การกักเก็บกลิ่นโดยใช้ส่วนผสมของอนุพันธ์อิมมีนของไคโทซานและอนุพันธ์เซลลูโลส

นางสาวราตรี ผูกชอบ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2555
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

FRAGRANCE ENCAPSULATION USING THE BLEND OF IMINE DERIVATIVES OF CHITOSAN AND CELLULOSE DERIVATIVES

Miss Ratree Phookchaub

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Biotechnology
Faculty of Science
Chulalongkorn University
Academic Year 2012

Thesis Title	FRAGRANCE ENCAPSULATION USING THE BLEND OF IMINE DERIVATIVES OF CHITOSAN AND CELLULOSE DERIVATIVES
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Field of Study	Biotechnology
Thesis Advisor	Associate Professor Supason Wanichwecharungruang, Ph.D.

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ราตรี ผูกชอบ : การกักเก็บกลิ่นโดยใช้ส่วนผสมของอนุพันธ์อิมมีนของไคโทซานและอนุพันธ์เซลลูโลส (FRAGRANCE ENCAPSULATION USING THE BLEND OF IMINE DERIVATIVES OF CHITOSAN AND CELLULOSE DERIVATIVES) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.ดร. ศุภศร วนิชเวชารุ่งเรือง, 73 หน้า.

งานวิจัยนี้มุ่งเน้นการสร้างระบบปลดปล่อยน้ำหอมแบบควบคุมที่มีความเข้มข้นสูงโดยไม่ต้องผ่านกระบวนการเพิ่มความเข้มข้น โดยเตรียมอนุภาคไมโครกักเก็บน้ำหอมที่มีระบบควบคุมการปลดปล่อยที่มีสองชั้นประกอบด้วย ชั้นของพันธะเคมีผ่านอนุพันธ์ชิฟเบสที่ถูกกราฟอยู่บนอนุพันธ์ซัคซินิลไคโทซาน และชั้นของการกักเก็บด้วยพอลิเมอร์ผสม โดยในงานนี้เป็นการทดลองกับกลิ่นแอล-คาโวนและเฮซิลซินนามอลแอลดีไฮด์ เริ่มต้นด้วยการสังเคราะห์ซัคซินิลไคโทซานโดยปฏิกิริยาของไคโทซานกับซัคซินิคแอนไฮไดรด์ จากนั้นนำผลิตภัณฑ์ที่ได้ไปใช้ร่วมกับเอทิลเซลลูโลสและเมทิลเซลลูโลสในการกักเก็บน้ำหอม ด้วยการโฮโมจีไนเซชัน ได้อนุภาคระดับไมโครที่มีแอล-คาโวน และเฮซิลซินนามอลแอลดีไฮด์ อยู่ 96.57±0.87% และ 81.43±0.67% (w/w) โดยกระบวนการที่ใช้มีประสิทธิภาพการกักเก็บ 82.17±0.41% และ 69.30±0.65% (w/w) ตามลำดับ และเมื่อนำไปศึกษาการปลดปล่อยน้ำหอมภายใต้สภาวะเร่ง พบว่าระบบนี้มีประสิทธิภาพในการปลดปล่อยคีโทนและแอลดีไฮด์ที่ยาวนานขึ้นได้เมื่อเปรียบเทียบคีโทนและแอลดีไฮด์อิสระ

สาขาวิชา.....เทคโนโลยีชีวภาพ.....ลายมือชื่อนิสิต.....

ปีการศึกษา.....2555.....ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

5372461123: MAJOR BIOTECHNOLOGY
KEYWORDS: IMINE DERIVATIVES / ENCAPSULATION/
CONTROLLED RELEASEE

RATREE PHOOKCHAB: FRAGRANCE ENCAPSULATION USING THE BLEND OF IMINE DERIVATIVES OF CHITOSAN AND CELLULOSE DERIVATIVES. ADVISOR: ASSOC. PROF. SUPASON WANICHWECHARUNGRUANG, Ph.D., 73 pp.

This work focuses on the fabrication process of fragrance controlled release system that gives high fragrance concentration product with no need of preconcentration step. The process involves the fabrication of micron-sized fragrance spheres that combine two release mechanisms, the barrier of Schiff base chemical linkage between fragrant aldehyde or ketone and succinylchitosan and the physical polymer blend barrier. L-carvone and hexylcinnamaldehyde were used as a model fragrance. The process involves the synthesis of *N*-succinylchitosan through the reaction between chitosan and succinic anhydride. Then the obtained product was used in combination with ethylcellulose and methylcellulose to encapsulate fragrance by homogenization to obtain L-carvone-loaded micro-particles and hexylcinnamaldehyde-loaded micro-particles at 96.57±0.87 % and 81.43±0.67% (w/w) loading. The process gave 82.17±0.41% and 69.30±0.6% (w/w) encapsulation efficiency respectively. The release study under accelerated condition showed that this system could retain the fragranced ketones and aldehyde more effectively than the free ketone and free aldehyde.

Field of Study: ……Biotechnology…… Student's Signature……
Academic Year: ……2012…….. Advisor's Signature……

ACKNOWLEDGEMENTS

First of all, I would like to express my sincere appreciation to my thesis advisor, Associate Professor Dr. Supason Wanichwecharungruang for her helpful supervision, invaluable assistance and generous encouragement to fulfill my achievement.

I also sincerely thank Assistant Professor Dr. Warinthorn Chavasiri, Associate Professor Dr. Chanpen Chanchao and Dr. Natthakitta Suwannateep for their time and suggestions as the committee members. And I would like to thank the Graduate School, Chulalongkorn University for financial support.

Finally, I would like to specially thank my family and research group members for their advice and encouragement throughout my master study.

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LIST OF ABBREVIATIONS

λ	wavelength
%	percent
°C	degree Celsius
DG	degree of grafting
DS	degree of substitution
cm^2	square centimeter
h	hour
s	second
µg	microgram
µl	microliter
mg	milligram
ml	milliliter
mW	milliwatt
min	minute
MW	molecular weight
mm	millimeter
nm	nanometer
ppm	parts per million
cm^{-1}	per centimeter
SEM	Scanning electron microscope
TEM	Transmission electron microscope
UV	ultraviolet
cm^{-1}	unit of wavenumber (IR)

CHAPTER I
INTRODUCTION

Fragrance has been used widely in many fields such as cosmetics, food, textile, detergents, medicine and pharmaceutical industries [1]. However, many of the fragrance molecules are unstable due to their reactive functionalities, such as, alcohols, terpenes, aldehydes and ketones [2], therefore the odor usually fades or changes during storage or use. Therefore, it is of interest to obtain a controlled delivery of the fragrance molecules. Current fragrance delivery technology can be categorized into two groups, those that use physical encapsulation and those that use chemical derivation [3]. The physical encapsulation in which active molecules are surrounded by a layer of material (shell) that prevents their release and the penetration of environmental factors until desired, provides the means to slowly release the active ingredients. Fragrance encapsulation systems can be prepared by (a) simple coacervation [4] (b) complex coacervation [5] (c) molecular inclusion [6] (d) spray drying [7] (e) spray chilling [8] (f) extrusion [9] and (g) freeze drying [10]. Many chemical derivatization have been designed for controlled release, e.g., Schiff base and acid-labile acetal bonds [11]. The preparations of Schiff base (imine bond) through reaction of aldehydes or ketones with amino groups, and of acetal through reaction with hydroxyl groups in polymeric chains have been demonstrated [12]. Recently, system that combines physical encapsulation and chemical derivatization has also been reported. Nevertheless, process that gives high concentration product without the preconcentration step is very rare. Here we have created a high loading fragrance encapsulation microparticles using immine derivatives of chitosan, ethylcellulose and methycellulose and demonstrates the controlled release of fragrance from the system.

1.1 Fragrance

Fragrance materials are either volatile organic compound isolated from plants and other natural sources or products from modern organic synthesis. Their odorous characteristics can be categorized into several groups and the popularly used ones include ketones, aldehydes, ethers, alcohols and esters.

Table 1.1 Examples of fragrance

Functional	Substances
Ketones	carvone, camphor, thuyone, cis-jasmone etc.
Aldehydes	vanillin, cinnamaldehyde, citronellal, citral, etc.
Ethers	Eucalyptol, anisole,1,8-cineole, menthofurane, etc.
Alcohols	linalool, geraniol, citronellol, menthol, etc.
Esters	benzyl acetate, amyl salicylate, menthyl, isobornyl acetate, etc.

Ketone and aldehydes represent a large percentage of odorous materials. Thus controlling the release of ketones and aldehydes will definitely benefits directly to the perfumery industry. The following paragraphs detail fragrance which are used in this study.

L-carvone

Other name are (−)-Carvone, (*R*)-(−)-Carvone, (*R*)-5-Isopropenyl-2-methyl-2-cyclohexenone, *p*-Mentha-6,8-dien-2-one and Carvol ($C_{10}H_{14}O$) [13]. L-carvone is a monoterpene ketone found as the main active component of various essential oils, such as spearmint (*Mentha spicata* L.), dill (*Anethum graveolens* L.), caraway (*Carum carvi* L.), and ginger grass (*Lippia* alba) [14]. It is obtained occurs naturally as the enantiomers (+)- and (−)-carvone. L-carvone is an aromatic ketone compound which is a yellow oil appearance with the molecular weight of 150.22 g/mol [15]. L-carvone is insoluble in water but soluble in ethanol, ether, chloroform and acetone. L-carvone is most well-known for its scent and taste of spearmint. Because of this, it is often used as the imitation spearmint flavor in toothpaste and chewing gum. It is also used in spice and floral fragrances and as a plant growth regulator.

