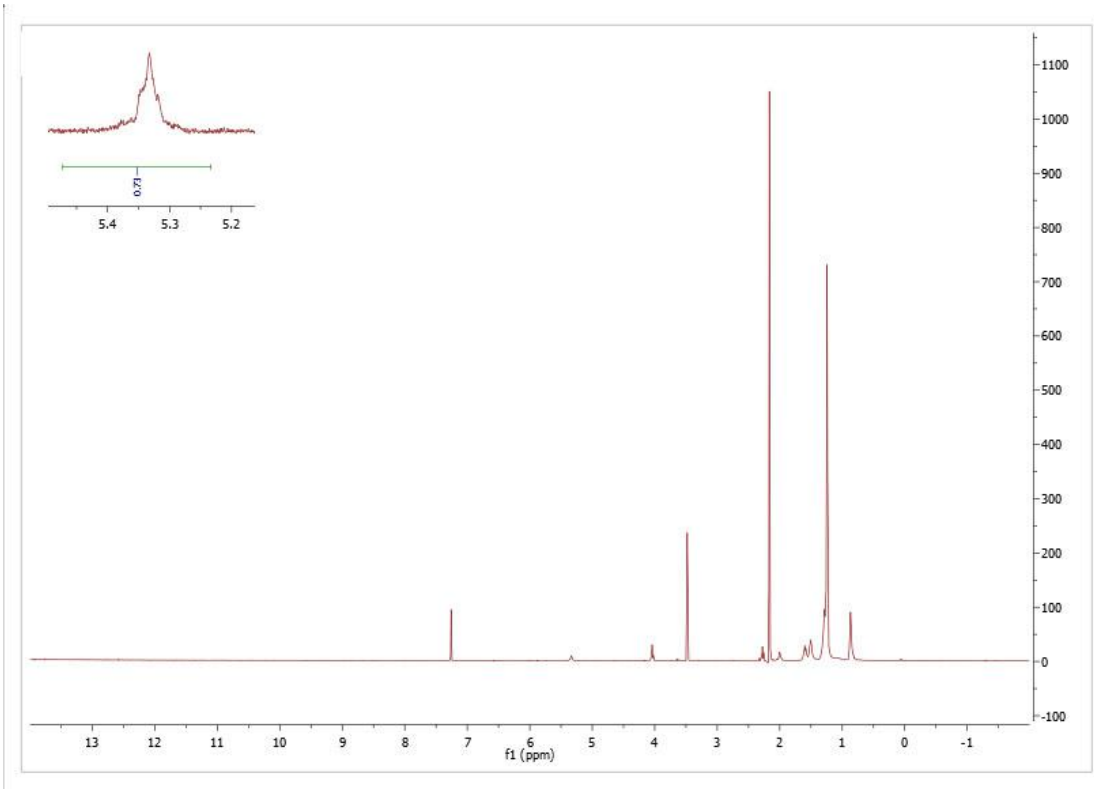

Figure 2.4A NMR Spectrum of the wax fraction eluted at 10 ^{o}C by using 710 µm sand support and isopropanol as the solvent.

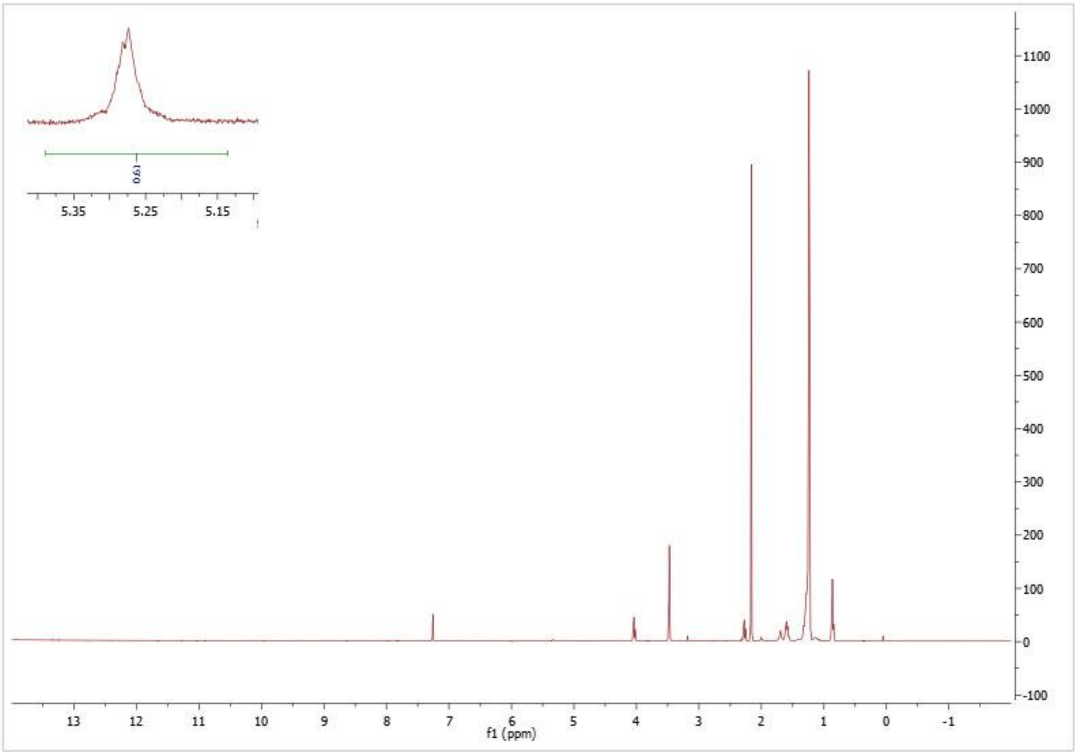

Figure 2.4B NMR Spectrum of the wax fraction eluted at 20 °C by using 710 µm sand support and isopropanol as the solvent.

