NANOENCAPSULATION OF EUGENOL BY BETA-CYCLODEXTRIN

BY

SHUNYA RODPOTHONG

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ENGINEERING (ENGINEERING TECHNOLOGY)
SIRINDHORN INTERNATIONAL INSTITUTE OF TECHNOLOGY
THAMMASAT UNIVERSITY
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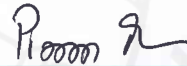
Advisor and Chairperson of Thesis Committee 
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Abstract

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Eugenol compound is one of the major components that can be extracted from the Thai sweet basil oil, *Ocimum basilicum*. Eugenol has been used for antibacterial activity and dental application due to its antiseptic and anesthetic properties. However, their products are facing the short shelf-life problem due to degradation of active compound from sunlight and oxidation. In order to overcome these problems, the active compounds from plant can be preserved by nanoencapsulation technique. β-cyclodextrin (β-CD) are used as a host for nanocapsule inclusion complex. Simulation result from molecular modeling indicated the formation of an inclusion complex with β-CD, which is water soluble cyclic oligomers of glucoses, is 1:1 molar ratio. The objectives are to evaluate the proper technique for inclusion complex nanocapsule formation and preservation efficiency of active compound in the complex. The formation of inclusion complex of β-CD and eugenol was carried out using three methods - normal grinding, cyclone mixing and microwave. The encapsulation efficiency of eugenol in β-CD inclusion complex from different methods was determined by UV-visible spectroscopy, GC, FT-IR and DSC. The results showed that, microwave is the most efficient technique of the inclusion complex nanocapsule formation. Moreover, the amounts of eugenol in the complex before and after the sunlight and air exposures were compared, using GC, in order to evaluate preservation efficiency.

Keywords: β-cyclodextrin, Encapsulation, Eugenol, Inclusion complex, Microwave

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Chapter 1
Introduction

Herbs are plants those provide benefits for living organisms. For thousands of year, human had studied about herbs and applied them in many fields of application both in interior and exterior to the body. Herbs can be used to consume for food and drug, smell for aroma therapy, and applied as a skincare. Since Thailand locates near the equator, the climate is warm, humid and the fertility of the soil, which is suitable for herbs planting. The topic of herbs has included in a part of the Eleventh National Economic and Social Development Plan of Thailand [1]. The health ministry of Thailand undergoes several campaigns about using Thai herb for curing people and enforces people to plant herbs that can encourage the using of herbs to save the money from importing medicinal drugs from abroad, which can provide high benefit to the country. Thai herbs are considered as industrial crops as they are beneficial through their various uses e.g. pharmaceutical, cosmetic, and food products. The high demand of herb is evident from Thai herb export data shown in Table 1.1.

Table 1.1: The Export value of Thai Herb and Spice from Information and communication Technology Center with Cooperation of the Customs Department [2]

Country	Value : Million Baht						
	2008	2009	2010	2011	2012	2013	2014
World (Total)	2498.0	2513.1	2577.7	2571.2	2814.7	2771.3	3397.5
ASEAN (9)	201.4	204.9	233.3	231.1	485.7	460.3	568.8
Japan	583.3	403.0	525.5	596.2	555.5	589.4	841.4
U.S.A	158.3	143.2	129.5	133.8	114.8	153.4	267.8
EU (27)	394.0	373.6	373.2	250.2	268.7	215.4	321.8

However Thai herb products are facing with short life time problem especially the degradation of an important active components in the products by air and sunlight. The problems include smell stability and the changes in physical properties of the products. These problems can cause the difficulty in storage, package and usage of products, which lead to a high investment cost. Moreover, the use of a variety of herbs

require high amount of herb as raw materials, which also cost more in raw material and transportation. For another problem of herbal product, a lot of existing herbal products requires a high number of frequent uses to obtain a desired effect. Expensive herbs or extracts from plants, including the herbs that are not in their crop season, cannot withstand an exposure to the environment and sunlight which make them to be limited availability.

Therefore, Nanoencapsulation [3], an inexpensive and effective technique, is introduced to overcome these problems in a way that it can extend the products shelf-life by increase the stability, from the air and sunlight exposure. Nanoencapsulation can encapsulate the active compound and also provide well distribution of active compound throughout the products. The technique can effectively preserve the active compounds from environment and reduce the amounts of herbs uses.

In order to encapsulate the active compound from herb, the host molecule must be selected. The host molecule should be nontoxic and be able to capture and preserve the compound inside the capsule. β-cyclodextrins [4] are nontoxic cyclic oligomers of glucose that can dissolve in water. β-cyclodextrin molecule has a truncated-cone shape structure with diameter around 1 nanometer. The inner surface cavity is hydrophobic, and outer surface is hydrophilic. The diameter of β-cyclodextrin is large enough for the non-polar herbal compounds to enter into the cavity and be captured inside the capsule. A nano-sized capsule of β-cyclodextrin can increase water solubility of the substances, the stability in term of storage or encapsulation, and improve the release behavior according to the desired time and place. Nanoencapsulation using β-cyclodextrin would increases the surface area of the product to enter the target or reach the desired effect more effectively. β-cyclodextrin is widely used in many pharmaceutical, drug delivery, and chemical industries across the countries [4]. Therefore β-Cyclodextrin is used in this project according to the least expensive and safety among other cyclodextrins.

The problem of nanoencapsulation using cyclodextrins with Thai herbs is the lack of current researches that specifically investigate the overall technique procedure of production, including high yield production of the inclusion complex. Unfortunately, this technique has been overlooked due to the understanding that it can be performed in a lab scale basis and cannot be applied to the local products. This project directly

emphasizes on encapsulation procedure that will improving the yield of inclusion complex formation with low cost technique and the complex stability testing in different storage conditions to apply to herbal product in the market.

The interested Thai herb for this project is Thai sweet basil which can be easily found and contained a distinct odor. The essential compound of sweet basil used in this project is in the form on essential oil. Many important compounds in the form of oil has been researched in the relation to Thai sweet basil oil and will be used as supports and references for this project. One of major components in Thai sweet basil oil, eugenol, is selected to be the guest molecule for the nanoencapsulation in this study. Eugenol is an active compound that widely used in dentistry with zinc oxide due to its antimicrobial properties [5].

From the study of host-guest interaction and nanoencapsulation of eugenol using β-Cyclodextrin, the possible application can be used in other active compounds from plant in many products such as spa product, cosmetic, fragrance textile and etc. This study also cover the application of nanoencapsulation in fragrant textile. Several literatures on textile industry exhibit different methods of attaching β-CD with textile fiber. In order to preserve the fragrant on the fabric, the essential component is captured inside the capsule or host and release at certain condition. From that concept, this study is focus on some attachment method of cyclodextrin and the fabric fiber, and performs in order to keep the essential oil in the cavity of cyclodextrin that attach on the fabric fiber. The concept is shown in the figure below. Cyclodextrin, as the host, is attached with the fabric fiber first. Follow by the nanoencapsulation of eugenol and some other essential oil. The concept is shown in figure 1.1.

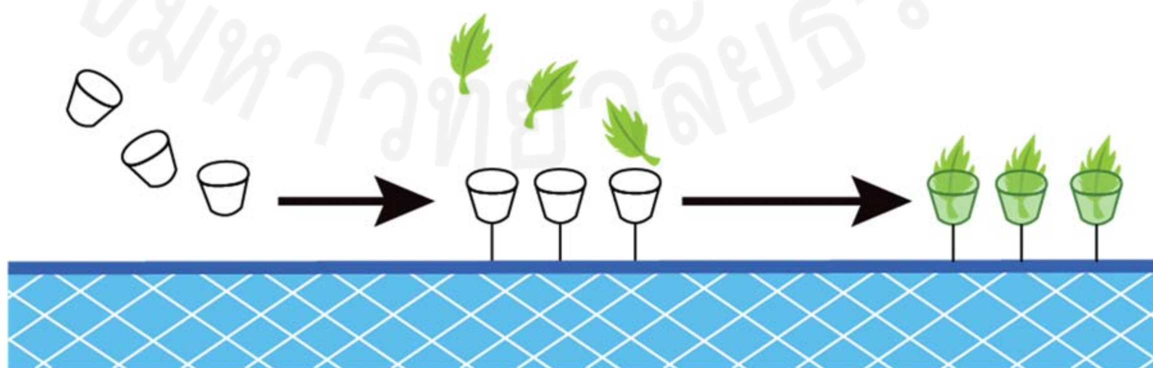

Figure 1.1: The concept of cyclodextrin attachment on the fabric fiber, and follow by the nanoencapsulation of essential oil from herb.

Objective

The primary objective of this thesis is the development of eugenol encapsulation procedure. Different inclusion complex preparation methods are studied, and the most effective method will be selected due to the highest percentage of eugenol encapsulation. The method selected should be ease, inexpensive and less complicated as much as possible. The inclusion complex of eugenol and β-cyclodextrin, which is the result of the preparation method, is study on the releasing rate and the tolerance of inclusion complex under different storage conditions. The objectives of this study are:

1. Study on nanoencapsulation of inclusion complex formation between β-cyclodextrin and sweet basil oil, specifically eugenol.
2. Research and develop the nanoencapsulation of inclusion complex formation procedure of eugenol oil and β-cyclodextrin, in order to improve nanoencapsulation technique for a high capture capacity and chemical and physical stability.
3. Test on the stability of β-cyclodextrin and eugenol inclusion complex under difference possible storage conditions, that the encapsulation can withstand the environment exposure.

Scope of thesis

This study is focusing on the development of nanoencapsulation of extracted essential oil from sweet basil, which majority is eugenol, and the host in the form of nanocapsule that generally used in drug delivery application, which is β-cyclodextrin.

Several techniques on nanoencapsulation is studied, and the most 3 possible techniques will be selected and compare the effectiveness under the same conditions. The encapsulation technique that gives the highest percentage of encapsulation will further develop to obtain the best optimal conditions for the production on local industry, which is ease and inexpensive. As well as the stability of the end product, it will be tested on the different environment exposure, to confirm the advantages of using nanoencapsulation.

Chapter 2
Literature Review

2.1 Encapsulation Techniques

Nanoencapsulation is the study to improve the performance and safety of chemicals [6]. Encapsulation is the sub-category of controlled release technology. Encapsulation concept is aim to encapsulated the active compound in the capsule of barrier polymer, which is designed to control the rate of release of active material at the active site. There are 3 different of release mechanisms. First is the constant release over time. Second is the release rate that diminishing over time. The last one is the burst release, that all of active material is suddenly released at particular time. The simple form of the capsule is consists of a small ball surround by a homogenous coating, as in figure 2.1(a). Another form of encapsulation is the nanosphere; where the active materials are disperse in the matrix of wall material, as in figure 2.1(b).

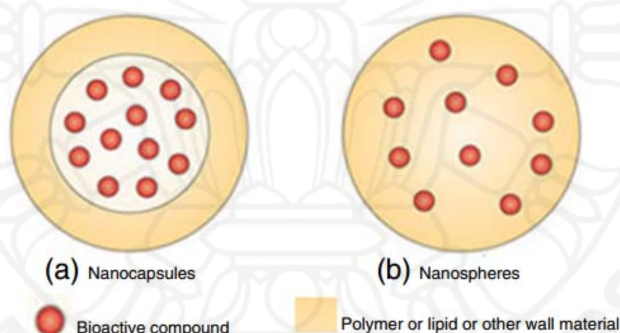

Figure 2.1: Schematic structure of (a) nanocapsule and (b) nanosphere [3]

Choice of shell or wall material is depended on the purpose of encapsulation. Examples of wall material are agar, albumin, cellulose and its derivatives, gelatin, paraffin, etc. All the wall material must able to keep the core inside. It implies that coating materials are met the requirement of core material. Good wall material must prevent the entrance of undesirable material and also release the core material at the right time, right place at the right releasing rate. Different techniques have been developed for the production of nanocapsules. In general, these could be divided into three main groups [7]:

1. Physical processes: spray drying-coating, extrusion, and spray drying.
2. Physiochemical processes: coacervation, entrapment into liposome.
3. Chemical processes: interfacial polymerization and molecular inclusion.

In order to select the appropriate encapsulation process for a given purpose, it is necessary to know the required size of the core particles, the physicochemical properties of

the core material as well as the nature of the substance to encapsulate and the wall material. Final properties of particle are determined by all those parameters. For food and medicine molecule, mostly is hydrophobic molecules. Thus, amphiphilic structure is introduced to deliver hydrophobic drug to the body blood system that is hydrophilic environment. In this study, the cyclodextrins are consider as the wall material or the host, and eugenol, the extracted essential oil of sweet basil, is consider as the core material or the guest molecule. In Table 2.1, the method of eugenol capture using different encapsulation techniques are shown with the percentage of eugenol captured.