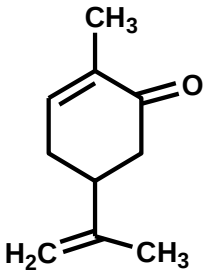


Figure 1.1 Chemical struture of L-carvone [15]

Hexylcinnamaldehyde

Other name are α-Hexylcinnamaldehyde; 2-(Phenylmethylidene)octanal, (2E)-2-Benzylideneoctanal ($C_{15}H_{20}O$) [16]. Hexylcinnamaldehyde is a widely used fragrance with a fresh floral scent of jasmine in particular. It is an aldehyde which is found in the essential oils of various plants. It is a pale yellow to yellow clear liquid to solid appearance with the molecular weight of 216.32 g/mol [17]. This compound contains phenyl group and unsaturated aldehyde (Figure 1.2). Hexylcinnamaldehyde is nearly insoluble in water but soluble in oils. Hexylcinnamaldehyde is the main component of jasmine perfumes. It is an ingredient in low concentrations of many cosmetic products (perfume, soap, deodorant, shampoo, etc.), as well as in products such as air fresheners, disinfectants and detergents. Hexylcinnamaldehyde is FDA approved and is approved by the cosmetics working group for topical use. However, the DIMDI (German Institute of Medical Documentation and Information) rates it as a Class B allergen when used in high concentrations (Source) [18]. Skin care products and cosmetics use Hexylcinnamaldehyde in low concentrations, and it is therefore considered a safe ingredient.

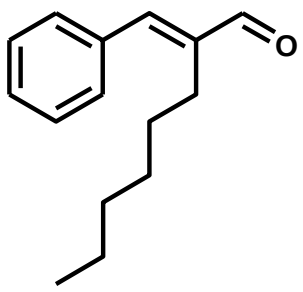


Figure 1.2 Chemical struture of Hexylcinnamaldehyde [18]

Table 1.2 Physical propertiea of some fragrances

Class	Chemical	Molar mass (g/mol)	m.p. (C°)	b.p. (C°)	Density (g/cm^3)
Ketone	L-Carvone	150.22	25.2	231	0.96
Aldehyde	Hexylcinnamaldehyde	216.32	4	308	0.95

1.2 Derivatives of fragrance

Fragrances are highly volatile organic compounds thus their odorous perceptions are short-lived. As mentioned earlier that many of them, especially ketone and aldehydes, are unstable functional group that can undergo degradation prior to their use in the products. To avoid these problems, pro-fragrances have been developed to prolong the fragrance release and stability. They release one or several volatile compounds from well-designed precursor molecules through the cleavages of selective labile-covalent bond during product usage. The following paragraphs contain details of Schiff base formation which used in this study.

Schiff base formation

Amongst many chemical derivatization reactions of aldehyde, reactions of aldehydes with amino groups to form imine bonds or Schiff bases have been popularly used so far for the reason that Schiff base is tolerant under basic conditions but hydrolysable under acidic conditions, it is easy to control the release of the original molecules by changing the environmental pH. The imine product could be stored under dry condition and brought into water contact whenever the aldehyde release was required [19]. Schiff base, a carbon-nitrogen double bond (C=N), could be obtained from the condensation reactions of amines and aldehydes/ketones. The Schiff base is nitrogen analogue of aldehyde or ketone with C=N in place of the carbonyl group (Figure 1.3).

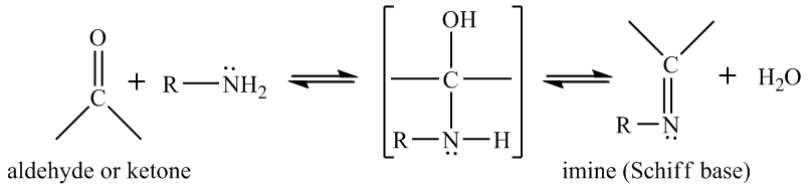
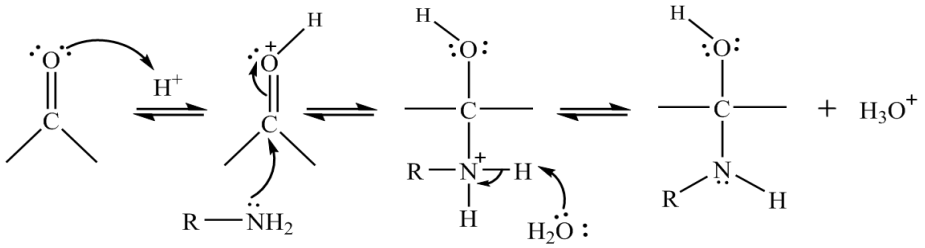


Figure 1.3 Schiff base formation

The mechanism of Schiff base formation starts with an acid-catalyzed nucleophilic addition of the amino group to carbonyl group, follow by deprotonation of nitrogen atom and finally loss of water to form a double bond (Figure 1.4).

Step 1: Acid catalyzed addition of the amine to the carbonyl



Step 2: Acid catalyzed dehydration

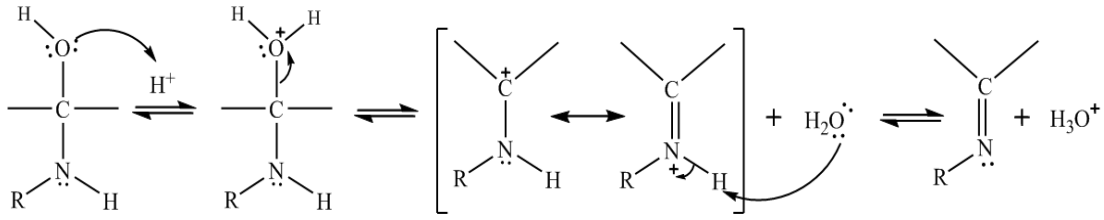


Figure 1.4 The mechanism of Schiff base formation [20]

1.3 Literature reviews of Schiff base

In 2005, Santos *et al*. [21] prepared Schiff base from chitosan and salicyladehyde derivative and from that the substitution degrees depended on the R group of salicyladehyde varying from 4.60 to 68.5%.

In 2009, Jin *et al*. [22] synthesized the Schiff base of chitosan by the reaction of chitosan with citral working under high-intensity ultrasound. The maximum yield achieved was 86.4%. The results confirmed that amino groups on chitosan reacted with citral to form the Schiff base. The antimicrobial activities of chitosan and Schiff base of chitosan were investigated against *Escherichia coil*, *Staphylococcus aureus* and *Aspergillus niger*. The results indicate that the antimicrobial activity of the Schiff

base increases with an increase in the concentration. It was also found that the antimicrobial activity of the Schiff base was stronger than that of chitosan.

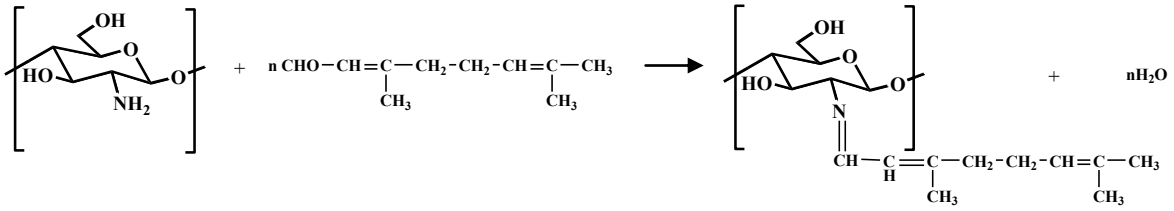


Figure 1.5 Synthesis Schiff base chitosan [22]

In 2011, Tree-udom *et al.* [23] prepared the fragrant chitosan nanospheres using ultrasonication. The fabrication process involves a chemical reaction to covalently link the fragrant aldehydes with the amine functionalities of the *N*-succinylchitosan (NS-chitosan) carriers that simultaneously leads to an ultrasonic aided reorganization of the spheres in such a way that the grafted entities are at the particles' core. Localization of the grafted imine moieties at the core of the imine-NS-chitosan particles was confirmed. The obtained fragrant chitosan nanospheres not only showed up to 85-fold fragrance prolongation but also dispersed well in water.

1.4 Improvement of fragrance

Odorous ketones and aldehydes although are popularly used in everyday products, they often lose their odor due to both their volatility and their reactive nature which leads to reaction with other components during product storage or usage. Volatility and reactivity of other groups of fragrance chemicals such as terpenes, were also a serious problem. Therefore it has become one of the main issues in the fragrance technology to inherit slow release. These problems could be solved by encapsulation technologies.

Encapsulation is the technique by which one material or a mixture of materials is coated with or entrapped within another material or system. The coated material is called active or core material, and the coating material is called shell, wall material, carrier or encapsulant. Coating material is used to protect the encapsulated material from evaporation, reaction, oxidation or otherwise dissipating prior to use during processing and storage. Considerable scientific and commercial interest have been on the delivery of bioactive compounds using polymeric materials as carrier. A number of natural and modified carbohydrate have been shown to be useful control release.

Industries have also involved in development of fragrance-controlled release system for consumer application. From a design point of view, novel control release systems for consumer application should fulfill the following requirements: 1) can release over a long period of time, 2) possess controllable (preferably constant) release rate, 3) are made of the inert, non-toxic and non-carcinogenic polymer and 4) are relatively stable under various storage condition [24-26].

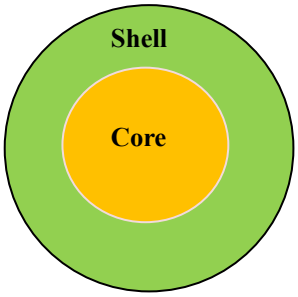


Figure 1.6 Core and shell of microparticles

Encapsulation is well known method to improve shelf life and prolong biological activities. Micro or nanocapsules consist of two disparate zones, the core zone and the coating zone. The core zone contains hydrophobic material such as fragrance, essential oils and their components, medicine. The coating zone (shell, wall, host) are made of various of materials such as cyclodextrin, starch, gum arabic, liposome and polymers.

Cyclodextrin

Cyclodextrin are modified carbohydrates that have been used for many years to modify the solubility properties of hydrophobic molecules by complexation. The widely used natural α-, β- and γ-cyclodextrins consist of six, seven, and eight D-glucopyranose residues, respectively. They differ on the size of the ring and their solubility. The central cavity of cyclodextrin is hydrophobic, while the rims of the surrounding walls are hydrophilic [27]. The hydrophobic guest molecules can stay at the core inside the ring to form inclusion complexes. This will alter physical, chemical, and biological properties of the encapsulated guest molecules [28]. The equilibrium of host-guest, however, depends on the pair and usually is hard to alter.