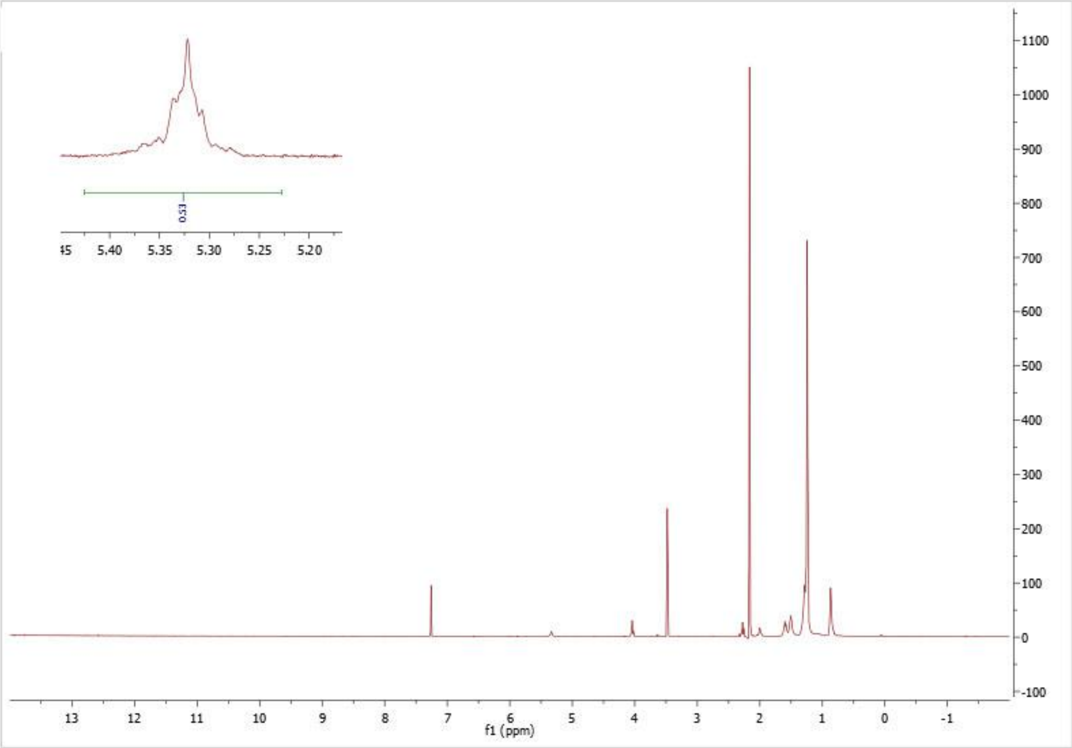

Figure 2.4C NMR Spectrum of the wax fraction eluted at 30 °C by using 710 µm sand support and isopropanol as the solvent.

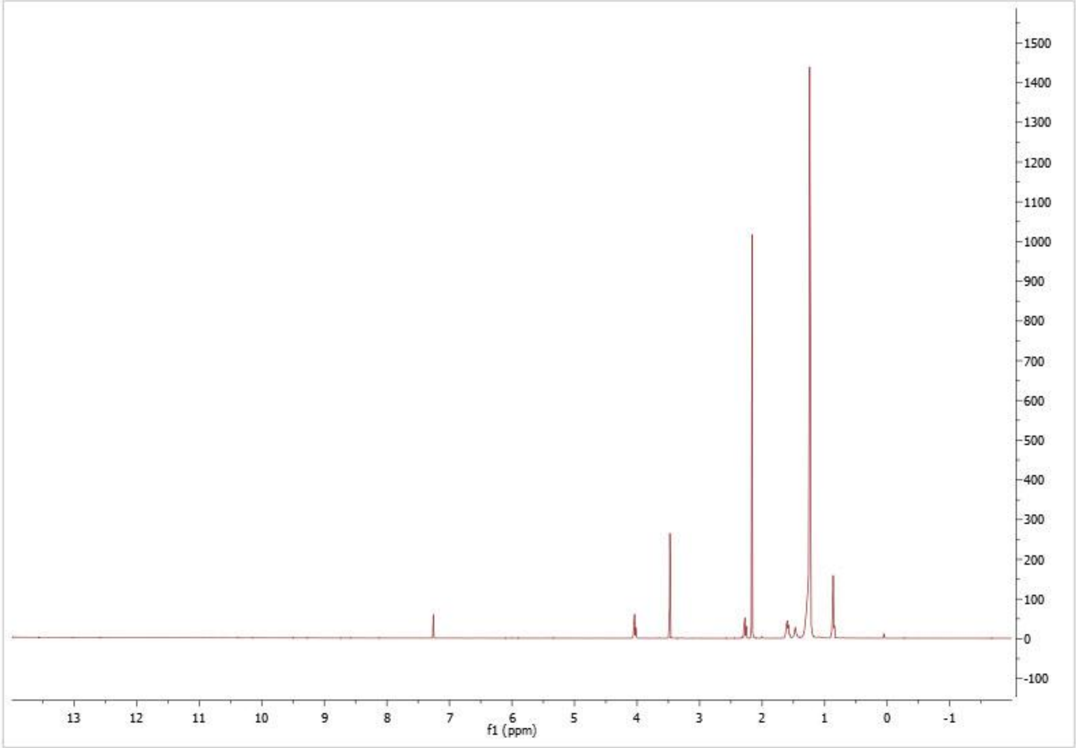

Figure 2.4D NMR Spectrum of the wax fraction eluted at 40 ^{o}C by using 710 µm sand support and isopropanol as the solvent.