Table 2.1: Methods of Eugenol Captures

Capture Method	Condition	% Eugenol Captured	Reference
Molecular inclusion	β-CD or 2-HP-β-CD was dissolved in 30 ml of distilled water at 55 ºC for 30 min, and then eugenol (host: guest molar ratio of 1:1) was added into the β-CD or 2-HP-β-CD solution. Then put into a shaking-incubator at 250 rpm, 25 ºC for 6 h for inclusion and freeze-dried to form a powder	β-CD/eugenol = 90.9 2-HP-βCD/eugenol = 80.9	[8]
Emulsion-Diffusion method	200 mg of PCL and 500 mg of eugenol were dissolved in 20 ml EA phase saturated with water, and emulsified by an aqueous solution using Ultra-Turrax T25 high-speed homogenizer at 9500 rpm for 5 min and freeze-dried at -55 ºC	100	[8]
Emulsification using Millard-type conjugates (whey protein isolate and maltodextrin) and spray-dried	Emulsion by homogenizer at 15,000 rpm for 3 min. The aqueous phase was dissolved with conjugates at an overall protein concentration of 3.7 g/100 g. A mixture of WPI:MD mass ratios of 1:2 was prepared by spray drying at 90 ºC for 2 h and spray dried at 150 ºC inlet temperature, 6.67 mL/min feed rate, 600 kPa compressed air pressure and 35 m3/h air flow rate prepared by spray drying at 90 ºC for 2 h and spray dried at 150 ºC inlet temperature, 6.67 mL/min feed rate, 600 kPa compressed air pressure and 35 m^3/h air flow rate	35.7	[9]

Table 2.1: Methods of Eugenol Captures (continued)

Capture Method	Condition	% Eugenol Captured	Reference
Solid inclusion complex	Mixing eugenol with α-, β-, γ- or DMb CD respectively in distilled water, and stirring 48 h at 293 K, and washed by using water and alcohol (95%) three times respectively and dried over 24 h at 383 K in vacuum	Not available	[10]
SCF method	50 ºC , 80 bar (which corresponds to conditions of highest solubility of essential oils in supercritical CO_2), and a contact time of 6 h	Not available	[11]
Coacervation	Solution of gelatin and sodium alginate in ratio 4:1 was prepared by hydration followed by stirring. Microcapsules were prepared at 40 ºC by drop wise addition of dilute HCl and chilled for 30 minutes. Formaldehyde solution, 37% (1 ml/g of gelatin) was added and stirred for 2 h at room temperature. Microcapsules were then separated by decanting the supernatant, filtered under vacuum, washed with water till free from formaldehyde and air-dried. Microcapsules were sieved through 22 mesh sieve and air-dried at room temperature.	38.2	[12]
Modified hot-homogenization ultrasonication	Stearic acid in combination with caprylic triglyceride was dissolved in 5 ml of acetone and then eugenol was added in the lipid solution. Solvent was evaporated on water bath with nitrogen purging. The resulting mixture of drug and melted lipids was dispersed in previously warmed (5 °C more than m.p of lipid) Poloxamer 188 (1%, w/v) solution by using high shear homogenizer (Ultraturrax, IKA Germany) for 5 min at 12,000 rpm. Resulting suspension was ultrasonicated (Dr. Heilscher, Germany) for 15 min at 40% amplitude and 0.5 s frequency	98.52	[13]

2.2 Beta-cyclodextrin (β-cyclodextrin or β-CD)

There are a family of cyclic oligosaccharides composed of alpha-1, 4 linked glucopyranose subunits, and glycosidic bond formed. They are produced as a result of intra-molecular transglycosylation reaction from degradation of starch by cyclodextrin glucanotransferase (CGTase) enzyme (about 3 g/kg of starch) [4]. It appears that in cyclodextrins the secondary hydroxyl groups (C_2 and C_3) are located on the wider edge of the ring and the primary hydroxyl group (C_6) on the other edge, and that the apolar C_3 and C_5 hydrogen and ether-like oxygen are at the inside of the torus-like molecules. The cyclodextrin structure is shown in Figure 2.2.

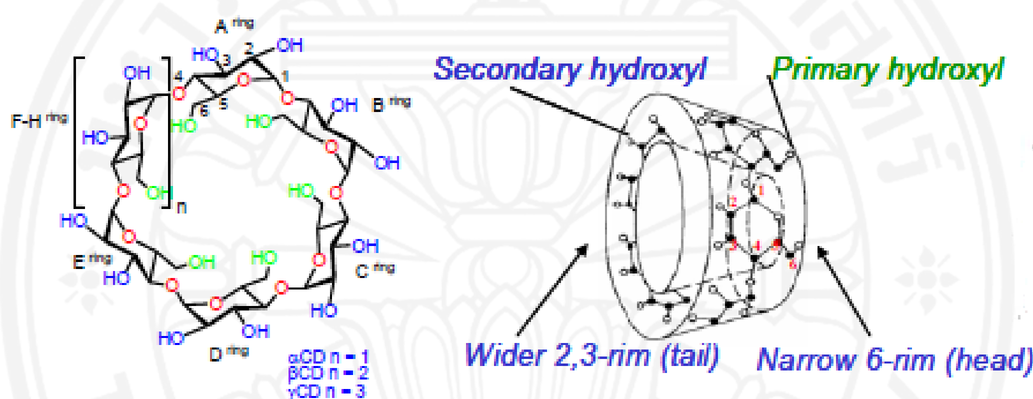

Figure 2.2: Cyclodextrin structures [14]

Alpha (α)-, beta (β)-, and gamma (γ)-cyclodextrins (CD) are parent cyclodextrins that composes of six, seven, and eight glucopyranose subunits, respectively [15]. β-CD is the most accessible, the lowest price, low water solubility and generally the most useful among of three CDs [16]. The comparison of three cyclodextrin is shown in table 2.2 and 2.3.

Table 2.2: Prices of Cyclodextrins (Sigma Aldrich, 2012)

Types	Prices of Cyclodextrins (SGD)		
	α	β	γ
Normal (≥98%)	481.5/ 25 g	111/ 25 g	2,005/ 5 g
Powder, Bioreagent, suitable for cell culture (≥98%)	252.5/ 5 g	72.7/ 5 g	786/ 1 g

Where A/B = price/packed size

Table 2.3 : Properties of Cyclodextrins [16]

Properties	Types of Cyclodextrin		
	α	β	γ
No. of glucose units	6	7	8
Molecular weight	972	1,135	1,297
Solubility in water, g 100 mL^{-1} at room temp.	14.5	1.85	23.2
Cavity diameter, Å	4.7-5.3	6.0-6.5	7.5-8.3
Height of torus, Å	7.9±0.1	7.9±0.1	7.9±0.1
Diameter of outer periphery, Å	14.6±0.4	15.4±0.4	17.5±0.4
Approx. volume cavity, $Å^3$	174	262	427
Approx. cavity volume in 1 mol CD, mL	104	157	256
Approx. cavity volume in 1 g CD, mL	0.10	0.14	0.20
Crystal forms (from water)	Hexagonal plates	Monoclinic parallelograms	Quadratic prisms
Crystal water, wt%	10.2	13.2-14.5	8.13-17.7
Diffusion constant at 40 °C	3.443	3.224	3.0
pK (by potentiometry) at 25 °C	12.332	12.202	12.081

Cyclodextrins can perform the host-guest inclusion complex, due to their torus-like structure which possesses hydrophobic cavity and hydrophilic at the outer surface [4]. The hydrophobic cavity of cyclodextrin molecules provides a microenvironment into which appropriately sized non-polar moieties can enter to form inclusion complexes. No covalent bonds are broken or formed during formation of the inclusion complex [4]. The driving force of the complex formation is the substitution of the high-enthalpy water molecules by an appropriate guest molecule. A more hydrophobic guest, comparing to water molecules displaces in the cavity then an apolar-apolar interaction occurs. The substitution decreases cyclodextrin ring strain resulting in a more stable lower energy state.

Inclusion complex benefits the modification of guest molecules which is not achievable by other methods. It enhances many properties which are [4, 17]:

- Solubility enhancement of highly insoluble guests
- Stabilization of labile guest against degradation effects of oxidation
- Control of volatility and sublimation
- Physical isolation of incompatible compounds
- Chromatographic separations
- Taste modification by masking off flavors

- Unpleasant odors and controlled release of drugs and flavors
- Directing of chemical reactions
- Control of fluorescence and light absorption

There are two key factors of the ability of a cyclodextrin to form an inclusion complex. The first is steric effect that depends on the relative size of the cyclodextrin to the size of the guest molecule or certain key functional groups within the guest [4]. The second one would be the thermodynamics interaction between the different components of the system (cyclodextrin, guest, solvent).

A displacement of the complex guest by another guest requires heating. Water can replace the guest. An equilibrium will be established between free and complex-cyclodextrin, the guest and the dissolved and non-dissolved complex.

β-cyclodextrin has been used in cosmetics industries, for instance, preserving the important contents in tea tree oil [17] and protecting hydroquinone in skin whitening [18]. It also improves the solubility on ingredients of the products such as Salicylic acid [19] and Retinoic acid (anti-aging compound) [20].

Table 2.4 summarize on the applications of using cyclodextrin to capture chemicals. The method of complex formation is also varied. From the literature reviews, the potential of using β-CD to encapsulate eugenol has a high possibility by the properties of cyclodextrin itself. However, the capturing method needs to be further investigated to achieve the high amount of eugenol which is encapsulated in the cyclodextrin.

Table 2.4: Guest Molecule Capturing of Cyclodextrin

Host	Guest	Method	Note	Reference
β-CD	Garlic Oil	Co-precipitation (1:1 molar ratio)	Release of guest by elevating temp from 25-50 ºC and in an acidic dissolution medium (pH 1.5)	[21]
α-CD	CO_2	Pressurization using High Pressure Micro reactor (N_{MAX} (mol CO_2/mol α-CD) = 1.41)	There are 4 condition of α-CD were used, Different initial moisture content (2, 10, and 30 wt%; 10 g each) and saturated α-CD solution (150 ml). Then CO_2 was filled to reactor under pressure of 3 MPa. After encapsulation the crystal were separated from liquid by vacuum filtration. The inclusion complex was washed with cold water and dehydrated acetone before being filtered again.	[22]
β-CD	Thymol and Cinnanaldehyde	Co-precipitation (1:1 molar ratio)	Guests were added to saturated solution of β-CD which heated at 55 °C. Stirred solution for 4 h, then cool to ambient temperature and stored overnight at 2 °C. Filtrated the precipitated complex and immersed in liquid nitrogen. Pulverized by mortar and stored under vacuum over $MgClO_4$.	[23]
β-CD	Fish Oil	Self-assembling aggregation using shaking-incubator at 250 rpm, 25 ºC for 4 h	0.3% w/w of β-CD was added to de-ionized water. Fish oil was added to β-CD solution with ratio 10:20 (β-CD:Fish Oil (w:w)). Then the mixtures were place in shaking-incubator for encapsulation by self-assembling aggregation at 250 rpm, 25°C for 4 h. The encapsulation efficiency 84.1%, Fish oil loading (62.7%), fish oil leakage after freeze-drying (11%).	[24]

Table 2.4: Guest Molecule Capturing of Cyclodextrin (continued)

Host	Guest	Method	Note	Reference
β-CD	Carvedilol	Microwave irradiation for 90 s in 60 ºC (1:2)	0.04 mmol of β-CD and 0.02 mmol of carvedilol were mixed in a glass container with minimum amount of solvent added (EtOH:water = 1:1 v/v). The mixture was reacted at 60°C for 90s in the microwave oven. After reaction, the solvent were added to remove the residual, and then precipitate was filtered. Drying in vacuum oven at 80 °C.	[25]
Hydroxypropyl-β-CD	Simvastatin	Supercritical antisolvent (SAS) process.	Dissolved with 1:1 molar ratio in the mixture solvent of dichloromethane and ethanol (1:2 v/v %). The SAS process was performed at 40 ºC and 120 bars. Spray to form the particle, removed the residual solvent by precipitation and flow SC-CO_2 to vessel for 120 min.	[26]
α-CD	Nylon 6	Solvent mixing and stirring under control temperature	0.15 g nylon-6 was dissolved in 3 ml of formic acid, and then 12 ml of acetic acid was added to the solution. 8 g of α-CD was dissolved in 40 ml of dimethyl sulfoxide, and then to nylon-6 solution. The mixed solution were stirred on hot plate for 2 h at 50 °C and then cooled to room temperature, while stirring for 6 h. Vacuum filtered the solid precipitate and dry with heating lamp in vacuum oven at 40 °C.	[27]
Hydroxypropyl-β-CD (HP- β-CD)	Hydrocortisone acetate (HC)	Spray-drying method	HC and HP-β-CD were dissolved separately in 400 ml of 96% ethanol and 400 ml of water. Then solutions were mixed together, sonicated for 15 min and subject to spray drying under the followed conditions: flow rate 0.25 l/h, inlet temperature 90 °C, outlet temperature 60 °C, and air flow rate is 700 NI/h.	[28]

2.3 Guest Molecule

The guest molecule used in this experiment is eugenol, which is a compound that found in sweet basil oil. Sweet basil (*Ocimum basilicum Linn.*) is a culinary herb that widely cultivated in Thailand. Certainly, sweet basil can be consumed directly as a fresh herb. Parts of sweet basil are benefit to our daily life basis. Its seed can be used to produce laxative [29]. Basil leaves are rich in beta-carotene which is used as a major constituent in cosmetics products and able to reduce risk of several chronic diseases including cancer and cardiovascular disease [30]. Its flowers and leaves are also used as carminative, galactogogue, stomachic, and antispasmodic medicinal plant in folk medicine [29]. Moreover, its extracted product can also be used in many industries especially in medical and perfumery.

Essential oil from sweet basil consists of many chemical components. The uses of its essential oil for antimicrobial and antibacterial in oral cavity are reported [31, 32]. These properties can be attributed to essential oil constituents which are euclyptol, estragole, eugenol, linalool, and methyl cinnamate [32-34]. The chemical structures are shown in figure 2.3. Major contents in the extracted oil are eugenol and linalool. However, chemical compositions depends on seasonal variations and its origin [32, 35]. Most components are polyphenol and in the class of flavonoids and anthocyanins.

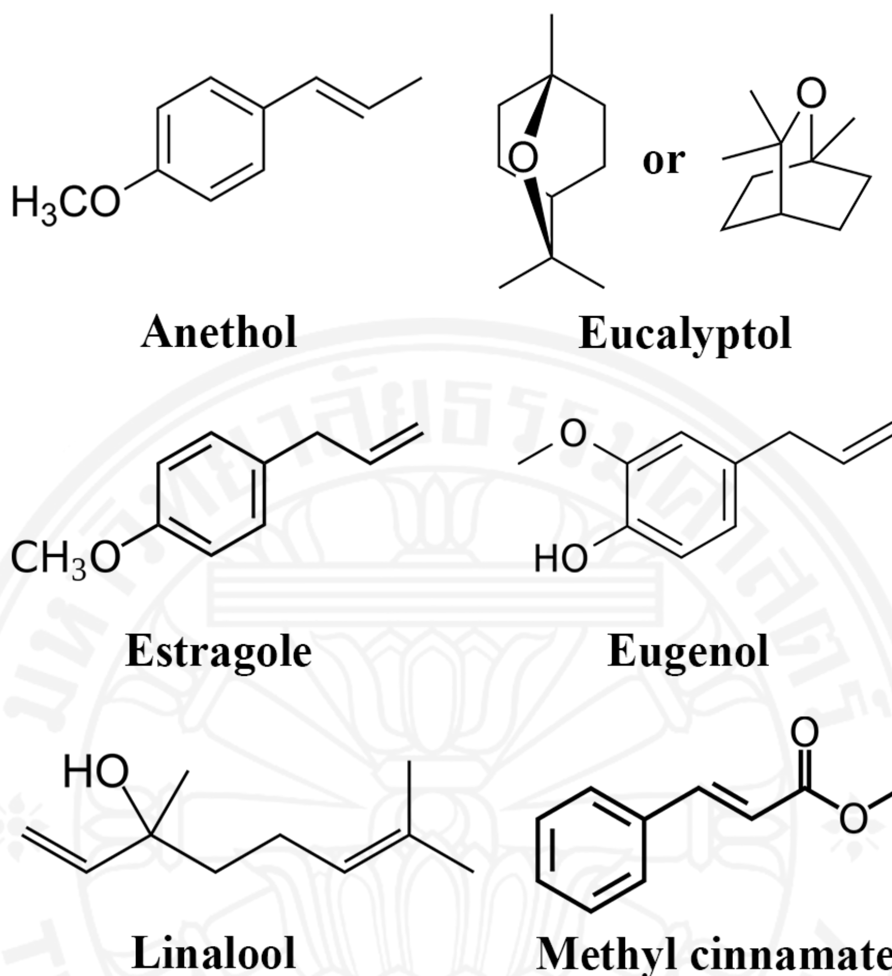

Figure 2.3 Structure of chemical components in Sweet basil oil

2.3.1 Eugenol

Eugenol ($C_{10}H_{12}O_2$), is phenylpropeen compound. It can be found in nutmeg, clove, cinnamon and sweet basil. Sometime it could be called as clove oil. Pure eugenol oil has clear and pale yellow color as show in figure 2.4. Eugenol has antiseptic and analgesic properties [5]. Therefore, it is used widely in medical and dentistry applications as ingredient of painkiller, toothpaste and filling material [5]. Moreover, eugenol is also applied in food, and perfumery industry. However, its hydrophobicity makes it easily oxidized by air and sunlight. Consequently, eugenol shelf life is very short and it limits the usage of eugenol.