Liposome

Liposomes are an artificially prepared vesicle composed of polar lipids (phosphatidylcholine or phosphatidylethanolamine) or mixtures of polar lipids with cholesterol or ergosterol [28]. The size of liposomes are in a range from 10-10000 nm depending on the method of manufacturing. The core of liposome could capture both hydrophobic and hydrophilic materials. Liposomes have a variety of different structures, such as unilamellar liposomes (single bilayer membrane), multilamellar vesicles (liposomes with multiple bilayer membranes), and multivesicular vesicles (liposomes contain other size of liposomes in their interior). The use of liposomes has many benefits such as improved penetration and diffusion of active ingredients, selective transport of ingredients, reduction of side effects, high biocompatibility and non-toxic. Despite of their potential value, the important trouble is the physical stability.

Polymer

Polymeric nano or microparticles offer a new level of physical stability comparing to the previously mentioned systems. Many polymers were used as shell materials for the encapsulation technology. Here the polymer used in this research will be reviewed

1.5 Literature reviews improvement of fragrance

In 2009, Liolios *et al.* [28] encapsulated carvacrol and thymol into liposome. Egg L-α-phosphatidylcholine (PC) and cholesterol were used to prepare liposomes. They were prepared by the mechanical shaking technique (thin film method). This method could successfully encapsulated essential oil components into liposomes and the improvement of their antioxidant and antimicrobial activities was observed. However, only a small amount of thymol and carvacrol could be incorporated in liposome, e.g. only 4.16% of carvacrol was encapsulated inside the liposome vesicle.

In 2010, Del Toro-Sa´nchez *et al.* [29] encapsulated thyme essential oil into β-cyclodextrin by the precipitation method. Thyme essential oil could be released from the cyclodextrin by exposing the capsules to high relative humidity.

In 2011, Coimbra *et al.* [30] demonstrated entraped caffeic acid (derivatives), carvacrol (derivatives), thymol, pterostilbene (derivatives), and N-(3-oxo-dodecanoyl)-l

homoserine lactone into the liposome bilayer. The liposome composed dipalmitoyl phosphatidylcholine (DPPC), egg-phosphatidylcholine-35 (EPC-35) and poly (ethyleneglycol) -2000-distearoylphosphatidylethanolamine (PEG2000-DSPE). The results showed that these compounds could be incorporated into liposomes. The N-3-oxo-C_{12}-homoserine lactone and pterostilbene derivatives could be loaded into liposome with efficiencies of 50–70%. However, liposomal caffeic acid could not prevent oxidation when used in various of products.

1.6 Carrier in encapsulated-fragrance system

Amphiphilic polymers are interesting to be used as polymer wall because they can entrap hydrophobic molecules. Their loading capacity depend on the matching of the loaded hydrophobic core and polymer. In this research, ethyl cellulose, methyl cellulose, and chitosan are experimented as shell material for fragrance encapsulation.

Ethylcellulose (EC)

Ethylcellulose (EC) is a cellulose derivative which some of the hydroxyl groups on the repeating glucose units are converted into ethyl ether groups. It is used mostly in pharmaceutical industry as drug binder such as thin-film coating material, and in food industry as a food additive. Ethylcellulose is one of the most stable cellulose derivatives. It is resistant to alkalis but sensitive to acids. Ethylcellulose takes up little water from moist air or during immersion, and water evaporation leaves the ethyl cellulose with unchanged property. Light such as the visible and ultraviolet radiant do not discolor ethyl cellulose. The glass-transition temperature of ethyl cellulose various with degree of ethoxy unit however it is around is 120-140 °C [31]. Ethyl cellulose is non-toxic and biocompatible [32], slightly soluble in water and soluble in ethanol.

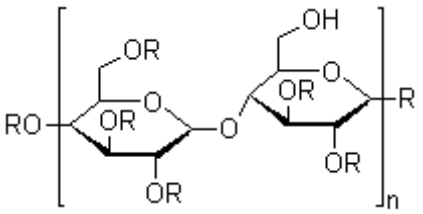


Figure 1.7 Structure of ethyl cellulose (R = H or C_2H_5) [32].

Methylcellulose (MC)

Methylcellulose (MC) is a chemical compound derived from cellulose which some of the hydroxyl groups on the repeating glucose units are converted into methyl groups. It is a hydrophilic polymer and it dissolves in water as the clear viscous solution or gel. It is used as a thickener and emulsifier in various food and cosmetic products. It is not toxic and not allergenic [33].

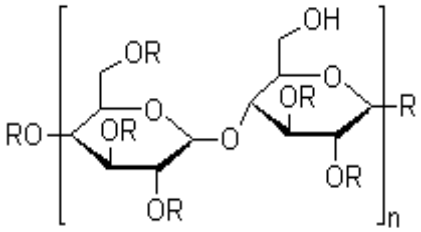


Figure 1.8 Structure of methylcellulose (R = H or CH_3) [33].

Poly(vinyl alcohol) (PVA)

Poly(vinyl alcohol), (PVA, PV(OH) or PVAL) is a well known synthetic polymer that soluble in water, while powder, tasteless and odorless. It is a highly crystalline which prepared by radical polymerization of vinyl acetate followed by saponification of poly(vinyl acetate). PVA has excellent film forming, emulsifying and adhesive properties. It is also resistant to oil, grease and solvents. It is odorless and nontoxic. It has high tensile strength and flexibility, as well as high oxygen and aroma barrier properties [34, 35]. PVA is biodegradable by a restricted number of aerobic bacteria, among them several species of Pseudomonas. The biodegradation of natural polymers.

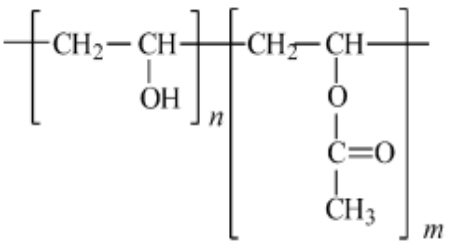


Figure 1.9 Structure of Polyvinyl alcohol (R = H or CH_3) [34].

Hydroxypropyl methylcellulose (HPMC)

Hydroxypropyl methylcellulose (HPMC) is an odorless and tasteless, white to slightly off-white, fibrous or granular, free-flowing powder that is a synthetic modification of the natural polymer, cellulose [36]. It is commonly used by the pharmaceutical, food, adhesive, paint, printing, and textile industries and has a variety of uses including: an enteric film coating, viscosity control agent, gelling agent, stabilizer, lubricant, binder, emulsifier and suspending agent. These added groups confer on the molecule its unique properties of being cold-water soluble, while at the same time exhibiting reversible gelation when heated and recooled [37].

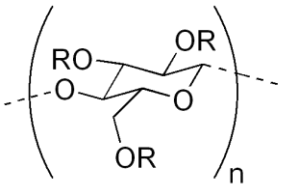
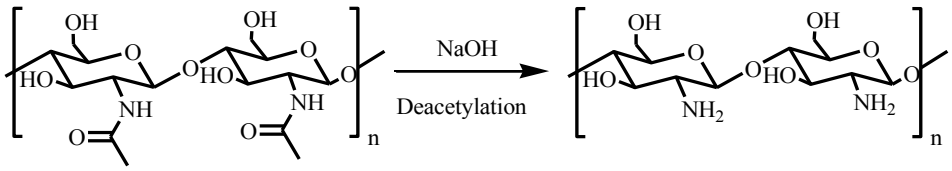


Figure 1.10 Structure of hydroxypropyl methylcellulose (R = H or CH3 or $CH_2CH(OH)CH_3$) [37].

Chitosan

Chitosan is a natural polymer derived from chitin by deacetylation (Figure 1.2). It consists of unbranched chain of β-(1-4)-2-acetamido-2-acetamido-2-deoxy-β-d-glucose and β-(1-4)-2-amino-2-acetamido-2-deoxy-β-d-glucose as a repeating units. It can be extracted from fungi, insect, lobster, shrimp and krill. This biopolymer and its derivatives have potential applications in the areas of biotechnology, biomedicine [38-41], agriculture, wastewater purification [42], environmental protection, food [43] and cosmetics. Chitosan is non-toxic, biocompatible, biodegradable and possesses anti-bacterial property [44]. Although chitosan should be useful for even more numerous applications, the compound suffers limitation on solubility, chitosan is soluble only in acid. Thus various chemical modifications of chitosan structure have been reported for the improvement of its solubility.



chitin chitosan

Figure 1.11 Deacetylation reaction of chitin [38].

Chemical modification of Chitosan

The structure of chitosan is widely modified in order to improve its solubility and applications [45-47]. *N*-succinyl-chitosan, a water dispersible chitosan derivative (Figure 1.12) was investigated in this research.

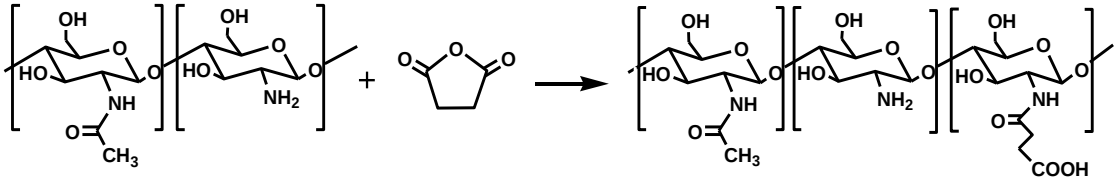


Figure 1.12 Preparation of *N*-succinylchitosan

In 2006, Aping *et al.* [48] synthesized N-succinyl-chitosan nanospheres (NSCS), succinyl was substituted at hydrogen atom of amino group in chitosan chains. In distilled water, NSCS easily dispersed into the stable and transparent dispersion. Examining of the dispersion indicated an assembling of polymer into spherical particles. The self-assembly of the obtained nanospheres probably induce from the decrease in intermolecular H-bonding thus lower the crystallinity of the chitosan. The average size of the NSCS nanospheres was about 50–100 nm (Figure 1.16).

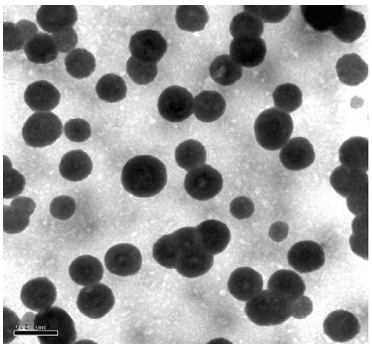


Figure 1.13 TEM micrographs of NSCS nanospheres [48]

1.7 Literature reviews of carrier in encapsulated-fragrance

Poor solubility in water of many fragrances usually causes difficulty in product formulation. Although surfactant can be used as stabilizing agent, it is still faced with other problems such as turbidity, irritation and stability. The encapsulation of fragrance into carrier provides both increased solubility and controlled release characteristic. Through encapsulation technology, easy handling, increased safety and enhanced stability have also been realized. Herein, the recent carriers for long-time lasting fragrance release with different types of polymer are being reviewed.