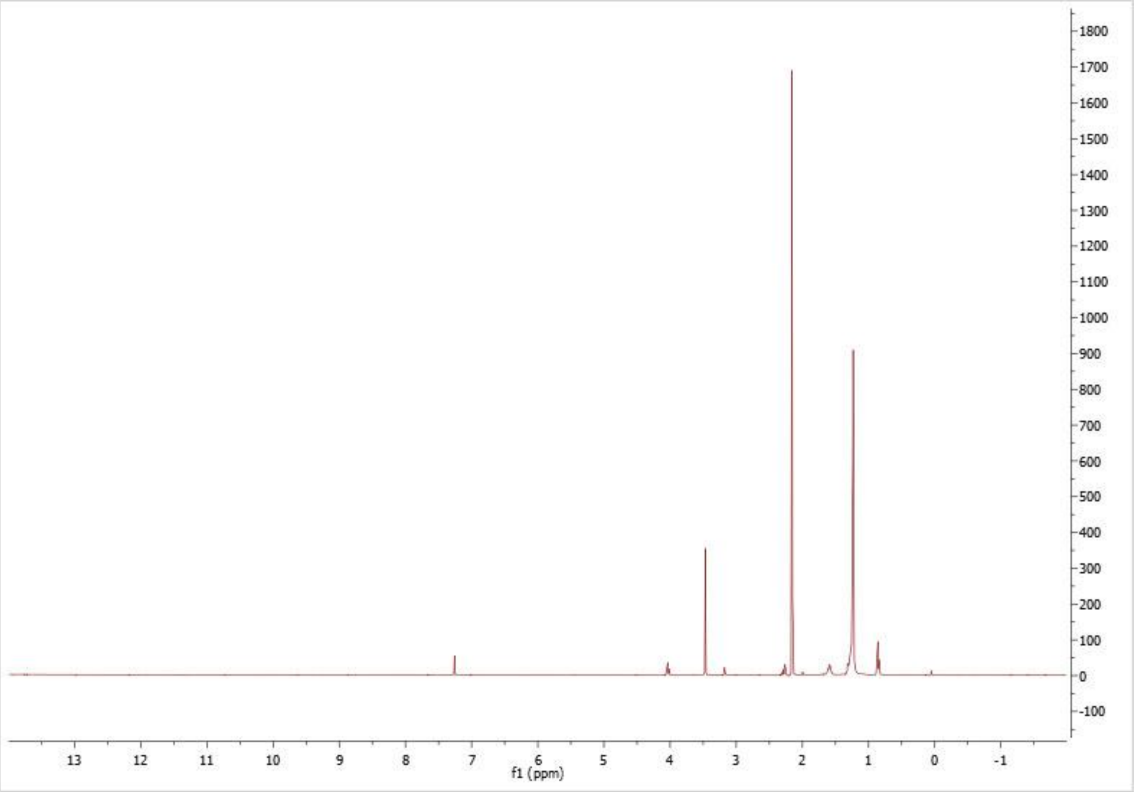

Figure 2.4E NMR Spectrum of the wax fraction eluted at 50 ^{o}C by condition used 710 µm sand and isopropanol as solvent.