Figure 2.4: Pure eugenol oil in the test-tube is pale yellow color liquid

From Hazardous Substance Data Bank or HSDB [36], eugenol vapor pressure is 0.0221 mm Hg at 25 °C when release to air. It indicated that eugenol will exist solely as a vapor in the atmosphere. Vapor phase of eugenol will be degraded in the atmosphere by photochemically-produced hydroxyl radical and its reaction with ozone. The half-life of both reactions are 5.9 hours and 23 hours respectively. If eugenol exposed to sunlight, photodegradation may occur on the surface of exposure. Therefore, eugenol is easy to be degraded when it is exposed to air and sunlight, and then eugenol may change in their properties.

Eugenol is considered as hepatotoxicity. The ingestion in a sufficient amount can lead to life-threatening complications, including acute effect on respiratory system and central nervous system. However, the 50 % lethal dose, 1,930 mg/kg of rat [37], is very much in dispute. Eugenol is rapidly absorbed and metabolized in the liver when ingested, and 95% of the dose is excreted within 24 hours [38]. The overdose of eugenol can causes shallow, rapid breathing, coughing up blood, blood in urine, burns in mouth and throat, abdominal pain, nausea, rapid heartbeat, dizziness, seizures, and even coma. The following tables show the uses of eugenol with different amount from literatures.

Table 2.5: Uses of Eugenol

Amount of Eugenol	Application	Condition	Reference
50% approx. 80 μM	Act as a metal ion in the inhibition of lipid peroxidation	Low density lipoprotein was diluted to the concentration of 60 µg/ml with 10 mM potassium phosphate buffer (pH 7.5) containing 1.5 µM of EDTA and 0.15 M NaCl. LDL oxidation was performed at 37 ºC in 1 ml of 10 mM phosphate buffer (pH 7.4) containing 2 µM $CuSO_4$, 0.15 M NaCl, and 1.5 µM eugenol or isoeugenol in the absence and presence of 5 µM a-tocopherol in a total volume of 1 ml.	[39]
varies from 40% to 71% in essential oil that extracted from several plant	Eugenol in Pharmacological action	Review on content of eugenol in in several plants.	[40]
40 μM for 4 h	-Induction of apoptosis of HL-60 cells.	MTT assay, adding eugenol to sample cell after 24h of culturing. Several concentration of eugenol was used, and incubated for an addition 4 h.	[41]
0.5-25 μM	Eugenol used for inhibit the proliferation of melanoma cells, which is a cancer cells.	0.5-2.5 µM eugenol in ethanol was treated for 18 hours. It causes the apoptosis to melanoma cells.	[42]

Table 2.5: Uses of Eugenol (continued)

Amount of Eugenol	Application	Condition	Reference
100, 200 and 400 mg/kg	Anti-inflammatory and antinociceptive activities of eugenol were test in rats and mice.	Male Swiss mice weighing 25 ± 5 g were used in the experiments on antinociceptive activity and acute toxicity. Male Wistar rats weighing 200 ± 30 g were used for the evaluation of the anti-inflammatory activity. Then the groups of rats were pre-treated with eugenol (100, 200 and 400 mg/kg, *p.o.*), indomethacin (5 mg/kg, *p.o.*) and celecoxib (10 mg/kg, *p.o.*) as the standard drugs, or water (0.1 mL, *p.o.*) as the control.	[43]
35, 50, 65, 85, 100 or 135 mg/L	The safety dose and duration of exposure of eugenol for use as anaesthetic on tambaqui, a kind of fish.	Eugenol was dissolved in alcohol at 10mL/20mL Ten juvenile and five sub-adults were individually exposed to either 35, 50, 65, 85, 100 or 135mg/L eugenol for a period of 10 min at 26-27 ºC. Second experiment vary with time from 10-30 min of eugenol exposure with concentration of 65 mg/L	[44]
10.05 mM (0.15%) and 16.75 mM (0.25%)	Determine how eugenol affects fermentation parameters and faecal coliforms in cattle and swine waste slurries stored anaerobically.	Wastes, faeces, urine and distilled water in the ratio 50: 35: 15 (weight basis) were blended for 1 min. The sample was mixed with 15 ml of 0.5 M H_2SO_4, centrifuged at 2000 *g* for 20 min at 4 ºC, and stored at 20 ºC until analyzed. Two concentration of eugenol were added and faecal coliform were eliminate within 1-2 days after adding.	[45]
Not available	Eugenol-containing sealer on marginal adaptation of dentine-bonded resin fillings	Teeth in group 1 were not contaminated with eugenol. Cavities in groups 2±4 were contaminated with a eugenol based sealer (Tubli-Seal, Kerr) and cleaned with: (i) sandblasting, (ii) bur finishing alone or (iii) bur finishing combined with swabbing with alcohol. These 4 groups were temporized for 6 weeks (Ketac, ESPE), whilst group 5 received no eugenol, was not temporized and acted as the control.	[46]

2.4 β-CD Attachment Method

Functional textile is the innovation that increases the value of textile to be not only the clothing. Self-cleaning textile, fragrance release textile and other are developed since the end of 20th century. The application of textile with β-CD is the capsule that carried desired effect molecule and release accordingly. The method of attachment that uses in this study is selected from the literature and applied the encapsulation method in the procedure. A. Rukmani had work on the inclusion complex of thymol and β-CD on cotton textile to create the antibacterial textiles [47]. Another functional textile and preparation method with β-CD will be show in the Table 2.5. The method of attach β-CD with the textile could be done by spraying, painting, padding, grafting, coating, sol gel and more [48]. However, the most suitable method is grafting of A.Rukmani, because this process required less special equipment and chemical that is safe and reasonable to apply in household industry.

Grafting is a method of fixation by using crosslinking agents as a connection between fabric and β-cyclodextrin. The crosslinking agent the mostly used are carboxylic acid, homobifunctional reactive dye and derivative β-CD.

Grafting with polycarboxylic acid

General used agent consists of a polycarboxylic acid, which is form the esterification between hydroxyl group of cellulose and hydroxyl group of β-CD as shown in figure 2.5. The fabrics used for this technique are cotton, wool, polyamide and polyester fiber.

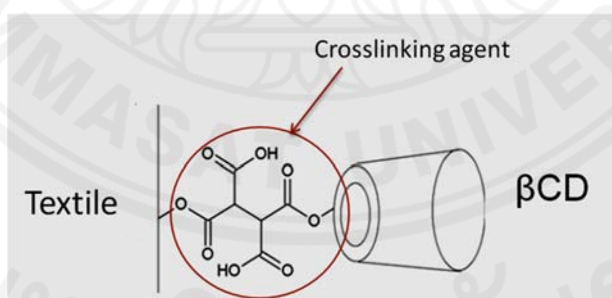

Figure 2.5: Grafting of β-CD via 1,2,3,4,butanetetracarboxylic acid (BTCA) onto a cotton textile [48].

Grafting with derivative β-CD

MCT-β-CD or monochlorotriazinyl-β-cyclodextrin is widely used on textile material [49]. The monochlorotriazinyl group is able to form covalent bonding with hydroxyl group in cellulose. The fixation method is under relative mild condition and uncomplicated. The ready to used MCT-β-CD are available in the market or self-preparation also possible. The literature

indicate the maximum attachment to cotton surface when compare with other grafting method [50]. In table 1, it can be seen that several literature are doing in the self-preparation of MCT-β-Cyclodextrin and other derivative by them because the price still high when compare with pure β-Cyclodextrin**.**

For fragrance releasing textile, the inclusion complex of β-CD with aroma molecule can reduce the vapor pressure of fragrance, result in reduction of strong smell, and delay the breakdown of molecules or long term fragrance releasing. At the same time, the empty β-CD could also trap the bad odors and become empty again during washing.

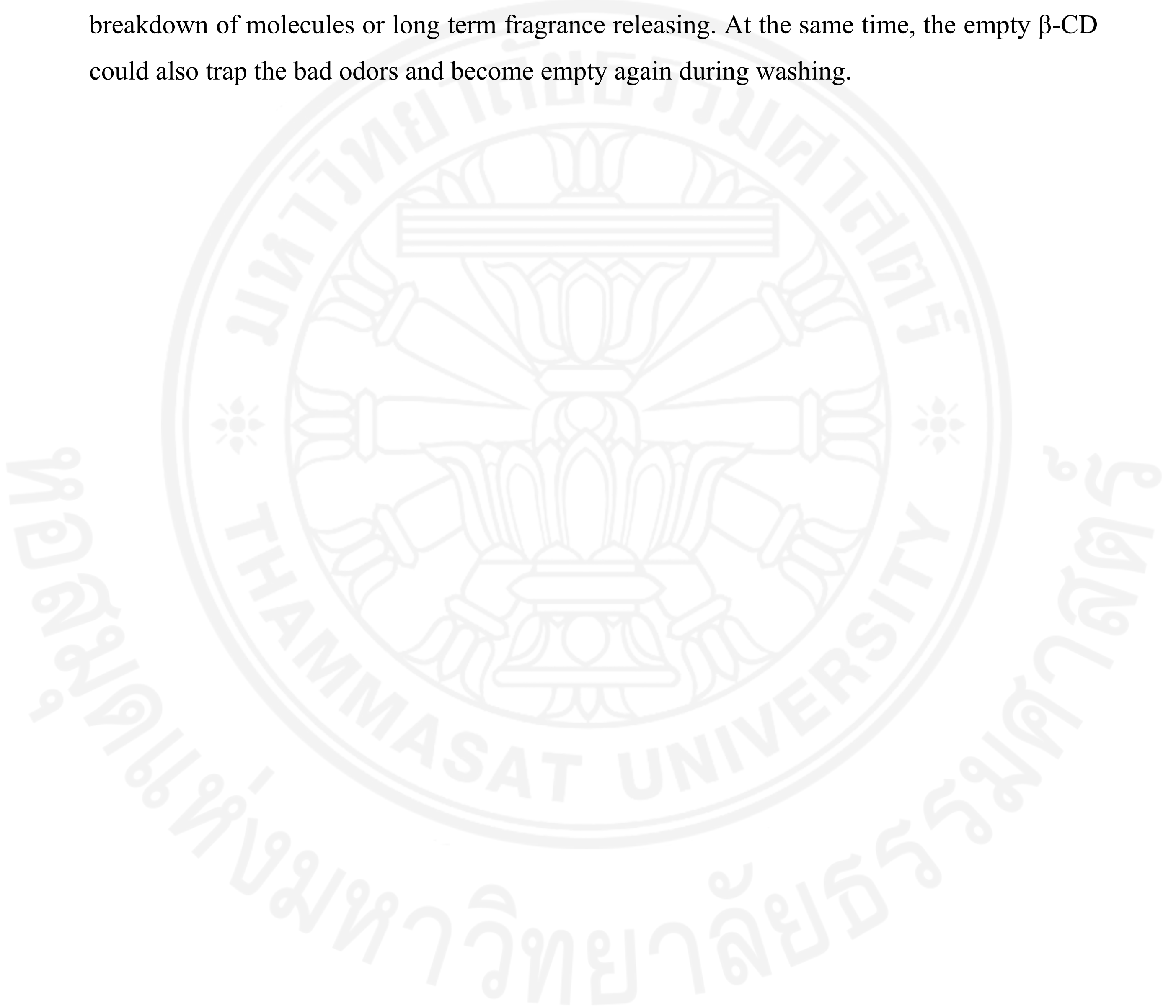

Table 2.6 Fixation method on fabric from literature

Technique	β-CD type	Substrate (fabric)	Material/Chemical	Condition	Fixation and immobility of β-CD
Crosslinking [47]	β-CD (305 SGD/100g)	Organic cotton	-Thymol (75.1 SGD/100 g) -Citric acid (CA) (81.3 SGD /100g) -Sodium hypophosphite (SHP) (catalyst) (249 SGD/ 1kg) -Ethanol	Immersed cotton in the bath that contain 0.8% β-CD, 0.2% CA and 0.15% SHP for 30 mins. Then squeeze and dried at 80°C. Increase the temperature to 150°C for 10 mins. After that immerse fabric in double distilled deionize water for 24 hrs. Squeeze and immerse in EtOH/water (70:30) with 5%Thymol, stirred for 2hr at 40°C Roll squeeze and wash with EtOH (40:60 water) 3 times and wash with tap water and dry.	UV-Vis, FTIR confirm the bonding between CA, β-CD and hydroxyl group of cotton. HPLC determined the content of thymol on fabric. It found that 0.403 % w/w of thymol, compare to 0.018% of ungraft fabric.
Crosslinking [51]	β-CD (305 SGD/100g)	Cotton (pretreat with boil Na_2CO_3)	Cyanuric chloride (86.3 SGD/1kg) NaOH(321S GD/1kg) Methanol (washing) (99.8%, 116.5 SGD/l)	1. Prepared cotton fabric Wash fabric with boiling aqueous Na_2CO_3 for 3 hr. and then dry in ambient condition. 2. Prepare β-CD polymer Dissolve β-CD and cyanuric chloride separately. Keep β-CD in ice bath and stir continuously. Add cyanuric acid dropwisely. Left for 4 hr. Stirring for 12 h at 30°C. Dialyzed and freeze dry powder of β-CD polymer. 3.Grafting in reactor Add 0.5 g of cotton, water and NaOH(to adjust pH) together. Add 0.5 g of β-CD polymer. Continue stirring at constant rate with temp 60-80°C for 12 hr. Dry at 80°C for 4 h with vacuum condition. Washing by water several times, then wash with methanol and dry at 80°C in vacuum condition.	- 10-12 h is the optimum duration for grafting process. - pH slightly have effect with grafting. - FTIR: present of C=N group, indicate the successful of grafting. - XRD: the modified β-CD loses its crystallinity compare to normal β-CD. - TGA: at 248°C the second mass loss occurred. It is the decomposition of modified CD unit. - DSC: β-CD polymer grafted cotton exhibited endothermic transition around 150°C. - 66% more dyeability than ungrafted cotton fabric