Use of Poly(vinyl alcohol)

In 2008, Fernanda *et al.* [49] studied effect of stirring on morphology and size distribution of lemongrass oil encapsulated with poly(vinyl alcohol) microcapsules (prepared by coacervation technique). The microcapsule obtained were spherical. The particle size of particles obtained rate. Increased stirring rate led to decreased size distribution. In addition, volume fraction (percentage of the lemongrass oil in the system) also affected the size of particles.

Use of Hydroxypropyl methylcellulose (HPMC)

In 2004, Popplewell *et al.* [50] disclosed the use of organically soluble cellulosic material selected from the group consisting of ethyl cellulose and hydroxylpropyl cellulose, to encapsulate organic fragrance chemicals and flavor chemicals with spay drying and extrusion method. The process also requires the use of emulsifiers. From this process, the particles obtained were 10-2000 microns

Use of polymer blend

In 2010, Sansukcharearnpon *et al.* [51] encapsulated the six fragrances, camphor, citronellal, eucalyptol, limonene, menthol and 4-tert-butylcyclohexyl into polymer blend based ethylcellulose (EC), hydroxypropylmethylcellulose (HPMC) and poly(vinylalcohol) (PV(OH)). The encapsulation process was carried out by solvent displacement method. This process gave nanospherical particles with the fragrance loading capacity and encapsulation efficiency of ≥40% and ≥80%, respectively, at weight ratio of the fragrance: polymer of 1:1. The release profiles of all encapsulated fragrances and pure fragrances were determined using thermogravimetric analysis

(TGA) and electronic nose (e-nose). The results from both techniques were agreeably and it was concluded that the release of all tested fragrances except limonene could be prolonged by encapsulation into the polymer blend (Figure 1.21).

Use of chitosan

In 2006, Wen *et al.* [52] prepared microcapsules for volatile essential oil encapsulation using oil-in-water (O/W) emulsion process, using chitosan as a wall material, citronella oil as an inner core material and coconut oil as a surfactant. The prepared sample at chitosan 0.5 wt%, NaOH 1.0 wt% with coconut oil possessed a good dispersion character and gave the encapsulation efficiency of 98.2%. The particle size of chitosan microcapsules decreased when the emulsification stirring rate increased. The release of citronella oil from the microcapsules was investigated by determining the time course of the microcapsules weight placed in an Infrared Moisture Determination Balance (IMDB). The release rate of citronella oil from smaller microcapsules was faster than that of bigger microcapsules because of larger surface area. The release behaviors of the active after thermal pretreatment at 40 ^{o}C and 60 ^{o}C for 1 min were similar, whereas that at 80 ^{o}C was significantly slower. The author explained the slower release rate at high temperature through the closing of the pores induced by the wall shrinkage at high temperature (Figure 1.17).

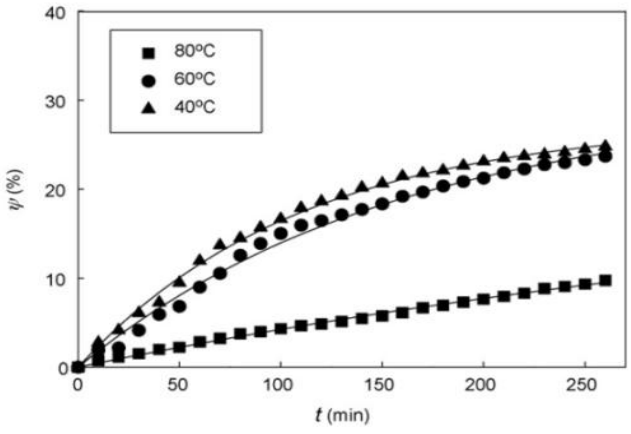


Figure 1.14 Time course of oil release from microcapsules at various temperatures [57].

1.8 Controlled release of fragrances

Controlled release is a mechanical which administer drug, fragrance, or other active agent were encapsulated in polymeric material or natural matrixes to release at a specific rate and specific condition, The release of active agents are depending on four important factors including initial loading of active agent in the polymer, the solubility of the active agent in the solvent, the equilibrium partitioning of the active agent between polymer and solvent and diffusion barriers. The advantages of controlled release including 1) decreased the losing of active agents between production processes at high temperature, 2) separate active agents from incompatible component to protect degradation of fragrance, 3) processes controllable release rate and 4) increased stability of fragrance storage under several condition [53]. The stability of encapsulated spheres is depending on chemical structure, volatility and polarity of fragrance, type of coating material and mechanical and condition of encapsulation. The mechanism of release, can be designed to involve solvent effects, such as diffusion, melting, pH, degradation and particle fracture [54].

Diffusion is a major mechanism of fragrance release from matrix system. The vapor pressure of volatile on each side of matrix is the important driving force influence diffusion. The volatile components slowly differs away from the matrix. The factors effect diffusion of fragrance include degree of swelling.

1.9 Literature reviews of control release

In 1999, Gunning *et al.* [55] encapsulated cherry and peppermint flavors into sucrose/maltodextrin carbohydrate matrixes by an extrusion process. The obtained products show some heterogrneity in tern of rod diameters, lengths and surface textures. The release was investigated at various water contents by headspace analysis in combination with gas chromatography (GC) and a flame ionization detector (FID). It was found that the release increased with increasing water content.

In 2006, Hwang *et al.* [56] prepared melamine resin microcapsules containing fragrant oil by oil in water emulsion technique and studied thermal properties. The obtained melamine microcapsules possessed a great surface smoothness. The particles size was below 10 μm with narrow size distribution. The obtained melamine resin

microcapsules were stable at high temperature up to 420 °C. The resultant melamine resin microcapsules possessed thermosetting wall

In 2007, Baranauskiene *et al*. [57] encapsulated the essential oil (EO) of peppermint (Mentha poperita L.) into different carbohydrate carrier materials consisting of several n-octenyl succinic anhydride (OSAN) = modified starches (maize and tapioca) and several hydrolyzed starches (dextrins), using spay-draying emulsification. The results revealed that the emulsification and encapsulation efficiencies of peppermint EO were higher for all n-octenyl succinic anhydride (OSAN) = modified starches as compared to those of hydrolyzed starches (dextrins). The compositions of pure, emulsified, and encapsulated peppermint in different starch matrices were quite similar when analyzed by GC and GC-MS. The release of peppermint was investigated by headspace analysis in combination with GC-FID method. It could be concluded that OSAN-modified starch gave in the highest retention of peppermint volatiles and the martrie could be recommended when slow release of volatiles is required.

1.10 Research goals

The aim of this research is to prepare the microparticles based biopolymer containing fragrance ketone and aldehyde molecules in order to effectively control the release of the fragrance ketone and aldehyde. The system consisted of polymer blend shell as a physical barrier and ketone and aldehyde linked to amine group of derivatives as a chemical barrier. The work included:

1. Preparation of N-succinylchitosan (N-SC).
2. Preparation of fragrance aldehyde and ketone derivative (imine-N-succinylchitosan microparticles).
    - Preparation of L-carvonenesuccinylchitosan
    - Preparation of hexylcinnamalidenesuccinylchitosan
3. Encapsulation fragrance into stable microparticles at high loading
4. Chemical structure, morphological observation and surface characterization of prepared microparticles.

5. Controlled release study of fragrance-encapsulated microparticles compared with free fragrance ketone and aldehyde.

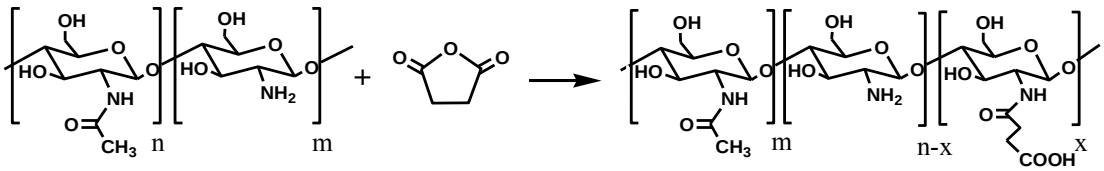
CHAPTER II

EXPERIMENTAL

2.1 Materials and Chemicals

Chitosan (M_V = 25,000 Da, 90% deacetylated) was purchased from Taming Enterprises Co., Ltd (Thailand). Succinic anhydride (analytical grade) was purchased from Acros Organics (Geel, Belgium). L-carvone, hexylcinnamaldehyde, ethylcellulose (EC), methylcellulose (MC), poly(vinyl alcohol) (PVA), were purchased from Sigma Aldrich (Steinheim, Germany). Hydroxypropyl Methyl cellulose (HPMC) was purchased from Dow Wolff Cellulosics (Bomlitz, Germany). Amberlyst^{TM} 15 Wet was purchased from Rohm and Hass Company (Philadelphia, U.S.A.). Ethanol and acetone were purchased from ACI Labscan Limited (Bangkok, Thaiand). Acetic acid was purchased from Merck (Darmstadt, Germany). Other reagents were analytical grade and were used without further purification.