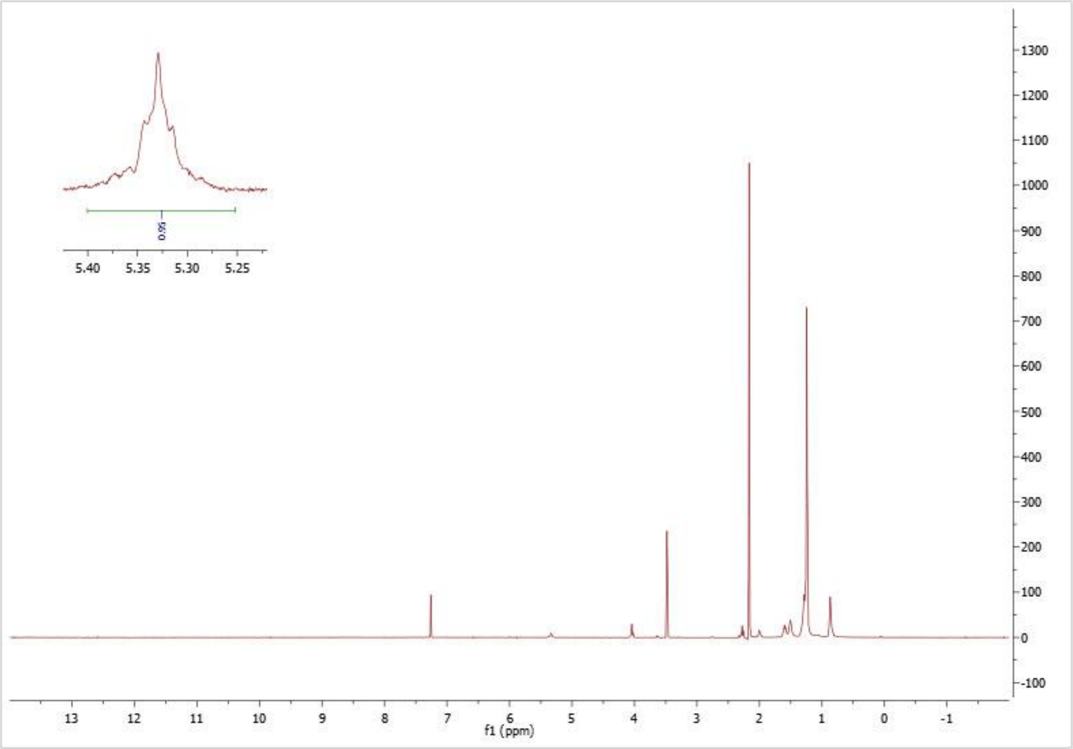

Figure 2.5A NMR Spectrum of the wax fraction eluted at 10 ^{o}C by using without inert support and isopropanol as solvent.

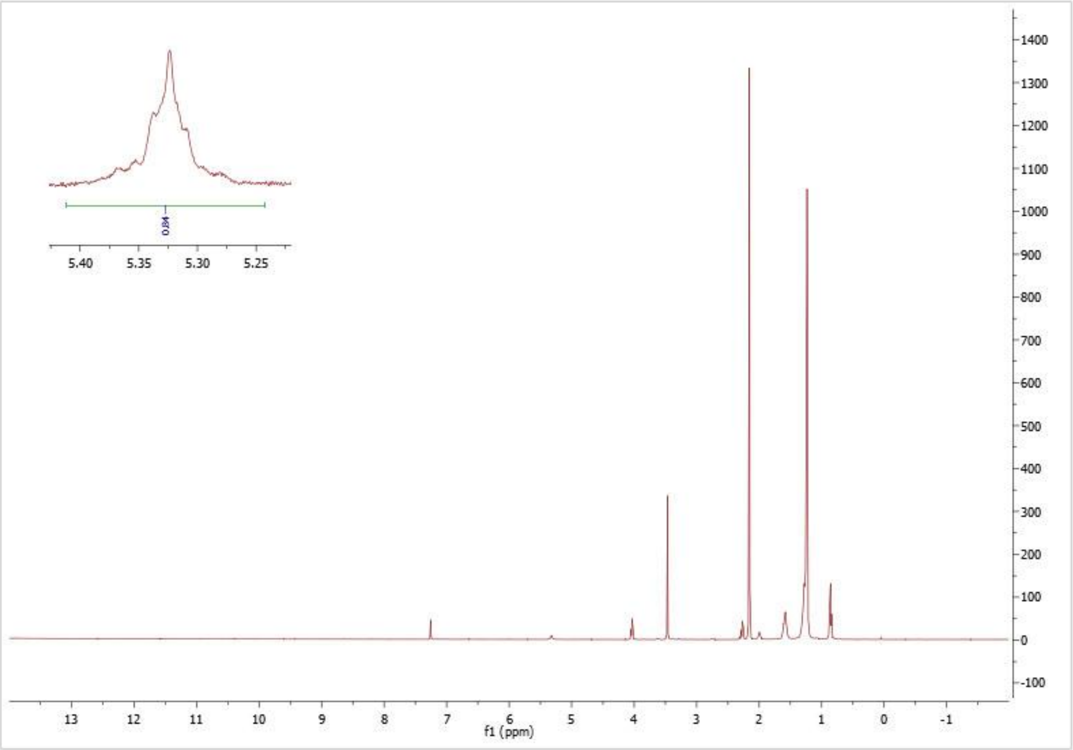

Figure 2.5B NMR Spectrum of the wax fraction eluted at 20 °C by using without inert support and isopropanol as solvent.