Table 2.6: Fixation method on fabric from literature (con't)

Technique	β-CD type	Substrate (fabric)	Material/Chemical	Condition	Fixation and immobility of β-CD
Radiation Grafting [52]	β-CD (305 SGD/100g) hydroxypropyl-β-CD (HP-β-CD)	Cotton Gauze	-Glycidyl methacrylate (GMA) -Nalidixic acid sodium salt -3-methylbenzoic acid (3-MBA) -Ethanol -Acetone -Methanol -Ultra pure water	Wash gauze in ethanol for 2 h and dried under vacuum. Exposed gauze to ^{60}Co γ-source in the present of air at room temp. The dose rate is 1-7.5 kGy. The irradiated gauze was placed in ampoule that contains 10% v/v (for 10ml) of GMA and purged with argon. Then sealed and heated at 60°C for 6 h as the reaction time. Wash with ethanol under stirring and replace the medium each hour for 5 times and once more after 24 hr. Dry gauze under vacuum for another 24 hr. Take gauze to 10ml of DMF for 2 h then transfer to mixture that contain 8ml of DMF, 0.5M NaCl (l) , β-CD and HP-β-CD in equimolar to GMA. Closed and keep under magnetic stirring at 70°C for 24hr. Wash with acetone and water under stirring for 4 days. Dry under vacuum at 50 °C	Suitable condition as describe in condition.
Thermal and Atmospheric Plasma Technique[53]	Per-(2,3,6-O-allyl)-β-CD	Cotton woven fabric	-Allyl bromide Sodium hydride (1065 SGD/250 g) -Oleic acid	Complex instruments are used, plasma machine. Fabrics are exposed to the plasma's active volume the located between two electrodes.	
Grafting [54]	β-CD (305 SGD/100g)	Cotton	2-p-toluidinyl-naphthalene-6-sulfonic acid (potassium salt; TNS) N-methylolacrylamide (NMA) (111 SGD/100g) HCl Formic acid (88.1SGD/100ml) Ceric ammonium nitrate (CAN)	1. CD-NMA synthesis β-CD, NMA and formic acid is purified and then mixed together in reactor with stirrer.80°C & 30 mins. The add acetone and stored at 5°C, then filtrate precipitate and wash with acetone. 2. Grafting The grafting was perform by add cotton fiber with nitric acid sol and stirred for 20 mins. Followed by adding CD-NMA and react at 40°C. After the optimum time, washing water. And dry at 110°C for 1h. 3. Inclusion complex formation	• Retain 100% of bacterial inhibition after 10 cycles of washing. • Higher than 40% grafting of benzoic acid grafted cotton exhibit above 99.5% of bacterial inhibition. • Vanillin fragrance retain after 7 days at room temp and another 7 days at 80°C

Table 2.6: Fixation method on fabric from literature (con't)

Technique	β-CD type	Substrate (fabric)	Material/Chemical	Condition	Fixation and immobility of β-CD
Biopolishing and grafting[55]	MCT-β-CD	Organic cotton	-Reactive blue dye (138 SGD /25g) -Sodium carbonate Na_2CO_3 (72.4 SGD/1L) -Thymol (75.1 SGD/100 g) -Acid cellulase (234.5 SGD/100g) -Sodium acetate (184.5SGD/1kg) -Acetic acid (122 SGD/500ml) -Deionized water	Biopolishing the fabric by immerse organic cotton in enzyme bath contain 3% of acid cellulose, 1hr 55°C,pH4.5, MLR1:10, then raised temp to 80 to deactivate enzyme. Wash and dry. Fixation of MCT- β-CD by soak fabrics in 10% of MCT- β-CD, and sodium carbonate, MRL 1:20, w/v with magnetic stirring at room temp. Squeeze and dry at 80°C and then cured in hot air oven at 150°C for 5 min. rinsed with deionized water.	Optimum condition is 10% of MCT-β-CD, 10% of sodium carbonate, 150°C and treatment duration is 6 mins. After 10 cycles of washing, the decrease of bacterial inhibition was shown.
Grafting [51]	MCT-β-CD	Cotton 100%	Na_2CO_3 (72.4 SGD/1L)	Fabric immersed in bath for 15 mins with β-CD at room temp in Na_2CO_3 solution. MCT-β-CD is added with varied concentration. Squeeze and treated at 150°C for 15 min in oven. Wash for 10 min at 60°C and dry at 80° for 1.5 hr.	Able to fix MCT-β-CD on the surface of cotton
Closslinking [51]	β-CD	Cotton 100%	Ramazol black 5 (90SGD/100g) NaCl (78.2 SGD/250g) Sodium carbonate (72.4 SGD/1L) NaOH(321 SGD/1kg)	Dye, β-CD, electrolyte and cotton fabric were added to the preset dye bath at 40°C for 15 mins. Then add sodium carbonate to the bath and raise temp to 50°C for 10 mins. The add NaOH (1M) and run the fixation cycle for 45 mins. Remove fabric, rinse with warm water and salt. The fabric is boiled in water bath for 20 mins, rinse and dry in air.	Able to fix MCT-β-CD on the surface of cotton

Table 2.6: Fixation method on fabric from literature (con't)

Technique	β-CD type	Substrate (fabric)	Material/Chemical	Condition	Fixation and immobility of β-CD
Esterification[56]	β-CD	Cotton	Itacoic acid Sodium hypophosphite (SHP) (catalyst) (249 SGD/ 1kg)	Prepare β-CD itanonate film. Ceric ammonium nitrate is added to fabric surface to generate free radical. Then graft copolymerization on cotton via follow 3 method. Pad- thermofixation, semi continuous pad-batch method and batch method. After that wash with hot and cold deionized water, and dry at 100°C for 2 hr.	The result shows the chemical bonding of CD on the cellulose surface.
Grafting [49]	MCT-β-CD	Cotton	-Sodium carbonate (72.4 SGD/1L) -Ethanol -Triclosan -Phenolphthalein	MCT-β-CD and Na_2CO_3 were added in water. Then dip fabric in solution for 5 min. Pass through roller padder and dry at 50°C for 10 minute and cured at 150°C for 10 min. then rinse with water and dry.	As amount of MCT-β-CD increase the % grafting also increase, but in non-liner relationship. By phenopthalein and triclosan inclusion complex forming, they imply that the cavity on the grafted fabric is available.
Grafting [57]	MCT-β-CD β-CD	Tencel fabric	Sodium carbonate (72.4 SGD/1L) Formic acid (88.1SGD/100ml) Nitric acid Ceric ammonium nitrate Benzoic acid N-methylolacrylamide (NMA) (111 SGD/100g) Ethanol HCl	Synthesis of NMA-CD first. By put β-CD, NMA and HCOOH in water react at 80°C for 30 min under magnetic stirring. Then add acetone (300ml) and store in refrigerator overnight. Then filtrate and wash with cold acetone and vacuum dried. Grafting tencel with NMA-CD Add tencel to solution that contain 200ml of ceric ammonium nitrate, and 1% of HNO_3. Left for 30 min with argon purge and magnetic stirring. Then add NMA-CD in the mixture and stir for 1 h at 40 °C. Wash with water and neutralize with 1% sodium carbonate. Wash again and dry at 110°C Grafting MCT-β-CD Dip tencel for 5 min in water of MCT-β-CD solution and sodium carbonate under magnetic stirring. Squeeze and dry in oven.	The cavities are available for forming inclusion complex.

Chapter 3
Methodology

3.1 Inclusion Complex Formation

The inclusion complex formation between β-cyclodextrin and eugenol utilizes the molar ratio of 1:1 of host and guest as suggested form the simulation in the previous study [58]. Eugenol is immiscible in water and β-cyclodextrin can dissolve in water so the eugenol solution and β-cyclodextrin solution were prepared separately. The two solution were mixed to form the complex with different methods of normal grinding, cyclone and microwave. Figure 3.1 shows the flow diagram of preparation method of inclusion complex between eugenol and β-cyclodextrin.

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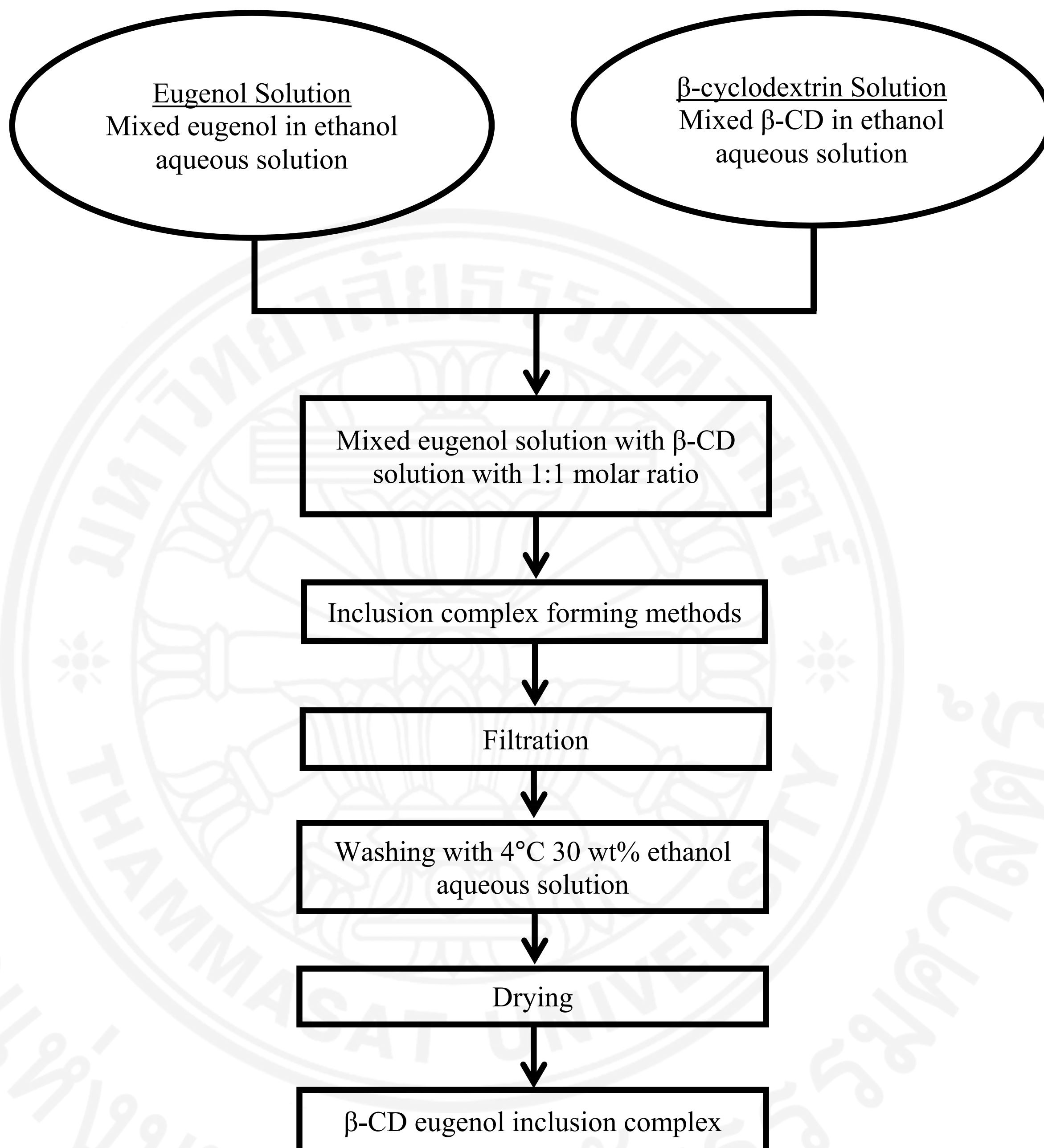

Figure 3.1 The schematic flow diagram of eugenol β-cyclodextrin inclusion complex formation

3.1.1 Calculation of Materials

The basis amount of β-cyclodextrin that used in the experiment is 300 mg, for the ease of collection and result measurement. The simply calculation to obtain the amount of eugenol are calculated using mole of β-CD, then convert to mole of eugenol by using 1:1 molar ratio of β-CD and eugenol. The detailed calculation is shown as following.

From the properties of host and guest, the molecular weight and the density of eugenol are 164.2 g/gmol and 1.06 g/mL, respectively. Molecular weight and the density of ethanol are 46.07 g/gmol and 0.789 g/mL, respectively. And molecular weight of β-CD is 1,135 g/gmol.

From the basis of 300 mg of β-cyclodextrin , to find the mole of β-CD, the calculation can be done as shown below;

$$\text{Mole of β-CD} = 300\text{ mg βCD} \times \left(\frac{1\text{ mg mol βCD}}{1{,}135\text{ mg βCD}}\right) \times \left(\frac{1\text{ g mol βCD}}{1{,}000\text{ mg mol βCD}}\right)$$

$$= 2.64\times10^{-5}\text{ mole β-CD}$$

$$= 26.4\text{ μmol β-CD}$$

Since the basis is set as 1:1 ratio, therefore mole of eugenol is equal to mole of β-CD, thus the mole of eugenol is equal to mole of β-cyclodextrin

Mole of eugenol = mole of β-CD

$$= 2.64\times10^{-5}\text{ mole eugenol}$$

$$= 26.4\text{ μmol eugenol}$$

After mole of eugenol is known, the amount of eugenol could determine by;

$$= 2.64\times10^{-5}\text{ gmol Eugenol} \times \left(\frac{10^{6}\text{μmol Eugenol}}{1\text{ gmol Eugenol}}\right) \times \left(\frac{164.2\text{ μg Eugenol}}{1\text{ μmol Eugenol}}\right) \times \left(\frac{1\text{ g of Eugenol}}{10^{6}\text{ μg Eugenol}}\right) \times \left(\frac{1\text{ mL Eugenol}}{1.060\text{ g Eugenol}}\right) \times \left(\frac{1000\text{ μL Eugenol}}{1\text{mL Eugenol}}\right)$$

$$= 40.84\text{ μL of Eugenol}$$

≈ 41 μL of eugenol is used for this experiment

3.1.2 Solution Preparation

Preparation of Eugenol Solution

Eugenol 41μL is added in ethanol aqueous solution. The percentage of ethanol in eugenol solution is 2% v/v, and total volume use for each batch is 1 mL.