2.2 Synthesis of N-succinylchitosan (N-SC)



Scheme 2.1 Synthesis of N-succinylchitosan (N-SC) [Tree-udom, 2010]

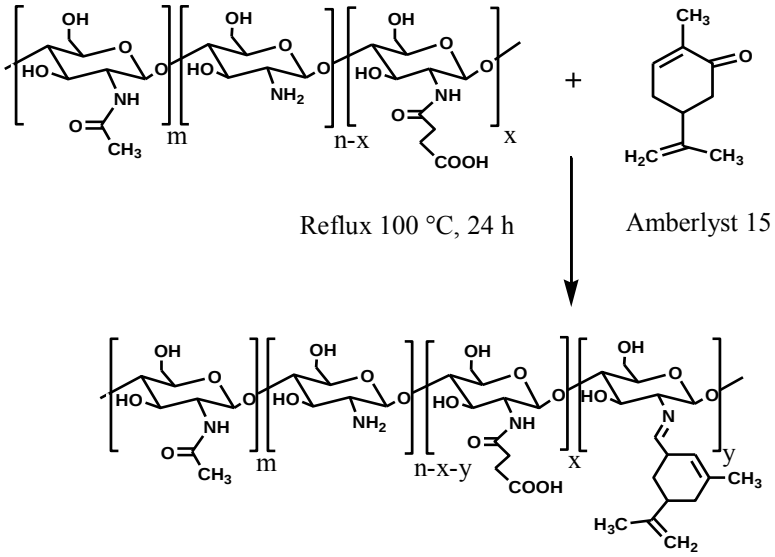
The N-succinylchitosan (N-SC) was synthesized according to Scheme 2.1 Succinic anhydride was allowed to react with each glucosamine unit of chitosan at mole ratios of 0.34:1, 0.25:1, 0.2:1 and 0.1:1. For each sample, 2.01 g of chitosan (12.5 mmole) was dissolved in 100 mL of 1%v/v acetic acid. The 0.414, 0.312, 0.25 and 0.125 g of succinic anhydride (4.2, 3.1, 2.50 and 1.25 moles, respectively) in 10 mL of acetone were slowly dropped into acidic chitosan solution while stirring, and the mixture was left at room temperature overnight. Then the reaction mixture was precipitated with excess acetone. The precipitate was collected by filtration and wash with acetone several times. Finally, a white powder was obtained. The product was characterized by Nuclear magnetic resonance (NMR), a Varian mercury spectrometer

(Variance Inc., Palo Alto, USA), operating at 400 MHz. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR), a Continuμm^{TM} infrared microscope equipped with a mercury-cadmium-telluride (MCT) detector and connected to a Nicolet 6700 FT-IR spectrometer (Thermo Electron Corporation, Madison, WI, USA) and ATR accessory consisting of a slide-on miniature germanium (Ge) as the internal reflection element was used, collecting with 64 scans in the mid-infrared region (4000 – 650 cm^{-1}).

N-succinylchitosan (N-SC): 88% yield of white powder and 0.19 degree of succinyl grafting. ^{1}H NMR (D_2O, 400 MHz, δ, ppm): 1.85 (H of acetyl groups), 2.26-2.33 (methylene protons of the succinyl), 2.65 (H2 of glucosamine, GlcN), 3.44-3.73 (H2′ of *N*-acetylglucosamine, GlcNAc, H3, H4, H5 and H6 of GlcNAc and GlcN), 4.38 (H1 of GlcNAc and GlcN). ATR-FTIR (cm^{-1}): 3284 (N-H and O-H stretching), 2874 (C-H stretching), 1639 (C=O stretching of amide I), 1557 (N-H bending of amide II), 1395 (symmetric stretching of COO^{-} and C-N stretching of amide III), 1319 (C-N stretching of amide III), 1149 (C-O-C stretching) and 1025 (C-O stretching of glucosamine unit).

2.3 Preparation of imine-N-SC derivatives

2.3.1 Finding the optimum ratio between N-SC derivatives and L-carvone

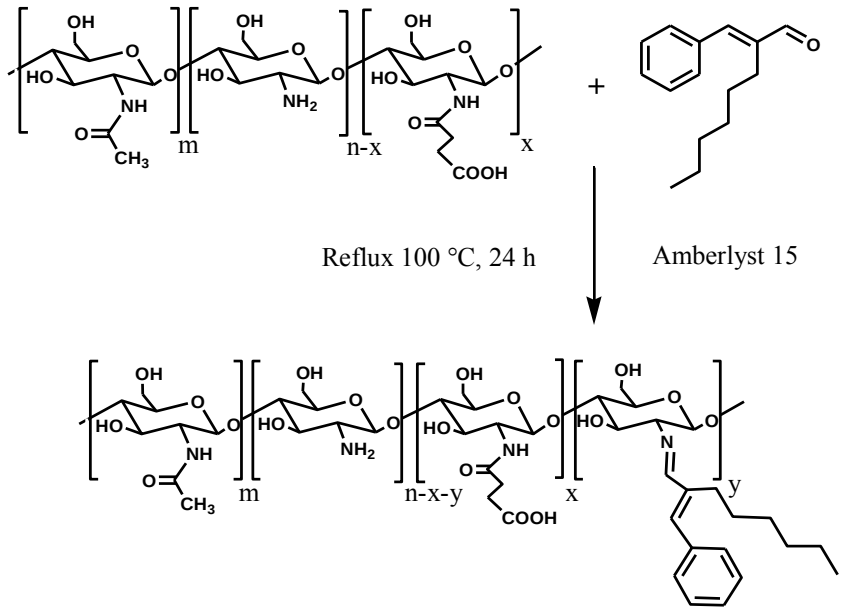


Scheme 2.2 Preparation of L-carvone imine-N-SC derivatives

L-carvone imine-N-SC derivatives (Schiff base's derivative) was prepared by heterogeneous phase-grafting reaction. N-SC (100 mg) was dispersed in 20 mL of distilled water. The alcoholic solution (100 mg of L-carvone in 5 mL of ethanol) was dropped into the aqueous phase and the amberlyst 15 Wet catalyst is added to a reaction system. Then the mixture was refluxed for 24 h at 100^{o}C under nitrogen atmosphere. The amine in N-SC derivatives was allowed to react with ketone (L-carvone) at the weight ratio of N-SC to L-carvone of 1:1, 2:1 and 3:1 (Scheme 2.2). The imine bond formation was characterized through ATR-FTIR.

L-carvonenesuccinylchitosan (LC-NSC) ATR-FTIR (cm^{-1}): 3282 (N-H stretching and O-H stretching vibration), 2867 (C-H stretching vibration), 1620 (C=N stretching vibration), 1555 (amide II), 1150 (C-O-C stretching vibration), and 1023 (C-O stretching vibration).

2.3.2 Finding the optimum ratio between N-SC derivatives and hexylcinnamaldehyde



Scheme 2.3 Preparation of hexylcinnamalidene-N-SC derivatives

Hexylcinnamalidene-N-SC derivatives (Schiff base's derivative) was prepared by heterogeneous phase-grafting reaction. N-SC (100 mg) was dispersed in 20 mL of distill water. The alcoholic solution (100 mg of hexylcinnamaldehyde in 5 mL of

ethanol) was dropped into the aqueous phase and the amberlyst 15 Wet catalyst is added to a reaction system. Then the mixture was refluxed for 24 h at 100^{o}C under nitrogen atmosphere. The amine in N-SC derivatives was allowed to react with aldehyde (hexylcinnamaldehyde) at the weight ratio of N-SC to hexylcinnamaldehyde of 1:1, 2:1 and 3:1 (Scheme 2.3). The imine bond formation was characterized through ATR-FTIR.

Hexylcinnamalidenesuccinylchitosan (HC-NSC) ATR-FTIR (cm^{-1}): 3284 (N-H stretching and O-H stretching vibration), 2871 (C-H stretching vibration), 1666 (C=N stretching vibration), 1583 (amide II), 1149 (C-O-C stretching vibration), and 1025 (C-O stretching vibration).

2.4 Microparticles formation

2.4.1 Encapsulation of L-carvone

2.4.1.1 Comparing the type of used polymer

Table 2.1 Amounts of materials used during the encapsulation of L-carvone

Order	Fragrance : Polymer 10 : 1				Polymer blend			
	LC 50,000 (ppm)	EC 4,000 (ppm)	MC 1,000 (ppm)	H_2O	PVA 5,000 (ppm)	HPMC 5,000 (ppm)	N-SC 5,000 (ppm)	Imine-NSC 5,000 (ppm)
1	10 g	20 ml	5 ml	75 ml	-	-	-	-
2	10 g	20 ml	5 ml	-	75 ml	-	-	-
3	10 g	20 ml	5 ml	-	-	75 ml	-	-
4	10 g	20 ml	5 ml	-	-	-	75 ml	-
5	10 g	20 ml	5 ml	-	-	-	-	75 ml

**LC : L-carvone*

Blend of EC and MC shell

L-carvone fragrance was encapsulated into the polymer-blend of EC and MC (EC: MC w/w ratio of 4:1) at weight ratio of L-carvone to polymer of 10:1 by

microemulsion homogenization. In brief, 400 mg of EC dissolved in 20 mL of ethanol were mixed with 100 mg of the MC dissolved in 5 mL of distilled water. The mixture was stirred until clear transparent solution was obtained. Then 5 g of L-carvone was added into EC/MC solution and then 75 mL of deionized water was slowly dropped into the mixture while the mixture was continuously homogenized by homogenizer (T25 digital ultra-turrax, Staufen, Germany) at 8000 rpm for 30 min. The final product was characterized by SEM.

Blend of EC, MC and Poly(vinyl alcohol) (PVA) shell

L-carvone fragrance was encapsulated into the polymer-blend of EC, MC and polyvinyl alcohol (PVA) (EC: MC w/w ratio of 4:1) at weight ratio of L-carvone to polymer of 10:1 by microemulsion homogenization. In brief, 400 mg of EC dissolved in 20 mL of ethanol were mixed with 100 mg of the MC dissolved in 5 mL of distilled water. The mixture was stirred until clear transparent solution was obtained. Then 5 g of L-carvone was added into EC/MC solution and then 500 mg of PVA dissolved in 75 mL of deionized water was slowly dropped into the mixture while the mixture was continuously homogenized by homogenizer at 8000 rpm for 30 min. The final product was characterized by SEM.

Blend of EC, MC and Hydroxypropyl Methylcellulose (HPMC) shell

L-carvone fragrance was encapsulated into the polymer-blend of EC, MC and HPMC (EC: MC w/w ratio of 4:1) at weight ratio of L-carvone to polymer of 10:1 by microemulsion homogenization. In brief, 400 mg of EC dissolved in 20 mL of ethanol was mixed with 100 mg of the MC dissolved in 5 mL of distilled water. The mixture was stirred until clear transparent solution was obtained. Then 5 g of L-carvone was added into EC/MC solution and then 500 mg of HPMC dissolved in 75 mL of deionized water and slowly dropped into the mixture while the mixture was continuously homogenized by homogenizer at 8000 rpm for 30 min. The final product was characterized by SEM.