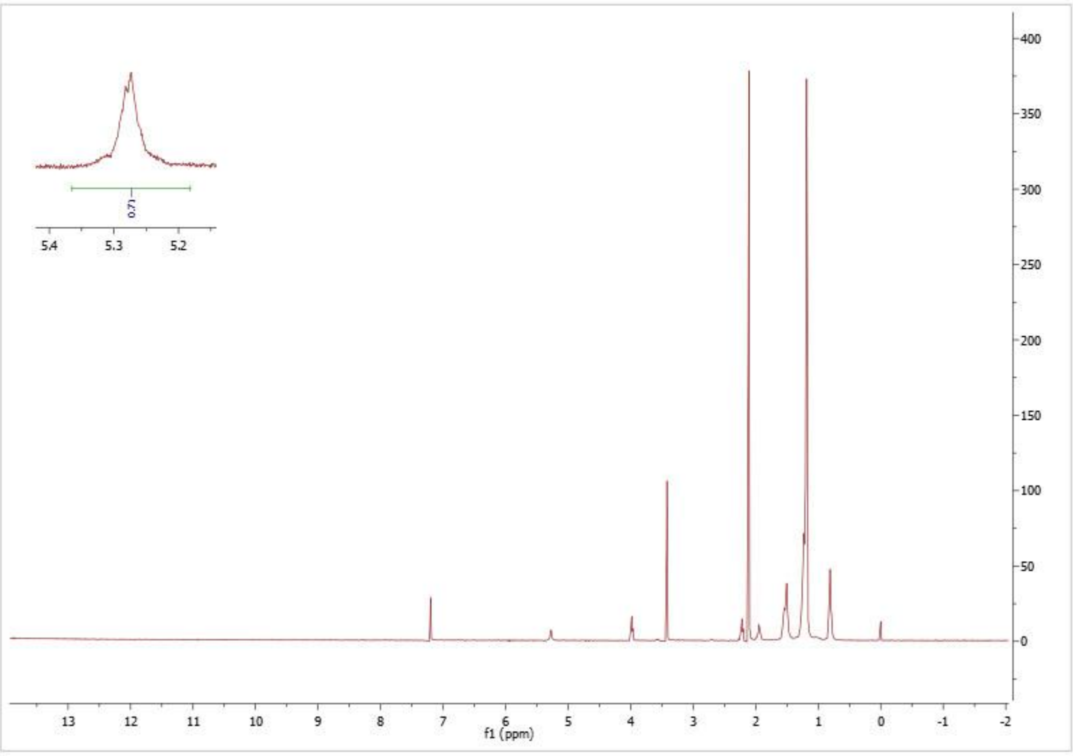

Figure 2.5C NMR Spectrum of the wax fraction eluted at 30 °C by using without inert support and isopropanol as solvent.

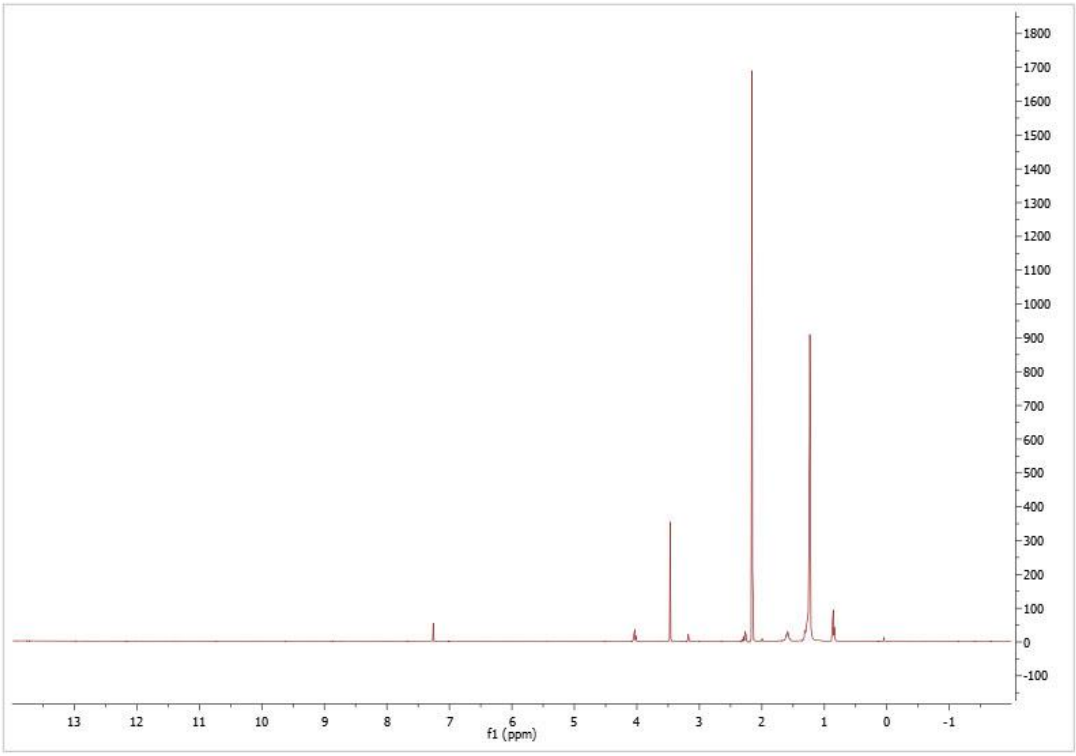

Figure 2.5D NMR Spectrum of the wax fraction eluted at 40 ^{o}C by using without inert support and isopropanol as solvent.