Preparation of β-cyclodextrin solution

300 mg of β-Cyclodextrin was mixed in ethanol aqueous solution. The amount of ethanol in the solvent is 10% v/v and make total is 1 mL.

3.1.3 Inclusion Complex Forming Techniques

In this study, the several inclusion complex forming techniques are selected from the literature reviews, due to the possibility to perform these techniques in the household industry, simple procedure and low investment cost. There are three methods of preparation inclusion complex use in the experiment: normal grinding, cyclone mixing and microwave method. All methods have equal amount of time of exposure and were performed under the same environmental condition.

Normal grinding

For normal grinding technique, two solutions from 3.1.2 were mixed together and were grinded using magnetic stirrer at 298 K or for one minute continuously. The mixture was allowed to stay in the container in order to reach equilibrium for five minutes.

Cyclone mixing

For cyclone mixing, the two solutions were mixed by vortex mixer (vortex mixer model: VELP-ZX3) at 15 rps (round per second) for ten seconds. The process was repeated for six times in order to achieve the total mixing time of one minute. The mixture was allowed to stay in the container in order to reach equilibrium for five minutes.

Figure 3.2 Vortex mixer (VELP - ZX3)

Microwave method

For microwave technique, the mixture was exposed to microwave radiation at 180 Watt for 3 seconds 20 times. In order to avoid the heat accumulation in the β-CD eugenol mixture, exposures were repeated every 15 seconds of resting interval. Total exposure time to the microwave radiation is one minute.

Microwave technique is introduced for forming the inclusion complex with total time limit of one minute. The appropriate operation time could determine by using water to test the temperature difference. The random operating times are ten seconds, five seconds, and three seconds.

Table 3.1: Result of temperature difference of solutions from microwave exposure time

Operating time	**Temperature difference (ºC)**
10 sec	15
5 sec	9
3 sec	3

The results of temperature difference of solution from different microwave exposure time is shown in Table 3.1. It is concluded that the operating time of ten

seconds and five seconds, cause temperature differences are extremely high. Therefore, the appropriate operation time is three sec with the temperature difference at 3ºC. The resting period is set to be 15 seconds. However, the resting period can be extend to two minutes if the temperature of solution is still high. The temperature of mixed solution is maintained below 60ºC throughout the experiment.

3.1.4 Preparation of Cleaning Solution

Since some of eugenol may not form the inclusion complex with β-CD, it is necessary to use the mixture of water and ethanol to wash out the rest of eugenol. In the cleaning solution, ethanol was mixed with distilled water 30 percent by weight or approximately 35.2% v/v. The temperature of using must be 4°C or lower. The total solution of each batch is 3 mL.

3.1.5 Filtration and Drying

After inclusion complex forming methods, the solution is filtrated by cellulose filters grade 1 from Whatman^{TM}. The particle retention liquid is 11μm. All samples were filtrated separately and wash with cold 30% w/w ethanol solution. The after washed samples were dried at the room condition for 24 hours.

3.1.6 Effluent Measuring

Since some eugenol may not form the inclusion complex with β-cyclodextrin, they will be removed by the washing solution and come out with effluent after filtration process. These effluents were measured and calculated back to eugenol encapsulation efficiency. As a primary result, UV-vis spectrophotometry technique will be used to measure the eugenol concentration.

3.1.7 Extraction of Eugenol from Inclusion Complex Powder

To measure concentration of eugenol inside the inclusion complex, eugenol is extracted. The method of extraction is solvent extraction. According to several literatures [59-61], 90-95% of ethanol solution is used for extract eugenol from plant. The same concept is then applied to this experiments, 90% of ethanol solution is used

for solvent extraction. The temperature during extraction is kept constant at room temperature. Duration of extraction is from 2-4 days, depend of measuring purpose.

3.1.8 Effect of Complex Storage Conditions

After the inclusion complex is obtained in powder form, by the most efficient technique, the storage condition effect was study. There are 3 conditions of study, dark storage, expose to air, and exposure to light. All samples were left in that certain environment for 4-5 days, or other equal amount of time. Each condition will discuss as followed:

Dark storage

For dark storage, the inclusion complex powder is kept in the closed container, and store in the dark environment (e.g. cupboard, chemical storage). The temperature is controlled to be the room temperature. This condition will be used as reference.

Expose to air

In this condition, the inclusion complex powder is kept in the open container. The air is allowed to contact with the inclusion complex powder, however, the temperature keeps constant at room temperature.

Expose to sunlight

For the expose to light sample, inclusion complex is kept in the open container. The sample is directly exposed to sunlight. The temperature is fluctuated with the intensity of light according to time, from 35-40°C. Average humidity is 55.29%.

3.2 Characterization

3.2.1 UV-Vis Spectroscopy

UV-Vis spectroscopy uses light in the visible region and ultraviolet region to be absorbed by the sample, to determine it quantitatively. A single beam UV-Vis spectrophotometer is used in the project to find the concentration of eugenol in the filtrate. The unknown concentration of eugenol is calculated by using a calibration curve of eugenol. Quartz cuvette is used in the project to obtain a more accurate result.

Figure 3.3: UV-Vis Spectrophotometer

3.2.2 Gas Chromatography

Gas Chromatography (GC) is used for analyzing compounds. Specifically, the amount of eugenol can be detected for computing the efficiency of the encapsulation. The GC model used in this study is PerkinElmer 680. Throughout the experiment, oven temperature is increased from 80 to 150°C for 10 minutes, and hold temperature at 150°C for 2 minutes. FID (Flame Ionization Detector) temperature is 200°C, while CAP (Capillary split injector) temperature is 275°C.

Figure 3.4: Gas Chromatography model PerkinElmer 680

In the study, gas chromatography is used to find the concentration of eugenol that extract from the inclusion complex powder by the ethanol solution in 3.1.7. Similarly, the result from storage experiment (3.1.8) and grafting experiment (3.3), are extracted and measured at the same GC conditions.

3.2.3 Diffuse Reflectance Infrared Fourier Transform Spectroscopy

Diffuse reflectance infrared Fourier transform spectroscopy or DRIFTS is an infrared spectroscopy technique. It uses to analyze powder or fine particles. Depending on the bulk sample structure, some lights are reflected and transmitted through the sample particles. The scattered IR is collected by the paraboloid mirror which is focused by the detector that characterizes the sample. The sample can be analyzed according to its size, shape, compactness, refractive index, reflectivity and absorption of the particles.

DRIFTS will confirmed the present of eugenol in the inclusion complex powder by compared with the available characterization data of eugenol.

Figure 3.5: Diffuse Reflectance Infrared Fourier Transform Spectroscopy

3.2.4 Differential Scanning Calorimetry

Differential scanning calorimetry or DSC is the instrument that measures the temperature and heat flow associated with transition in material as a function of time and temperature in a controlled condition. The measurements provide quantitative and

qualitative information about physical and chemical changes that involve endothermic or exothermic processes, or changes in heat capacity. Both sample and reference are maintained at nearly the same temperature throughout the experiment.

In the experiment, the DSC model is DSC1 from METTLER TOLEDO. Reference crucible contains nothing, while sample pan contain the samples. The sample weight is from 5-15mg with open crucibles. The same amount of eugenol, pure β-CD, inclusion complex and other samples are weighted and test in order. Temperature of experiment is from 30°C to 500°C with heating rate of 10°C/min. The gas flow to the system is pure nitrogen with 50 ml/min. The samples, inclusion complex of eugenol and β-cyclodextrin, and references will be heat with the specific rate of heating. The heat flow causes the temperature chance and the sample undergoes the physical transition as phase transition. DSC is able to measure the amount of heat absorbed or released during that transition by observing the different in heat flow between the sample and reference. The result from DSC experiment is a curve of heat flux versus the temperature or versus time. The exothermic or endothermic reaction of sample will show the positive of negative peak depend on the technology of instrument.

Figure 3.6 Differential Scanning Calorimetry (DSC)

3.3 Grafting Methodology

The grafting process used in this study must have low investment cost, in order to applied this process into local business. Also the safety on substances handling, toxicity should be avoid, since we want to use this function textile as clothing. Several possible methods are studied with the concern on the price of substance used. The methods that involve with high-cost equipment were eliminated first, follow by the high-value substance. Finally, the process that is not complicated processing will be select as a model and applied with the best inclusion complex method later. The first draft will be adjusted until the formulation is suitable with β-cyclodextrin-eugenol encapsulation graft on cotton fabric. The schematic below is from A.Rukmani [47], the selected process. From their study, β-cyclodextrin is grafted with cotton fabric, and follow by their inclusion complex with thymol.

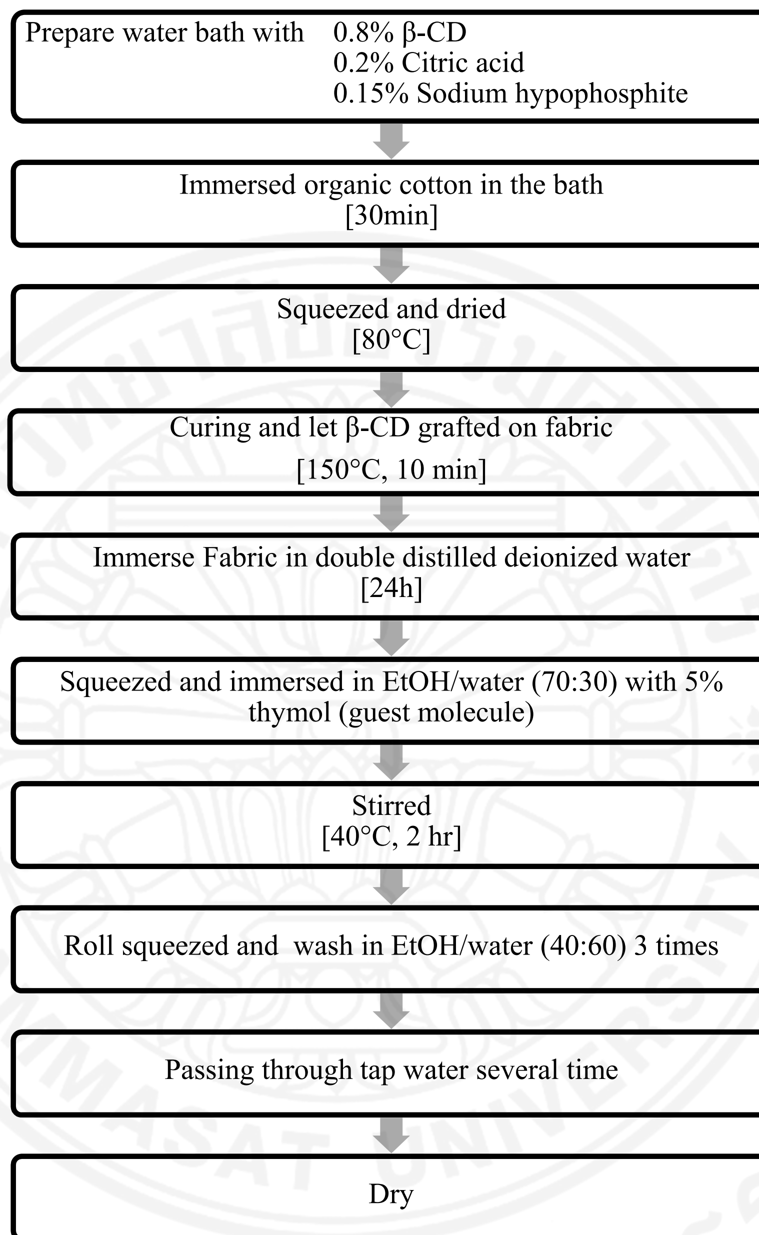

Figure 3.7: The process flow chart of inclusion of antibacterial agent thymol on β-cyclodextrin-grafted organic cotton summarized from their procedure.

In this thesis, the grafting method is based on their study and applied the successful encapsulation technique into the procedure as shown in figure 3.8.

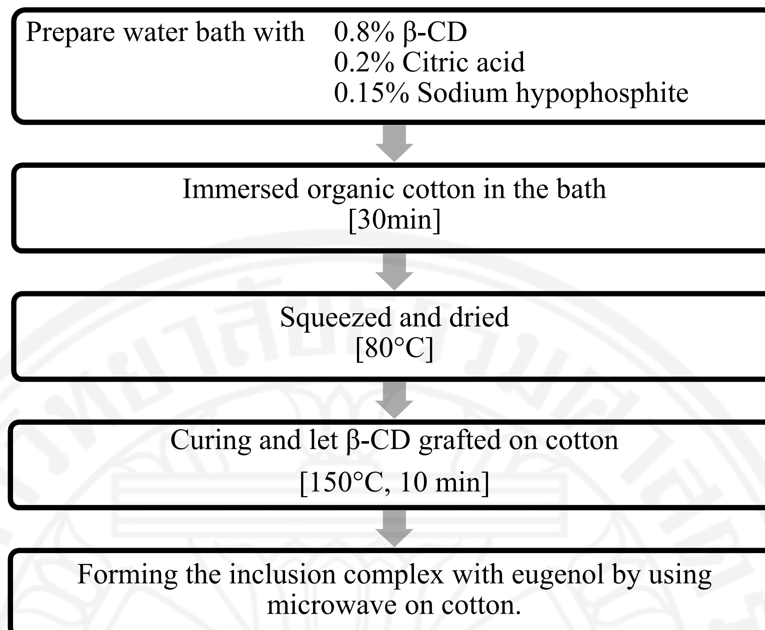

Figure 6 The process flow chart of encapsulation process on cotton

After the inclusion complex forming method, the grafted cotton and encapsulated cotton will be washing by 30% of ethanol solution at 4 °C to get rid of excess eugenol on the cotton without dissolve inclusion complex. The characterization by GC and DSC will be used to determine the amount of eugenol that grafted on the cotton, and calculate the effectiveness of this grafting method.