Blend of EC, MC and N-SC shell

L-carvone fragrance was encapsulated into the polymer-blend of EC, MC *and* N-SC (EC: MC w/w ratio of 4:1) at weight ratio of L-carvone to polymer of 10:1 by

microemulsion homogenization. In brief, 400 mg of EC dissolved in 20 mL of ethanol were mixed with 100 mg of the MC dissolved in 5 mL of distilled water. The mixture was stirred until clear transparent solution was obtained. Then 5 g of L-carvone was added into EC/MC solution and then 500 mg of N-SC dissolved in 75 mL of deionized water and slowly dropped into the mixture while the mixture was continuously homogenized by homogenizer at 8000 rpm for 30 min. The final product was characterized by SEM.

Blend of EC, MC and imine derivatives of N-SC shell

L-carvone fragrance was encapsulated into the polymer-blend of EC, MC and imine derivatives of N-SC (EC: MC w/w ratio of 4:1) at weight ratio of L-carvone to polymer of 10:1 by microemulsion homogenization. In brief, 400 mg of EC dissolved in 20 mL of ethanol were mixed with 100 mg of the MC dissolved in 5 mL of distilled water. The mixture was stirred until clear transparent solution was obtained. Then 5 g of L-carvone was added into EC/MC solution and then 75 mL of imine derivatives of chitosan solution (Scheme 2.2) was slowly dropped into the mixture while the mixture was continuously homogenized by homogenizer at 8000 rpm for 30 min. The final product was characterized by SEM.

2.4.1.2 Optimization of polymer : L-carvone

To find the optimum ratio between polymer (blend of EC, MC and imine derivatives of N-SC) and L-carvone, the weight ratio of polymer and L-carvone was experimented at 1:1, 1:3, 1:5, 1:10 and 1:20. The final products were characterized by SEM.

2.4.1.3 Finding the optimum speed of homogenization

L-carvone fragrance was encapsulated into the polymer-blend of EC, MC and imine derivatives of N-SC (EC: MC w/w ratio of 4:1) at L-carvone to polymer weight ratios of 10:1 by microemulsion homogenization. The suspension was homogenized by homogenizer at 6000, 8000, 10000, 12000 rpm respectively. Particles size and morphology of the obtained suspension were analyzed by SEM.

2.4.2 Encapsulation of hexylcinnamaldehyde

2.4.2.1 Comparing the type of used polymer

Table 2.2 Amounts of materials used during the encapsulation of hexylcinnamaldehyde

Order	Fragrance : Polymer 10 : 1				Polymer blend			
	HC 50,000 (ppm)	EC 4,000 (ppm)	MC 1,000 (ppm)	H_2O	PVA 5,000 (ppm)	HPMC 5,000 (ppm)	N-SC 5,000 (ppm)	Imine-NSC 5,000 (ppm)
1	10 g	20 ml	5 ml	75 ml	-	-	-	-
2	10 g	20 ml	5 ml	-	75 ml	-	-	-
3	10 g	20 ml	5 ml	-	-	75 ml	-	-
4	10 g	20 ml	5 ml	-	-	-	75 ml	-
5	10 g	20 ml	5 ml	-	-	-	-	75 ml

**HC : Hexylcinnamaldehyde*

Blend of EC and MC shell

Four hundred milligrams of EC was dissolved in 20 mL of ethanol then 100 milligrams of the MC were dissolved in 5 mL of distilled water and stirred until clear transparent solutions were obtained and the two solutions were mixed. Then 5 g of hexylcinnamaldehyde was added into EC/MC solution and then 75 mL of deionized water was slowly dropped into the mixture while the mixture was continuously homogenized by homogenizer at 8000 rpm for 30 min. The final product was characterized by SEM.

Blend of EC, MC and Poly(vinyl alcohol) (PVA) shell

Four hundred milligrams of EC was dissolved in 20 mL of ethanol then 100 milligrams of the MC were dissolved in 5 mL of distilled water and stirred until clear transparent solutions were obtained and the two solutions were mixed. Then 5 g of hexylcinnamaldehyde was added into EC/MC solution and then 500 milligrams of PVA dissolved in 75 mL of deionized water was slowly dropped into the mixture

while the mixture was continuously homogenized by homogenizer at 8000 rpm for 30 min. The final product was characterized by SEM.

Blend of EC, MC and Hydroxypropyl Methylcellulose (HPMC) shell

Four hundred milligrams of EC was dissolved in 20 mL of ethanol then 100 milligrams of the MC were dissolved in 5 mL of distilled water and stirred until clear transparent solutions were obtained and the two solutions were mixed. Then 5 g of hexylcinnamaldehyde was added into EC/MC solution and then 500 milligrams of HPMC dissolved in 75 mL of deionized water was slowly dropped into the mixture while the mixture was continuously homogenized by homogenizer at 8000 rpm for 30 min. The final product was characterized by SEM.

Blend of EC, MC and N-SC shell

Four hundred milligrams of EC was dissolved in 20 mL of ethanol then 100 milligrams of the MC were dissolved in 5 mL of distilled water and stirred until clear transparent solutions were obtained and the two solutions were mixed. Then 5 g of hexylcinnamaldehyde was added into EC/MC solution and then 500 milligrams of N-SC dissolved in 75 mL of deionized water was slowly dropped into the mixture while the mixture was continuously homogenized by homogenizer at 8000 rpm for 30 min. The final product was characterized by SEM.

Blend of EC, MC and imine derivatives of N-SC shell

Four hundred milligrams of EC was dissolved in 20 mL of ethanol then 100 milligrams of the MC were dissolved in 5 mL of distilled water and stirred until clear transparent solutions were obtained and the two solutions were mixed. Then 5 g of hexylcinnamaldehyde was added into EC/MC solution and then 75 mL of imine derivatives of N-SC solution (Scheme 2.3) was slowly dropped into the mixture while the mixture was continuously homogenized by homogenizer at 8000 rpm for 30 min. The final product was characterized by SEM.

2.4.2.2 Optimization of polymer : Hexylcinnamaldehyde

To find the optimum ratio between polymer (Blend of EC, MC and imine derivatives of N-SC) and hexylcinnamaldehyde, the weight ratio of polymer and

hexylcinnamaldehyde were experimented at 1:1, 1:2, 1:3, 1:5, 1:10 and 1:20. The final products were characterized by SEM.

2.4.2.3 Finding the optimum speed of homogenization

Hexylcinnamaldehyde fragrance was encapsulated into the polymer-blend of EC, MC and imine derivatives of N-SC (EC: MC w/w ratio of 4:1) at hexylcinnamaldehyde to polymer weight ratios of 10:1 by microemulsion homogenization. The suspension was homogenized by homogenizer at 6000, 8000, 10000, 12000 rpm respectively. Particles size and morphology of the obtained suspension were analyzed by SEM.

To determine encapsulation efficiency percentage (% EE), and loading capacity (% loading), 1 ml of the suspension (concentration of EC/MC of 5000 ppm) was added with 15 mL of hexane and shacked vigorously for 30 min, The hexane layer was then subjected to UV-visible spectrophotometer (Shimadzu Corporation, Kyoto, Japan) to quantify L-carvone and hexylcinnamaldehyde with the aid of appropriate calibration curve. Percent encapsulation efficiency (%EE) and % loading capacity (%LC) were determined as following:

$$\% \text{ EE} = \frac{\text{weight of encapsulated fragrance}}{\text{weight of fragrance used initially}} \times 100$$

$$\% \text{ LC} = \frac{\text{weight of encapsulated fragrance}}{\text{weight of encapsulated fragrance} + \text{weight of polymer}} \times$$

2.5 Morphology and Zeta potential of the microparticles

The morphology of fragrance-loaded microparticles SEM and TEM. Particle size distribution and the ζ potential were observed by SEM.

SEM photographs were obtained using a scanning electron microscope (JEM-6400, JEOL, Tokyo, Japan). A drop of the encapsulation of fragrance suspension was placed on a glass slide and dried. The sample was coated with a gold layer under vacuum at 15 kV for 90 s.

An average particle size (z-average size) was measured by Zetasizer (Nano Series Model) (Malvern Instruments, Worcestershire, UK) equipped with a He-Ne laser beam at 632.8 nm (scattering angle of 173º) at 25 ± 2ºC. Each measurement was repeated three times and an average value was reported.

2.6 Determination of release profiles

2.6.1 The release of L-carvone from microparticles

Five milliliters of the microparticles aqueous suspensions (pH of 5, prepared at L-carvone to polymer weight ratio of 10:1, final concentration of L-carvone of 54g/100ml), were put into a 2 ml flat bottom headspace vial (eight vials for each sample). The vials were left uncovered at 55 °C. At the indicated times (0, 1, 2, 3, 5, 8, 11 and 14 days), one of the eight initial vials per sample was pH adjusted to 1.0 with 1 M HCl, filled with 5 ml of hexane and then capped with headspace aluminum crimp caps with PTFE/silicone septa. The hexane layer was then subjected to L-carvone quantification using UV–Vis spectroscopy with the aid of a calibration graph constructed from freshly prepared standard L-carvone solutions.

2.6.2 The release of Hexylcinnamaldehyde from microparticles

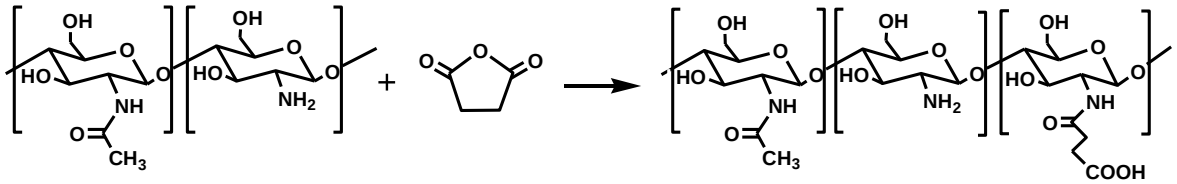
Five milliliters of the microparticles aqueous suspensions (pH of 5, prepared at hexylcinnamaldehyde to polymer weight ratios of 10:1, final concentration of hexylcinnamaldehyde of 54g/100ml), were put into a 2 m l flat bottom headspace vial (eight vials for each sample). The vials were left uncovered at 55 °C. At the indicated times (0, 1, 2, 3, 5, 8, 11 and 14 days), one of the eight initial vials per sample was pH adjusted to . with M HCl, filled with 5 ml of hexane and then capped with headspace aluminum crimp caps with PTFE/silicone septa. The hexane layer was then subjected to hexylcinnamaldehyde quantification using UV–Vis spectroscopy with the aid of a calibration graph constructed from freshly prepared standard hexylcinnamaldehyde solutions.