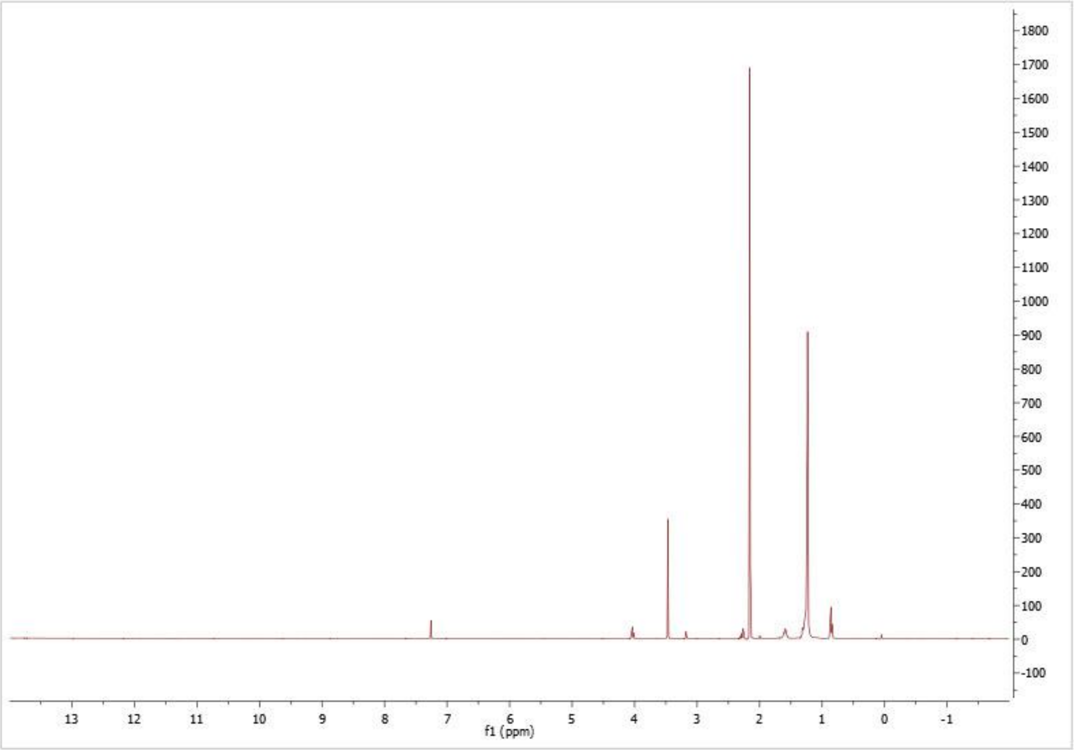

Figure 2.5E NMR Spectrum of the wax fraction eluted at 50 ^{o}C by using without inert support and isopropanol as solvent.

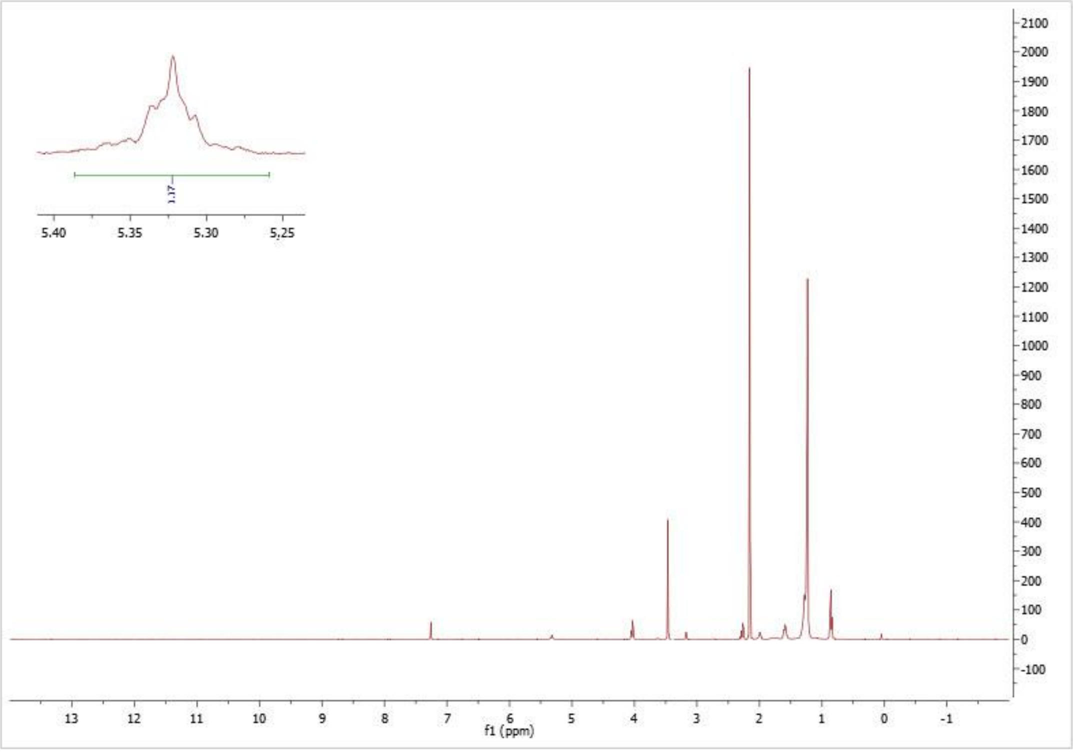

Figure 2.6A NMR Spectrum of the wax fraction eluted at 10 ^{o}C by Fractionation Crystallization technique.