Chapter 4
Result and Discussion

4.1 Evalution of Inclusion Complex Forming Techniques

In order to form the inclusion complex, eugenol and β-cyclodextrin need to dissolve separately in ethanol solution. Pure ethanol cannot used as solvent because eugenol can completely dissolve in ethanol, then eugenol cannot form the inclusion complex with β-cyclodextrin. Similarly, pure water cannot use as solvent, because eugenol will form the phase separation by forming the droplet in the water instead of inclusion complex. Therefore, some amount of ethanol is added to disperse eugenol to be mixable with water.

The ethanol concentration in the solvent for β-CD solution is higher than eugenol solution as shown in Appendix 2. Once eugenol solution is mixed with this solution, eugenol can easier get into β-Cyclodextrin and form the inclusion complex with the assist of ethanol.

The washing should be cold around 4°C because high concentration of ethanol is used. If the solution is used at room temperature, the high ethanol concentration could dissolve β-CD and eugenol, and they will flow with the effluence instead of remaining as a complex at the filter.

4.1.1 UV-Visible Analysis

Table 4.1 shows the result of eugenol encapsulation from different encapsulation techniques in form of percentage of eugenol encapsulation. The concentration of eugenol in the effluent solution after encapsulation process were determined by UV-vis spectrophotometer by comparing with calibration curve. The remaining eugenol in the effluent can indicate the amount of eugenol that can enter into β-CD and form the complex. A small amount of remaining eugenol implies the high percent encapsulation technique. The percentage of encapsulation of each technique can be calculated as following:

$$\% \text{ eugenol encapsulation} = \frac{[\text{eugenol}]^{\text{initial}} - [\text{eugenol}]^{\text{remain}}}{[\text{eugenol}]^{\text{initial}}} \times 100\%$$

where $[\text{eugenol}]^{\text{initial}}$ is an initial concentration of eugenol
$[\text{eugenol}]^{\text{remain}}$ is remaining concentration of eugenol in effluent
Initial concentration of eugenol = 88.23 µmol/g of β-cyclodextrin

Table 4.1: Encapsulation percentages of eugenol in each technique from UV-vis Spectrophotometry

Technique	**Remaining Eugenol Concentration in Effluent (µmol/g of β-CD)**	**$[\text{eugenol}]^{\text{initial}} - [\text{eugenol}]^{\text{remain}}$**	**% Eugenol Encapsulation**
Normal grinding	34.87	53.36	60.47
Cyclone mixing	54.93	33.30	37.74
Microwave	24.73	63.50	71.90

Note: Initial concentration of eugenol is 88.23 µmol/g of β-cyclodextrin

The results in table 4.1 shows that a cyclone mixing technique has the lowest % Eugenol Encapsulation, since this technique to use the viscous shake to mix eugenol aqueous solution and β-CD aqueous solution together. The two ethanol aqueous solutions dissolve excessively well. Besides that they are more likely to be a solution rather than forming the inclusion complex.

Normal grinding, that requires no special technique, gives higher percent encapsulation of eugenol than the cyclone mixing under the same condition.

However, microwave is a preferred technique as it provides the highest % eugenol encapsulation. Since the microwave ray rotate the eugenol molecule be the position that inclusion complex are able to form with the lowest binding energy. Therefore the microwave technique will be used for optimize the formula to obtain the better percentage of eugenol encapsulation.

4.1.2 GC Analysis of Eugenol Extracted from the Inclusion Complex

10 mg of inclusion complexes from different techniques were extracted by using of the powders are dissolved in 50 ml of 90% ethanol solution, separately. For extraction, the dissolved solutions were left for 2 days and then inject solutions to GC. Table 4.2 shows amount of eugenol that was extracted from the inclusion complex from different technique in the unit of μmol/g of complex.

Table 4.2 The amount of eugenol that extract from the inclusion complex of different forming techniques

Encapsulation method	Amount of Eugenol after Extraction (μmol/g of inclusion complex)
Normal grinding	28.8±0.21
Cyclone mixing	16.5±0.14
Microwave	42.0±0.01

Microwave technique obtains the highest result, followed by normal grinding and cyclone mixing, respectively.

4.2 Confirmation of Eugenol in the Inclusion Complex

The results from section 4.1 suggest the microwave exposure technique obtain the most effective result. Therefore, microwave technique was selected to study further. To confirm the present of eugenol in the form of inclusion complex, infrared spectroscopy and DSC are used.

For infrared spectroscopy, the characteristic peaks of free eugenol are at 1,610 cm^{-1} and 1,637 cm^{-1} for carbon-carbon bonding on aromatic ring stretching and carbon-carbon bond on alkene stretching, respectively. The background of this measurement is pure β-CD, therefore no peak of β-CD in the result. The result will be shown only the peak of eugenol in both solid inclusion complex form and free eugenol form.

The result from DRIFTS is shown in the figure 4.1. The plot between absorbance and the wavenumber is shown. The shift of characteristic peak of eugenol at the wavenumber from 1,610 $cm^{-1}$to 1,613 cm^{-1}, and from 1,637 cm^{-1} to 1,654 cm^{-1}, indicate the aromatic bonding stretching which is the carbon double bond of eugenol in the inclusion complex.

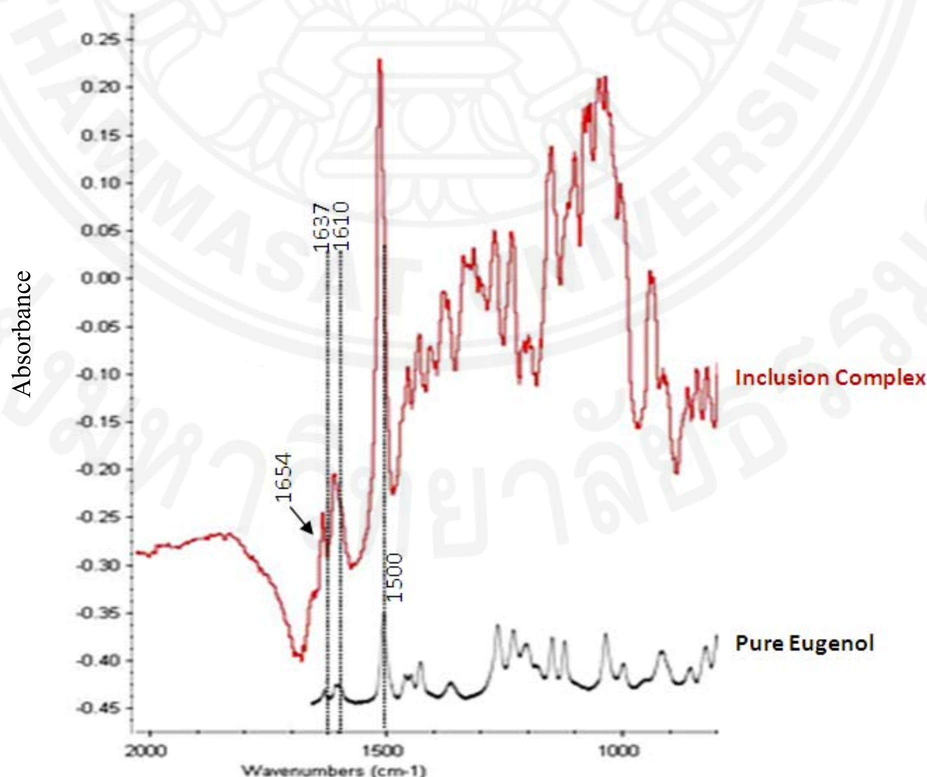

Figure 4.1: DRIFTS result of inclusion complex using pure β-CD as background

The inclusion complex powder after filtrate and dry are kept in plastic container, which also used in the DSC measurement. The result is shown in figure 4.2. DSC is used in dynamic method to see the amount of heat flow with the increasing of temperature. The heating rate is 10 K/min, measure from 25 to 400°C. The reference is blank sample and the gas flow in the environment is oxygen gas at 50 ml/min. Results are shown in the arbitrary of heat used in Y-axis versus the temperature of measurement on X-axis.

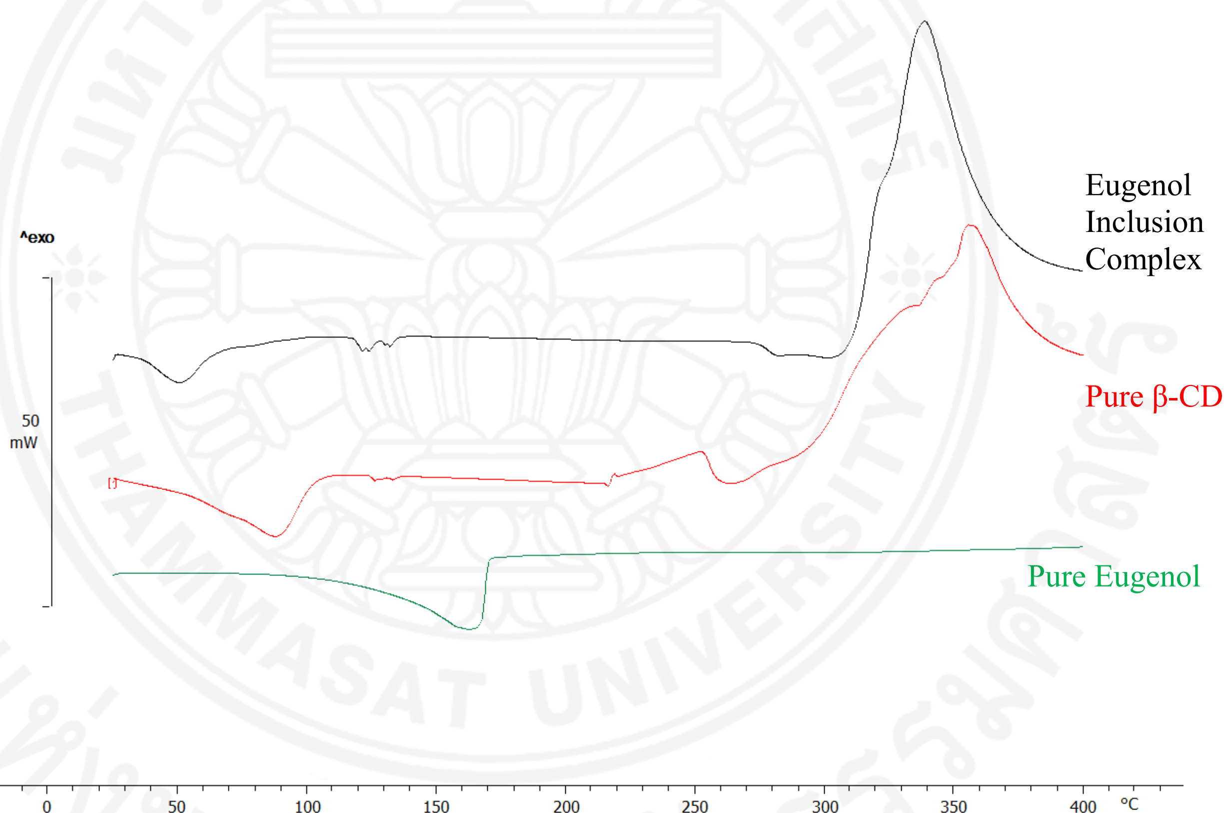

Figure 4.2: DSC curves of eugenol β-CD system, pure eugenol, pure β-cyclodextrin, and eugenol-β-CD inclusion complex with oxygen gas flow environment.

DSC curve shows decomposition of eugenol around 140°C. For pure β-cyclodextrin, it starts to decompose at temperature around 270°C. The endothermic peak for pure eugenol around 160°C and exothermic peak at 230°C for pure β-CD both do not exist in inclusion complex curve. At temperature around 300°C of inclusion complex cure, it begins to decompose with the elevation of temperature. The different of onset, or the starting temperature of decomposition, indicates existent of eugenol-β-cyclodextrin inclusion complex.

Another result in Figure 4.3 is the same sample as Figure 4.2 except the gas flow to the system is nitrogen gas at the same velocity. Pure eugenol starts to decompose around 120-180°C. Pure β-cyclodextrin powder have water removal at 100°C and start to decompose around 320-340°C. Compare with eugenol-β-CD inclusion complex powder, the decomposition peak is shift due to lower the eugenol in the form of complex with β-CD.

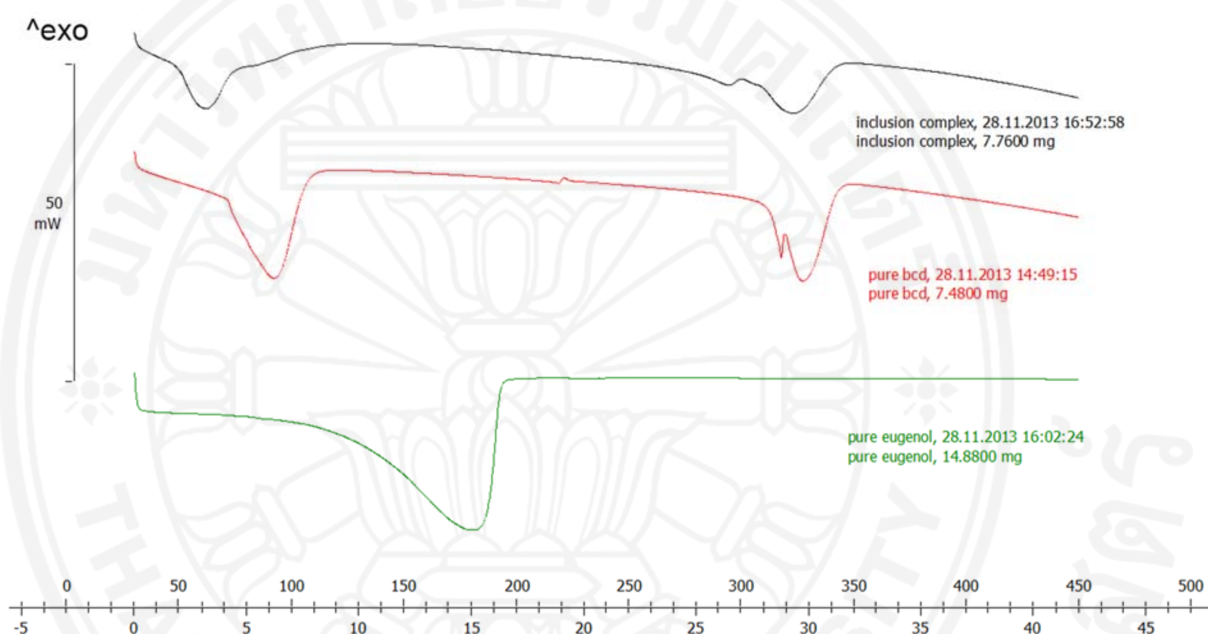

Figure 4.3 DSC curves of heat flow (a.u.) versus temperature of eugenol β-CD system, pure eugenol, pure β-cyclodextrin, and eugenol-β-CD inclusion complex with nitrogen gas flow environment.