CHAPTER III

RESULTS AND DISCUSSION

We have created a high loading fragrance encapsulation microparticles using various polymeric shell materials. The shell materials included ethyl cellulose (EC), methyl cellulose (MC), poly(vinyl alcohol) (PVA), hydroxypropyl methylcellulose (HPMC), N-succinylchitosan (N-SC) and imine derivatives of N-succinylchitosan (imine-N-SC). The obtained fragrance-loaded particles were evaluated for their release profiles.

3.1 Synthesis and characterization of N-succinylchitosan (N-SC)



Scheme 3.1 Synthesis of N succinylchitosan (N-SC)

The reaction for derivatization of chitosan is present in Scheme 3.1. The chitosan derivative was prepared via a succinylation reaction between amino group and succinic anhydride to form N-succinylchitosan (N-SC).

The physical appearance of the synthetic N-SC obtained from reactions conducted at mole ratios between the succinic anhydride : amino group of the chitosan of 0.1:1, 0.2:1, 0.25:1, and 0.34:1, were pale yellow (N-SC1 and N-SC2) and white (N-SC3 and N-SC4) powder, respectively (Figure 3.1).

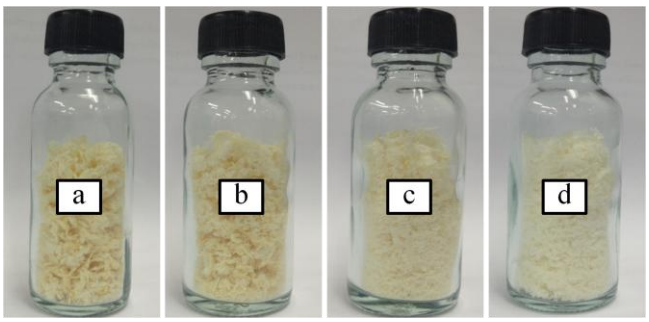


Figure 3.1 N-SC products obtained at the mole ratios of succinic anhydride: chitosan of (a) 0.1:1 (N-SC1), (b) 0.2:1 (N-SC2), (c) 0.25:1 (N-SC3) and (d) 0.34:1 (N-SC4)

Since one of the main objectives to first prepare N-SC was to overcome the water insolubility of chitosan, thus the water dispersibility of the product was seriously considered. And it was found that the N-SC could disperse well in water. The hydrogen bonding of the obtained N-SC should become less pronounced comparing to the original chitosan, thus N-SC could easily disperse in deionized water. Well-defined derivatives existed in the colloidal aqueous solution as shown in Figure 3.2.

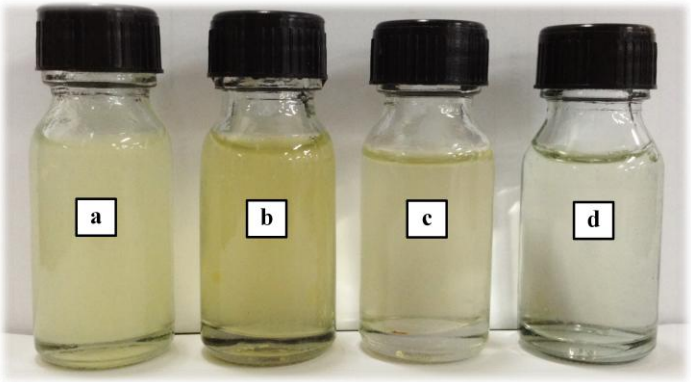


Figure 3.2 The 1 mg/mL colloidal dispersion of (a) N-SC1, (b) N-SC2, (c) N-SC3 and (d) N-SC4

Comparing between N-SC1, N-SC2, N-SC3 and N-SC4 a better transparent colloidal suspension in water was observed for N-SC4 (Figure 3.2). This result indicated that the dispersibility increased when the amount of succinyl group of N-SC increased.

The successful succinyl moiety grafting was confirmed by NMR and ATR-FTIR. For ^{1}H NMR spectrum of N-SC in D_2O (Figure 3.3), signal appearing at 2.26 -2.33 ppm assigned to dimethylene protons of grafted succinyl was observed. Using the integral ratio between 4H from ethyl group of succinyl and 1H from C2 of glucosamine unit (at 2.65 ppm) with 90% deacetylation degree, the degree of grafting could be estimated as 0.19. We found that the obtained chitosan derivative could be apparently miscible in water even when not all amino groups were functionalized. Therefore, there were amino groups on polymer chain left to be coupled with fragrances afterward. ATR-FTIR spectrum (Figure 3.4) showed new absorption band at 1557 and 1395 cm^{-1} corresponding to N-H bending vibration of amide II and symmetric stretching vibration of COO^{-} and C-N stretching vibration of amide III, respectively.

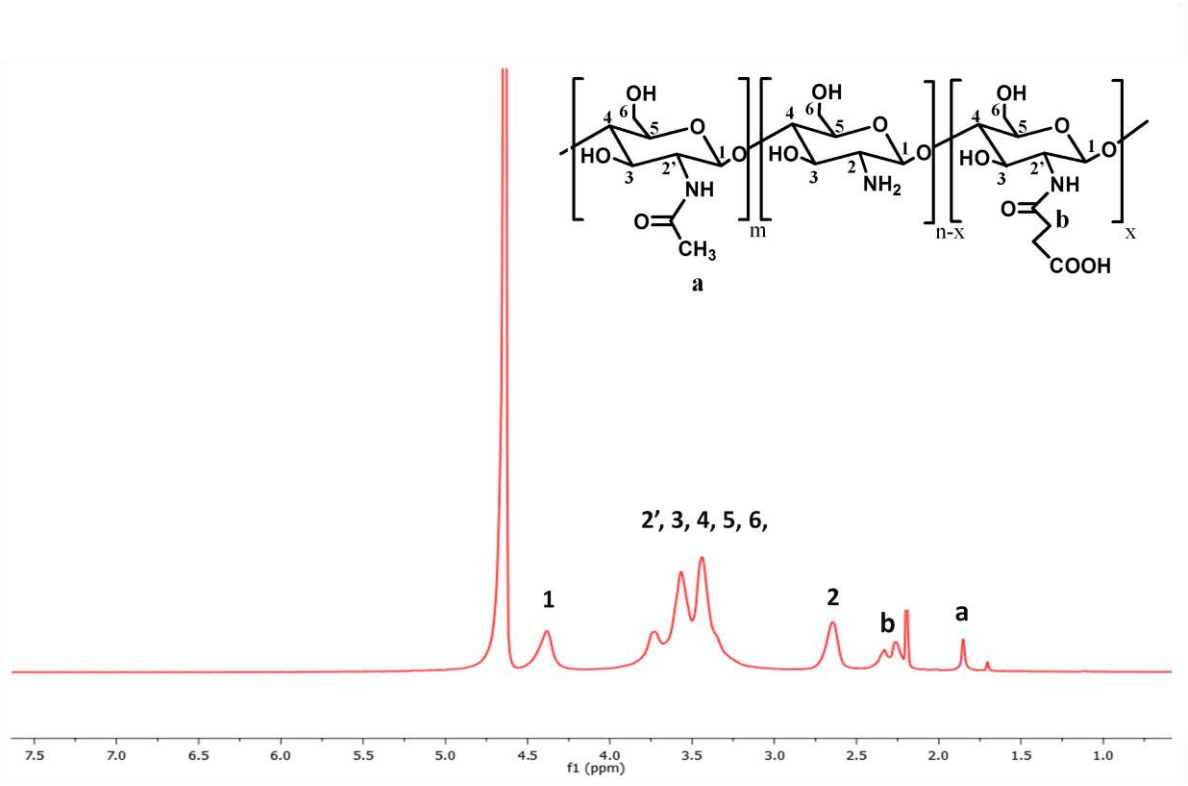


Figure 3.3 ^{1}H NMR spectrum of N-SC4

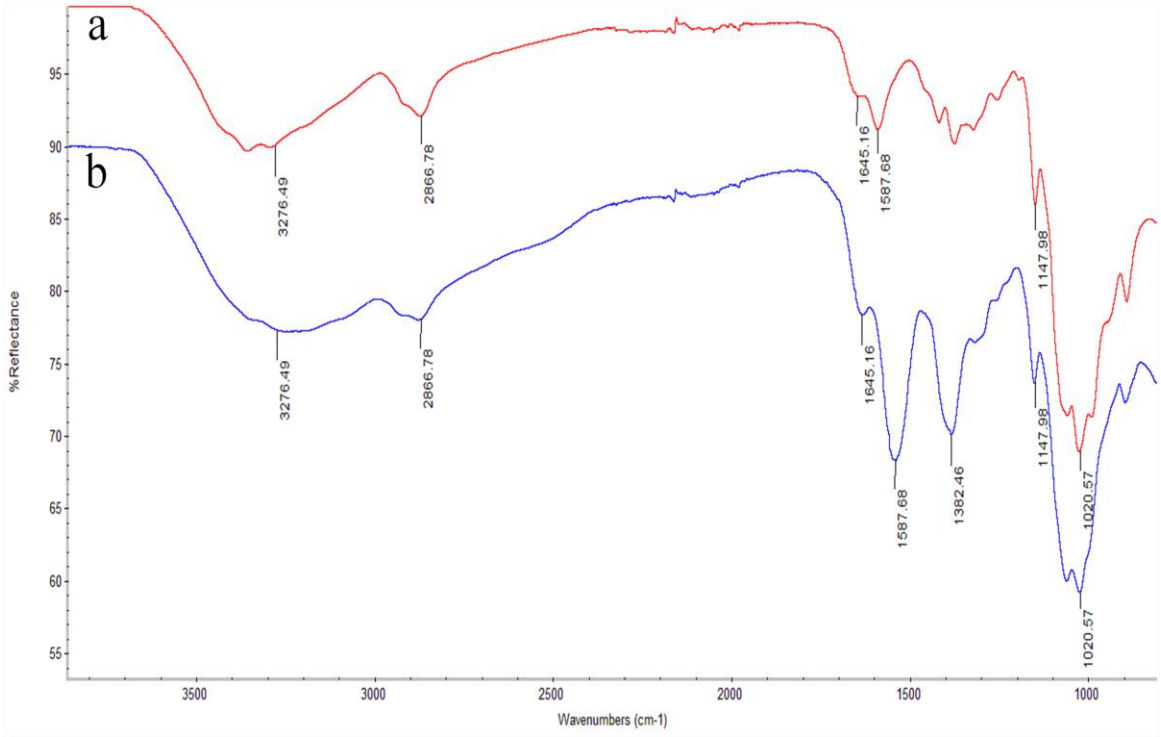
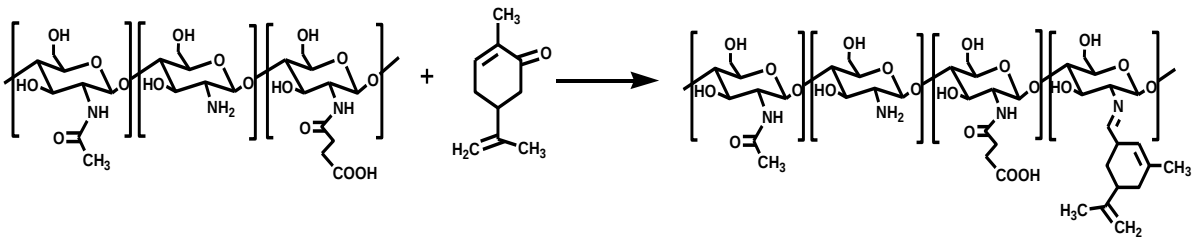