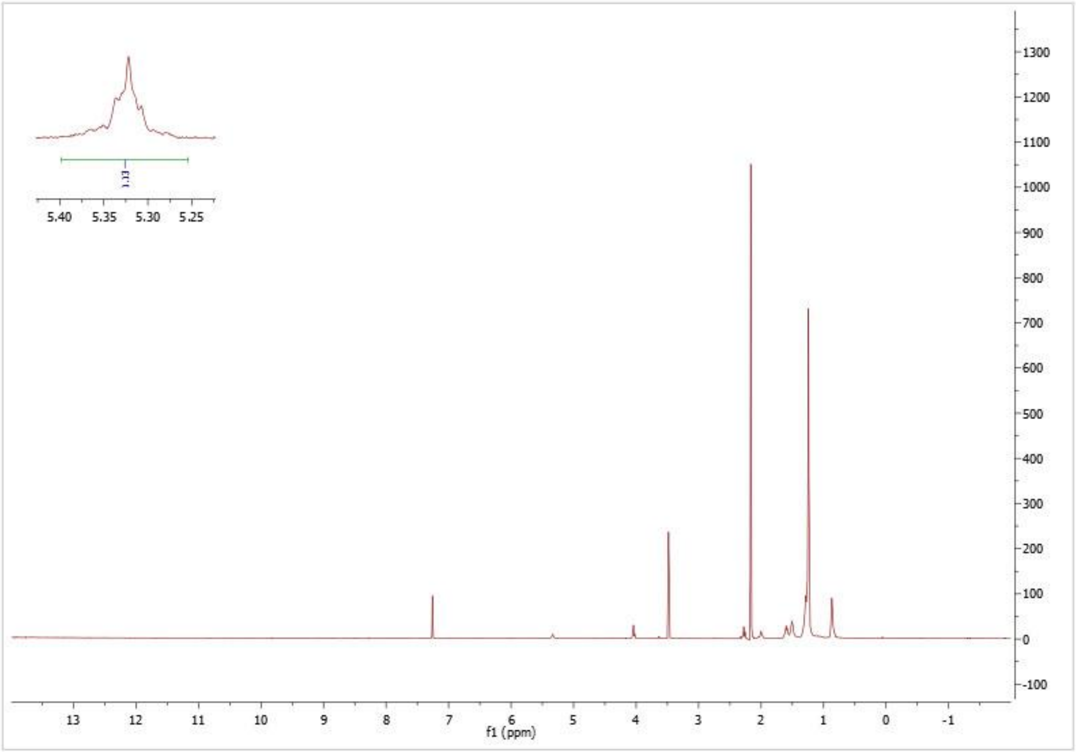

Figure 2.6B NMR Spectrum of the wax fraction eluted at 20 °C by Fractionation Crystallization technique.

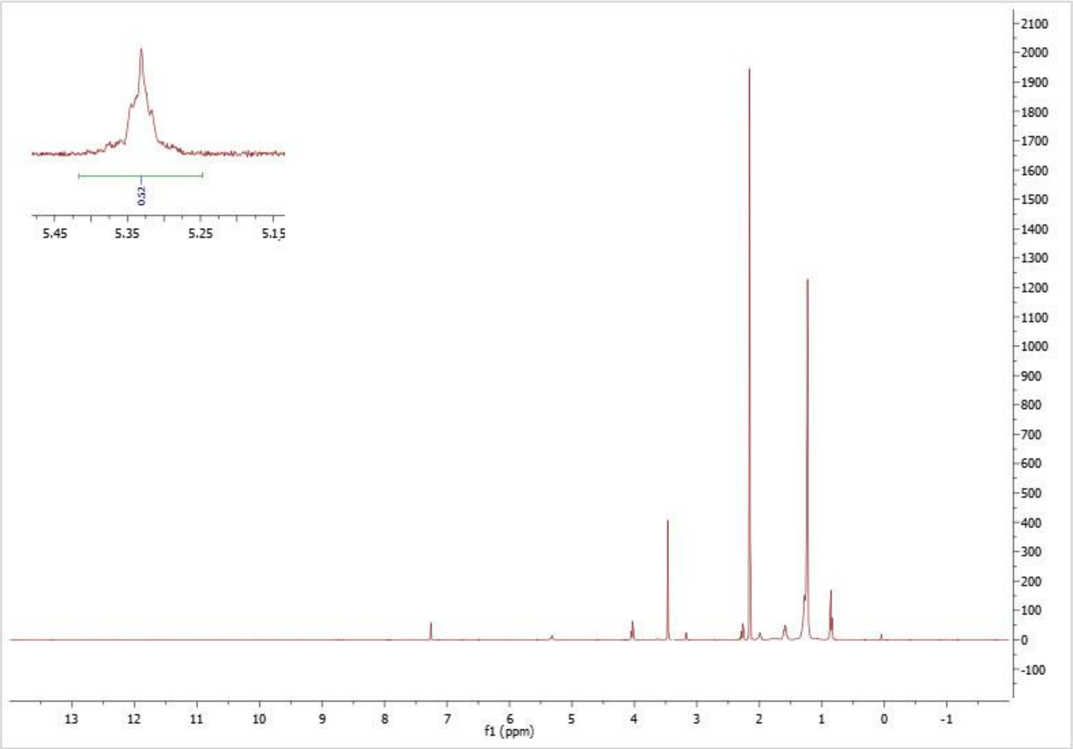

Figure 2.6C NMR Spectrum of the wax fraction eluted at 30 °C by Fractionation Crystallization technique.