4.3 Test on Different Inclusion Complex Powder Storage Conditions

4.3.1 IR Characterization on Different Storage Conditions

All sample from different storage conditions were measured by IR. They are measure directly from the inclusion complex powder. The inclusion complex powders are compared with pure β-cyclodextrin. All conditions are performed for 7 days before extracted and measured the result. The results are shown in Figure 4.4. Since the pure eugenol have the peak of carbon-carbon double bond stretching around 1500-1600 cm^{-1}, its shows the peak in all sample except the references, which is pure β-cyclodextrin. Therefore, it is implied that eugenol still exist in the inclusion complex even in the different storage conditions.

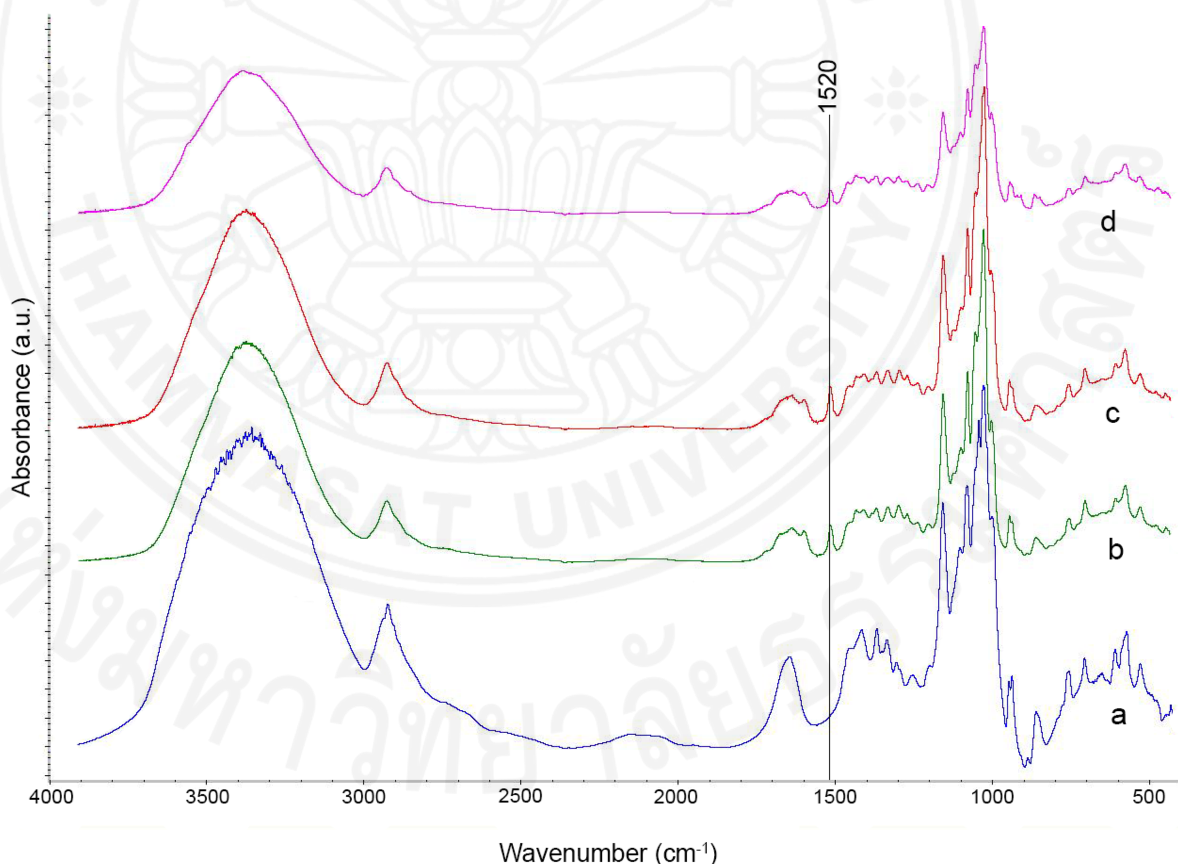

Figure 4.4: IR Result of 3 different storage conditions, test for 7 days: a) pure β-cyclodextrin, b) dark storage, c) sunlight exposure, d) air exposure.

4.3.2 DSC Characterization on Different Storage Conditions

The powder of inclusion complex after stored at certain conditions, were measure by DSC characterization. The result of comparison between 3 storage conditions is shown in Figure 4.5. The water content of inclusion complex powder in dark storage sample and air exposure sample were removed around 45°C, while the sunlight exposure sample does not show the peak, since the evaporation of water might occur during the exposure. The eugenol decomposition peaks are shown around 110-130°C, these peak are considered as eugenol evaporation. The final peaks of decomposition are shown around 320°C at all storage conditions. It can be seen that the peak is shifted to the left when compare to the peak of pure β-CD in Figure 4.3.

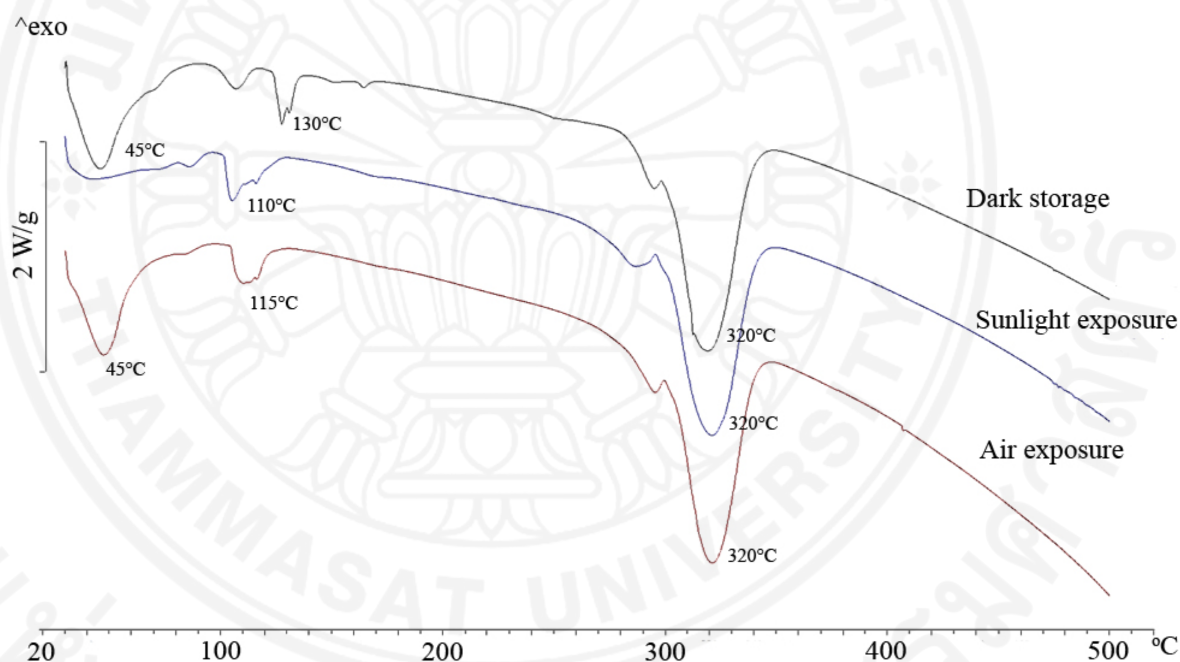

Figure 4.5: The DSC curve of temperature and heatflow (a.u.) of different storage conditions.

4.3.3 GC Characterization on Different Storage Conditions

For measuring by GC, eugenol is extracted from the powder by high concentration of ethanol solution. Results are shown in table 4.3. The powder used is the same batch as for DSC experiment. Area results were obtained and then calculated back to amount of eugenol. When compare with dark storage, as the reference, the light exposure inclusion complex powder is decreased by 31%, while the air exposure decrease on eugenol concentration about 21%. The results show that some of eugenol still remains in the

form of inclusion complex, and some of eugenol can be volatile from the inclusion complex by the change temperature and environmental condition.

Table 4.3 Eugenol concentration that is extracted from different storage conditions

Storage condition	**Extracted eugenol [µmol/g of inclusion complex]**	**% Decrease of eugenol**
Dark storage (Ref.)	42.00±0.01	0.00
Air exposure	33.09±0.12	21.21
Sunlight exposure	28.99±0.13	31.00

Another experiment on the temperature of exposure was also studied. In this study, each 10 mg of inclusion complex powder were exposed for 3 hours, under room temperature 25°C , 40°C , 60°C , 80°C and 100°C. Eugenol were extracted and measured by GC, and results are shown in Table 4.4. The result shows that the higher temperature, the lower eugenol remains. Obviously, from room temperature to 80°C, the eugenol remained are not much different, however at 100°C, eugenol concentration is decrease about 35% compare to the reference room temperature.

Table 4.4 Eugenol concentration at different temperature exposure and percentage decresing

Temperature	**Extracted eugenol [µmol/mg of inclusion complex]**	**% Decrease**
Reference (25°C)	42.00±0.01	0.00
40°C	40.30±0.02	4.05
60°C	38.00±0.19	9.52
80°C	37.12±0.08	11.61
100°C	27.05±0.11	35.60

4.4 Grafting Experiment on the Cotton Fabric

In the experiment, β-cyclodextrin is introduced to cotton sheet by 2 different methods and follow by the inclusion complex forming technique. The first method is the directly added, which will be called as ungrafted. The second one is grafted cotton, which is followed the procedure in chaption 3.3 and figure 3.8.

For the proceduce of inclusion complex forming technique, the cottons with grafted and ungrafted with β-cyclodextrin were dipped in the eugenol in 2% v/v ethanol

solution at room temperature. Then the microwace technique is applied with both cottons separately for 3 seconds 20 times with 15 seconds waiting interval. Followed by the drying process at room temperature for 24 hours.

4.4.1 GC Characterization on the Washing Experiment

In this part, the eugenol concentrations were compared between graft and ungrafted cotton, which are obtained from different the washing times, from 0 time washing to 2 times washing. The washing solution consist of water and detergent, and cottons were washed for 5 mins at room temperature. Then eugenol was extracted from the cottons by 90% ethanol water solution. The amount of eugenol in each cottons were measured by gas chromatography.

The first set of cotton are used as the references, the grafted and ungrafted cotton with 0 time washing. The second set of cottons were separated to 2 batches, each batch consist of grafted and ungrafted cotton. The first batch is washed 1 time and the second batch is washed 2 times. All cottons were washed separately. After washing, the eugenol was extracted from both cottons and compare the amount of eugenol lost from washing. The results show that the grafted cotton can keep more eugenol than ungrafted cotton as shown in Table 4.5

Table 4.5 Extracted eugenol concentrations from the sample before and after washing.

Conditions of Cotton Fabric	Extracted Eugenol Concentration (µmol/g cotton)	% of Eugenol lost
Graft (ref.)	1.26	0.00
Graft + 1 Wash	0.39	69.38
Graft + 2 Wash	0.18	85.71
Ungraft (ref.)	1.04	0.00
Ungraft + 1 Wash	0.04	96.00

Chapter 5
Conclusions and Recommendations

Eugenol can be encapsulated by using β-cyclodextrin in the form of inclusion complex to preserve the compound from the environment. Microwave technique is the most efficient method to form 1:1 molar ratio host-guest complex among other inclusion complex preparation methods in this study, based on UV-visible spectroscopy analysis and Gas chromatography technique, microwave technique has the capability of encapsulating eugenol up to 63%. The FT-IR, DRIFTS analysis confirmed the presence of aromatic carbon double bonds, alkene carbon, and carbon-hydrogen bonds of the aromatic ring which are the major parts of eugenol molecule structure in the inclusion complex. As well as DSC, the result confirmed the present of eugenol in the form of inclusion complex with β-cyclodextrin.

The result, inclusion complex powder, from the microwave technique were tested further on different storage conditions, expose to atmosphere and expose to sunlight. IR, DSC and GC are confirmed the present of eugenol in the inclusion complex form. Thus, the inclusion complex formation by microwave exposure could preserve eugenol for several environments.

The application of eugenol-β-cyclodextrin inclusion complex with fabrication also studied, and washed several times to find the duration of eugenol in the complex. The result were extracted by ethanol solution and tested with GC, it show that grafted cotton can preserve more eugenol that the ungrafted one. For future study in fabric application, to increase the amount of encapsulated eugenol in the β-cyclodextrins that grafted on the cotton, the improvement on procedure of grafting process and formula is needed.

References

1. National Economic and Social Development Board, O.o.t.P.M. (2011) *THE ELEVENTH NATIONAL ECONOMIC AND SOCIAL DEVELOPMENT PLAN (2012-2016)*. Retrieved October 20, 2013, from http://www.nesdb.go.th/Portals/0/news/plan/p11/Plan11_eng.pdf.
2. Information and Communication Technology Center, T.C.D. (2014) *The Export value of Thai Herb and Spice* Retrieved March 14, 2015, from http://www.ops3.moc.go.th/menucomth/export_market/#.
3. Ezhilarasi, P., et al. (2013). Nanoencapsulation techniques for food bioactive components: a review. *Food and Bioprocess Technology*, Vol. 6, 628-647.
4. Del Valle, E.M.M. (2004). Cyclodextrins and their uses: a review. *Process Biochemistry* Vol. 39, 1033-1046.
5. Moreira-Lobo, D.C., et al. (2010). Eugenol modifies the excitability of rat sciatic nerve and superior cervical ganglion neurons. *Neuroscience letters*, Vol. 472, 220-224.
6. Galán, M.A. and E.M. Del Valle. (2005). *Chemical engineering: trends and developments*. John Wiley & Sons.
7. Quintanilla-Carvajal, M.X., et al. (2010). Nanoencapsulation: a new trend in food engineering processing. *Food Engineering Reviews*, Vol. 2, 39-50.
8. Choi, M.-J., et al. (2009). Physical and light oxidative properties of eugenol encapsulated by molecular inclusion and emulsion–diffusion method. *Food Research International*, Vol. 42, 148-156.
9. Shah, B., P.M. Davidson, and Q. Zhong. (2012). Encapsulation of eugenol using Maillard-type conjugates to form transparent and heat stable nanoscale dispersions. *LWT-Food Science and Technology*, Vol. 49, 139-148.
10. Yang, Y. and L.X. Song. (2005). Study on the inclusion compounds of eugenol with α-, β-, γ-and heptakis (2, 6-di-O-methyl)-β-cyclodextrins. *Journal of inclusion phenomena and macrocyclic chemistry*, Vol. 53, 27-33.
11. Locci, E., et al. (2004). 13C-CPMAS and 1H-NMR Study of the Inclusion Complexes of β-Cyclodextrin with Carvacrol, Thymol, and Eugenol Prepared in Supercritical Carbon Dioxide. *Chemistry & biodiversity*, Vol. 1, 1354-1366.