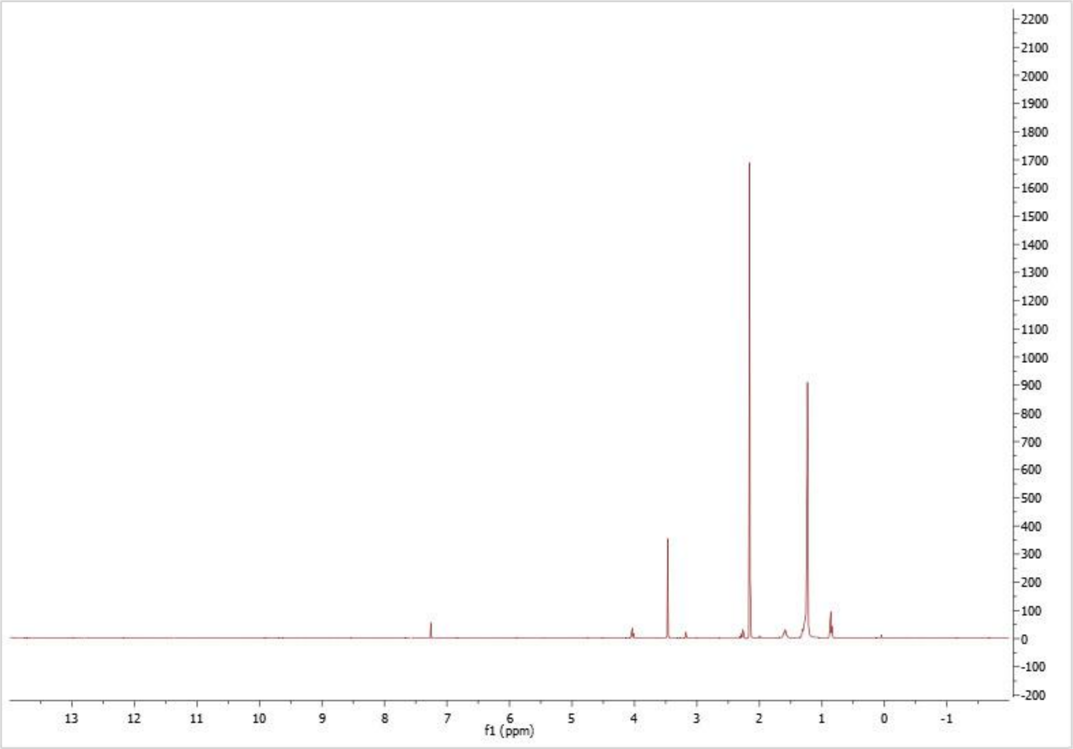

Figure 2.6D NMR Spectrum of the wax fraction eluted at 40 °C by Fractionation Crystallization technique.

4.8 NMR results

All separated wax fractions were analyzed by NMR. The integration of the characteristic peaks and % of the components for each fraction are presented in Table 4.8.1.1 to 4.83.

4.8.1.1 Hexane as solvent, 510 µm sand.

Table 4.8.1.1 Integration and % of the components for each fraction separated by TREF

Elution temperature	Peak integration			% of component	
	-CH=CH	$-CH_2-$	$-CH_3$		
	5-6	1.2-1.7	0.9-1	SO	SD and SP
10	1.58	79.32	9.39	33.65	66.35
20	1.32	22.46	12.94	20.40	79.60
30	-	25.53	14.33	0	100
40	-	32.96	5.14	0	100

4.8.1.2 Isopropanol as solvent, 510 µm sand.

Table 4.8.1.2 Integration and % of the components for each fraction separated by TREF

Elution temperature	integrating of each spectrum			% wt. in waxes	
	-CH=CH	$-CH_2$	$-CH_3$		
	5-6	1.2-1.7	0.9-1	SO	SD and SP
10	0.33	34.57	4.03	16.42	83.58
20	0.50	27.46	9.02	5.76	94.24
30	0.26	31.91	7.47	6.96	93.04
40	-	36.68	4.03		100.00
50	-	34.57	9.17		100.00

4.8.1.3 Mixed solvent (hexane:propanol) as solvent, 510 µm sand.

Table 4.8.1.3 Integration and % of the components for each fraction separated by TREF

Elution temperature	integrating of each spectrum			% wt. in waxes	
	-CH=CH	$-CH_2$	$-CH_3$		
	5-6	1.2-1.7	0.9-1	SO	SD and SP
10	0.96	38.08	6.76	28.40	71.60
20	0.85	33.25	11.96	14.38	85.62
30	-	45.38	6.25		100
40	-	38.8	5.32		100
50	-	32.22	0.84		100

4.8.2.1 Isopropanol as solvent, 710 µm sand.

Table 4.8.2.1. Integration and % of the components for each fraction separated by TREF

Elution temperature	integrating of each spectrum			% wt. in waxes	
	-CH=CH	$-CH_2$	$-CH_3$		
	5-6	1.2-1.7	0.9-1	SO	SD and SP
10	0.73	53.86	8.76	16.67	83.33
20	0.61	87.45	9.85	12.39	87.61
30	0.53	57.90	8.69	12.20	87.80
40	-	91.32	10.39	0	100
50	-	65.74	6.43	0	100

4.8.2.2 Isopropanol as solvent, without inert support.

Table 4.8.2.2. Integration and % of the components for each fraction separated by TREF

Elution temperature	integrating of each spectrum peak			% wt. in waxes	
	-CH=CH	$-CH_2$	$-CH_3$		
	5-6	1.2-1.7	0.9-1	SO	SD and SP
10	0.96	57.8	9.46	20.30	79.70
20	0.84	72.34	13.57	12.38	87.62
30	0.71	61.59	12.69	11.19	88.81
40	-	85.62	7.39	0	100
50	-	58.2	9.46	0	100

4.8.3 Isopropanol as solvent, Fractionation Crystallization technique.

Table 4.8.3. Integration and % of the components for each fraction separated by TREF

Elution temperature	integrating of each spectrum peak			% wt. in waxes	
	-CH=CH	$-CH_2$	$-CH_3$		
	5-6	1.2-1.7	0.9-1	SO	SD and SP
10	1.17	66.75	7.76	30.15	69.85
20	1.13	78.9	10.67	21.18	78.82
30	0.92	62.95	11.69	15.74	84.26
40	-	93.72	7.39	0	100
50	-	56.13	6.43	0	100.

According to the data in tables and Appendix A, all fractions eluted, the peaks at 4.6-5.9 exhibit vinylic proton (C=C-H) in stearyl oleate structure and the peaks at 0.9-1.7 show proton of the methyl (CH_3) and methylene ($-CH_2-$) groups. Percentage of each component was calculated from the integration of the characteric peaks.

BIOGRAPHY

Miss Taveeporn Siamsakul was born on October 28, 1987 in Nakhonsawan. She received the B.Eng. Degree in petrochemical and Polymeric Materials at Silpakorn University in 2010. Since then, she has been a graduate student studying in the program of Petrochemistry and Polymer Science at Faculty of Science, Chulalongkorn University and finished her study in 2012.

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