12. Shinde, U. and M. Nagarsenker. (2011). Microencapsulation of eugenol by gelatin-sodium alginate complex coacervation. *Indian journal of pharmaceutical sciences*, Vol. 73, 311.
13. Garg, A. and S. Singh. (2011). Enhancement in antifungal activity of eugenol in immunosuppressed rats through lipid nanocarriers. *Colloids and Surfaces B: Biointerfaces*, Vol. 87, 280-288.
14. Pham, D.-T. (2007).*BETA-CYCLODEXTRIN MODIFICATION AND HOST GUEST COMPLEXATION*, in *School of Chemistry and Physics*: The University of Adelaide).
15. Song, L.X., et al. (2009). Inclusion complexation, encapsulation interaction and inclusion number in cyclodextrin chemistry. *Coordination Chemistry Reviews*, Vol. 253, 1276-1284.
16. Szejtli, J. (1998). Introduction and general overview of cyclodextrin chemistry. *Chemical reviews*, Vol. 98, 1743-1754.
17. Koehler, P., R.-D. Petersen, and S. Borchert. (1999). Stabilisation of tea tree oil. *SÖFW-journal*, Vol. 125, 10-13.
18. Tsomi, V. (1991).*COSMETIC SKIN-LIGHTENING COMPOSITION BASED ON A HYDROQUINONE/2,6-DIMETHYL-β-CYCLODEXTRIN COMPLEX*: Google Patents).
19. Deckner, G.E. and C. Sang. (2000).*Cosmetic compositions*: Google Patents).
20. Pitha, J. (1982).*Water soluble form of retinoids*: Google Patents).
21. Wang, J., et al. (2011). Physicochemical and release characterisation of garlic oil-β-cyclodextrin inclusion complexes. *Food Chemistry*, Vol. 127, 1680-1685.
22. Neoh, T.-L., H. Yoshii, and T. Furuta. (2006). Encapsulation and release characteristics of carbon dioxide in α-cyclodextrin. *Journal of inclusion phenomena and macrocyclic chemistry*, Vol. 56, 125-133.
23. Ponce Cevallos, P.A., M.P. Buera, and B.E. Elizalde. (2010). Encapsulation of cinnamon and thyme essential oils components (cinnamaldehyde and thymol) in β-cyclodextrin: Effect of interactions with water on complex stability. *Journal of food engineering*, Vol. 99, 70-75.

24. Choi, M.-J., et al. (2010). Physical characteristics of fish oil encapsulated by β-cyclodextrin using an aggregation method or polycaprolactone using an emulsion–diffusion method. *Food chemistry*, Vol. 119, 1694-1703.
25. Wen, X., et al. (2004). Preparation and study the 1: 2 inclusion complex of carvedilol with β-cyclodextrin. *Journal of pharmaceutical and biomedical analysis*, Vol. 34, 517-523.
26. Jun, S.W., et al. (2007). Preparation and characterization of simvastatin/hydroxypropyl-β-cyclodextrin inclusion complex using supercritical antisolvent (SAS) process. *European journal of pharmaceutics and biopharmaceutics*, Vol. 66, 413-421.
27. Wei, M., et al. (2002). Manipulation of Nylon-6 crystal structures with its α-Cyclodextrin inclusion complex. *Macromolecules*, Vol. 35, 8039-8044.
28. Filipović-Grčić, J., et al. (2000). Chitosan microspheres with hydrocortisone and hydrocortisone–hydroxypropyl-β-cyclodextrin inclusion complex. *European journal of pharmaceutical sciences*, Vol. 9, 373-379.
29. Duke, J.A. (2010). *Handbook of medicinal herbs*. CRC press.
30. Mayne, S.T. (1996). Beta-carotene, carotenoids, and disease prevention in humans. *The FASEB Journal*, Vol. 10, 690-701.
31. Didry, N., L. Dubreuil, and M. Pinkas. (1994). Activity of thymol, carvacrol, cinnamaldehyde and eugenol on oral bacteria. *Pharmaceutica Acta Helvetiae*, Vol. 69, 25-28.
32. Suppakul, P., et al. (2003). Antimicrobial properties of basil and its possible application in food packaging. *Journal of agricultural and food chemistry*, Vol. 51, 3197-3207.
33. Lee, S.-J., et al. (2005). Identification of volatile components in basil (< i> Ocimum basilicum</i> L.) and thyme leaves (< i> Thymus vulgaris</i> L.) and their antioxidant properties. *Food Chemistry*, Vol. 91, 131-137.
34. Calín-Sánchez, Á., et al. (2012). Volatile composition of sweet basil essential oil (< i> Ocimum basilicum</i> L.) as affected by drying method. *Food Research International*, Vol. 48, 217-225.

35. Hussain, A.I., et al. (2008). Chemical composition, antioxidant and antimicrobial activities of basil (< i> Ocimum basilicum</i>) essential oils depends on seasonal variations. *Food Chemistry*, Vol. 108, 986-995.

36. NETWORK, T.T.D. *HSDB: EUGENOL*. Retrieved April 30, 2015, from http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@rn+@rel+97-53-0.

37. Sober, H.A., F. Hollander, and E.K. Sober. (1950). Toxicity of Eugenol Determination of LD50 on Rats. *Experimental Biology and Medicine*, Vol. 73, 148-151.

38. Fischer, I., G. Von Unruh, and H. Dengler. (1990). The metabolism of eugenol in man. *Xenobiotica*, Vol. 20, 209-222.

39. Ito, M., K. Murakami, and M. Yoshino. (2005). Antioxidant action of eugenol compounds: role of metal ion in the inhibition of lipid peroxidation. *Food and chemical toxicology*, Vol. 43, 461-466.

40. Prakash, P. and N. Gupta. (2005). Therapeutic uses of Ocimum sanctum Linn (Tulsi) with a note on eugenol and its pharmacological actions: a short review. *Indian journal of physiology and pharmacology*, Vol. 49, 125.

41. Yoo, C.-B., et al. (2005). Eugenol isolated from the essential oil of< i> Eugenia caryophyllata</i> induces a reactive oxygen species-mediated apoptosis in HL-60 human promyelocytic leukemia cells. *Cancer letters*, Vol. 225, 41-52.

42. Ghosh, R., et al. (2005). Eugenol causes melanoma growth suppression through inhibition of E2F1 transcriptional activity. *Journal of Biological Chemistry*, Vol. 280, 5812-5819.

43. Daniel, A.N., et al. (2009). Anti-inflammatory and antinociceptive activities A of eugenol essential oil in experimental animal models. *Revista Brasileira de Farmacognosia*, Vol. 19, 212-217.

44. Roubach, R., et al. (2005). Eugenol as an efficacious anaesthetic for tambaqui, Colossoma macropomum (Cuvier). *Aquaculture Research*, Vol. 36, 1056-1061.

45. Varel, V.H. and D. Miller. (2004). Eugenol stimulates lactate accumulation yet inhibits volatile fatty acid production and eliminates coliform bacteria in cattle and swine waste. *Journal of applied microbiology*, Vol. 97, 1001-1005.

46. Peters, O. and F. Lutz. (2000). Effect of eugenol-containing sealer on marginal adaptation of dentine-bonded resin fillings. *International endodontic journal*, Vol. 33, 53-59.

47. Rukmani, A. and M. Sundrarajan. (2012). Inclusion of antibacterial agent thymol on β-cyclodextrin-grafted organic cotton. *Journal of Industrial Textiles*, Vol. 42, 132-144.

48. Bhaskara-Amrit, U.R., P.B. Agrawal, and M. Warmoeskerken. (2011). Applications of β-cyclodextrins in textiles. *AUTEX Research Journal*, Vol. 11, 94-101.

49. Cabrales, L., et al. (2012). Cotton fabric functionalization with cyclodextrins. *surfaces*, Vol. 6, 8.

50. Agrawal, P. and M. Warmoeskerken. (2012). Permanent fixation of β-cyclodextrin on cotton surface—An assessment between innovative and established approaches. *Journal of applied polymer science*, Vol. 124, 4090-4097.

51. Shown, I. and C. Murthy. (2009). Grafting of cotton fiber by water-soluble cyclodextrin-based polymer. *Journal of applied polymer science*, Vol. 111, 2056-2061.

52. Hiriart-Ramírez, E., et al. (2012). Radiation grafting of glycidyl methacrylate onto cotton gauzes for functionalization with cyclodextrins and elution of antimicrobial agents. *Cellulose*, Vol. 19, 2165-2177.

53. Nada, A.A., P. Hauser, and S.M. Hudson. (2011). The Grafting of Per-(2, 3, 6-O-allyl)-β Cyclodextrin onto Derivatized Cotton Cellulose via Thermal and Atmospheric Plasma Techniques. *Plasma Chemistry and Plasma Processing*, Vol. 31, 605-621.

54. Lee, M.H., K.J. Yoon, and S.W. Ko. (2000). Grafting onto cotton fiber with acrylamidomethylated β-cyclodextrin and its application. *Journal of Applied polymer science*, Vol. 78, 1986-1991.

55. Sundrarajan, M. and A. Rukmani. (2013). Biopolishing and cyclodextrin derivative grafting on cellulosic fabric for incorporation of antibacterial agent thymol. *Journal of the Textile Institute*, Vol. 104, 188-196.
56. Nazi, M., R.M.A. Malek, and R. Kotek. (2012). Modification of β-cyclodextrin with itaconic acid and application of the new derivative to cotton fabrics. *Carbohydrate Polymers*, Vol. 88, 950-958.
57. Nostro, P.L., et al. (2003). Surface treatments on Tencel fabric: Grafting with β-cyclodextrin. *Journal of applied polymer science*, Vol. 88, 706-715.
58. Lawtrakul, L., K. Inthajak, and P. Toochinda. (2014). Molecular calculations on β-cyclodextrin inclusion complexes with five essential oil compounds from Ocimum basilicum (sweet basil). *SCIENCEASIA*, Vol. 40, 145-151.
59. Lugemwa, F.N. (2012). Extraction of Betulin, Trimyristin, Eugenol and Carnosic Acid Using Water-Organic Solvent Mixtures. *Molecules*, Vol. 17, 9274-9282.
60. Myint, S., W.R. Wan Daud, and A.B. Mohamad. (1995). Determination of optimal conditions for extraction of alcohol-soluble eugenol containing material from cloves. *Pertanika Journal of Science & Technology*, Vol. 3, 96-106.
61. Myint, S., et al. (1996). Gas chromatographic determination of eugenol in ethanol extract of cloves. *Journal of Chromatography B: Biomedical Sciences and Applications*, Vol. 679, 193-195.

Appendices

Appendix A
Properties of Subtances

Eugenol

Chemical formula:	$C_{10}H_{12}O_2$
Brand:	MERCK
Purity:	≥99
Molar Mass:	164.2 g/mol
Density:	1.064-1.066
Boiling Point:	254°C

β-cyclodextrin

Chemical formula:	$C_{42}H_{70}O_{35}$
Brand:	SIGMA
Purity:	≥98
Molar Mass:	1,135.01 g/mol
Melting Point:	290°C

Ethanol

Chemical formula:	C_2H_5OH
Brand:	MERCK
Purity:	99.9
Molar Mass:	46.07 g/mol
Density:	0.790 - 0.793 g/cm3 (20 °C)
Flash Point:	12°C

Appendix B
The Concentration of Ethanol Used in the Solutions

Ethanol concentration in eugenol solution is 2 %vol and ethanol in β-cyclodextrin solution is 10%vol. The amount of solution used is 1 mL as shown in Table A2.

Table A2: Propose formula of preparation of eugenol solution and β-cyclodextrin solution

	Eugenol solution	**β-CD solution**
Percent of Ethanol (% v/v)	2	10
Amount of Solvent (mL)	1	1

Appendix C
Analysis Equipment

UV-Visible spectroscopy

UV-Vis spectroscopy uses light in the visible region and ultraviolet region to be absorbed by the sample, to determine it quantitatively. It basically consists of a light source, a sample holder, a diffraction grating to separate the different wavelengths of light, and a detector. It uses the principle of electrons transition in which the electrons are transited from the ground state to the excited state. Therefore, molecules with π-electrons are easily excited by the visible light energy. As a result, the easier they can get excited, the longer wavelength they can absorb. The color of the chemicals involved directly affects the absorption of light.

The light absorbance can be described using the Beer-Lambert Law;

$$A = \log 10\left(\frac{I_0}{I}\right) = \varepsilon \cdot c \cdot L$$

Where A = Measured absorbance

I_0= Intensity of the incident light at a given wavelength

I = Transmitted intensity

ϵ = Molar absorptivity constant

c = Concentration of the absorbing species

L = The path length through the sample

It is stated that the measured absorbance is directly proportional to the concentration of the absorbing species. The UV-Vis spectroscopy can tells how quickly the absorbance changes with the concentration by constructing a calibration curve. The calibration curve can be used to find out the unknown concentration of the desired sample by back calculating.

Gas Chromatography

Gas Chromatography (GC) is used for analyzing compounds. Specifically, the amount of eugenol can be detected for computing the efficiency of the encapsulation. It composes of two phases in gas chromatography, mobile phase is a carrier gas; helium is used as it is an inert gas. The stationary phase is the column itself. The compound is vaporized to be analyzed by the oven as the gas phase passes by. The retention time of each compound gives the analytical value of gas chromatography.

Figure 3.4: Gas Chromatography model PerkinElmer 680

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