Figure 3.4 ATR-FTIR spectra of (a) chitosan and (b) N-SC4

3.2 Preparation of imine-N-SC

3.2.1 Finding the optimum ratio between N-SC derivatives and L-carvone



Scheme 3.2 Preparation of L-carvone imine-N-SC derivatives

The imine-N-SC was prepared as shown in scheme 3.2. Three weight ratios of N-SC to L-carvone (1:1, 2:1 and 3:1) were experimented. The IR spectrum of L-carvone-grafted succinylchitosan (Figure 3.5) shows an absorption peak at 1634 cm^{-1} and 1589 cm^{-1} corresponding to the C=N stretching of imine bond (Schiff base) and the C=C stretching vibration of the aromatic ring, respectively.

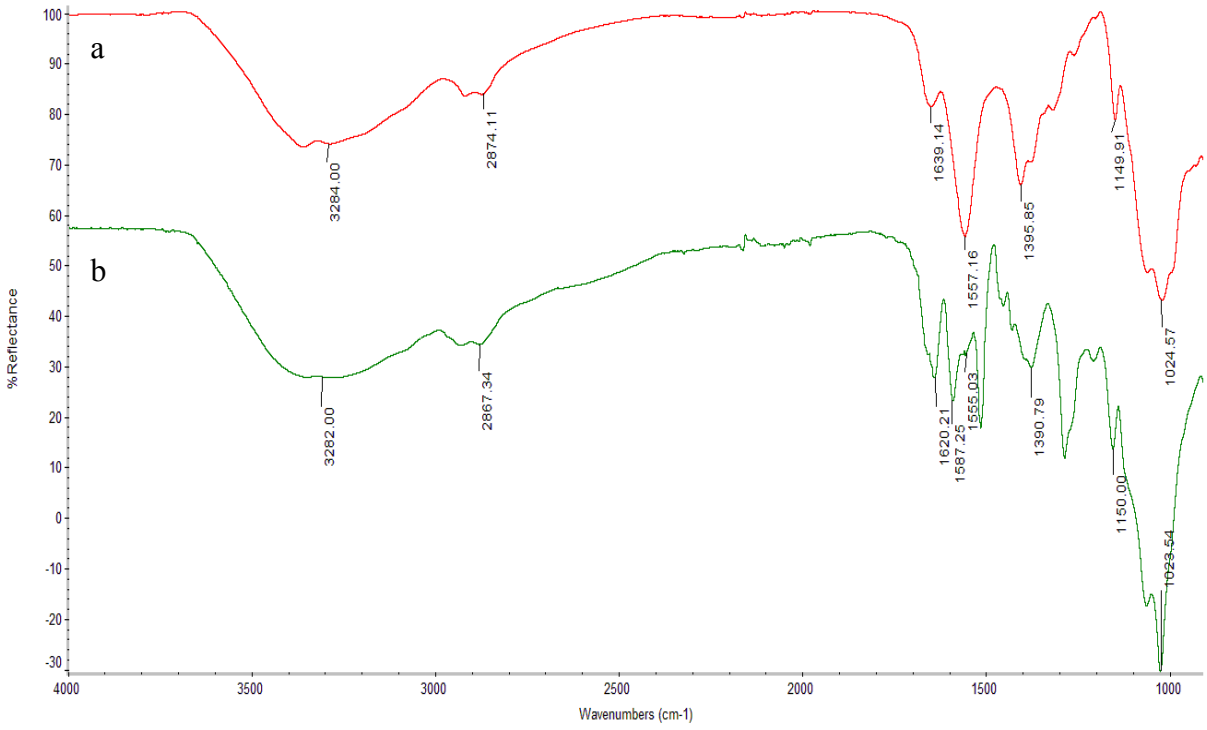
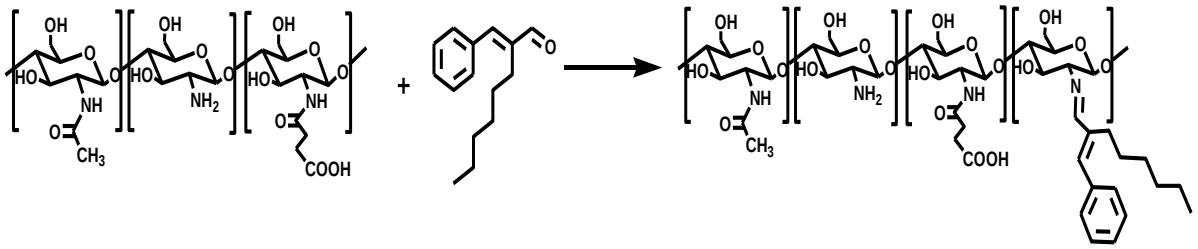


Figure 3.5 ATR-FTIR spectrum of (a) N-SC and (b) L-carvonenesuccinylchitosan prepared at N-SC: L-carvone weight ratio of 1:1.

3.2.2 Finding the optimum ratio between N-SC derivatives and hexylcinnamaldehyde



Scheme 3.3 Preparation of Hexylcinnamalidene-N-SC derivatives

The imine-N-SC was prepared as shown in scheme 3.3. Three weight ratios of N-SC to hexylcinnamalidene (1:1, 2:1 and 3:1) were experimented. The IR spectrum of hexylcinnamalidene-grafted succinylchitosan (Figure 3.6) shows an absorption peak at 1642 cm^{-1} and 1593 cm^{-1} corresponding to the C=N stretching of imine bond (Schiff base) and the C=C stretching vibration of the aromatic ring, respectively.

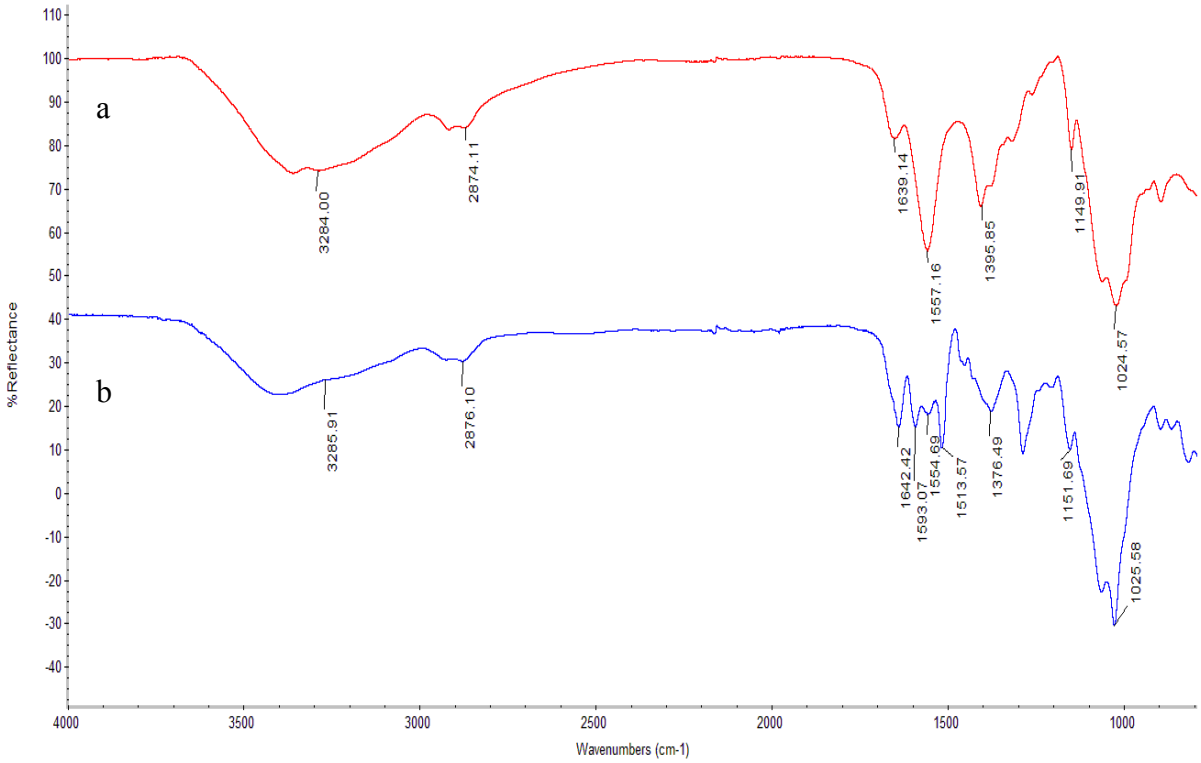


Figure 3.6 ATR-FTIR spectrum of (a) N-SC and (b) hexylcinnamalidenesuccinylchitosan prepared at N-SC: Hexylcinnamaldehyde weight ratio of 3:1

3.3 Microparticles formation

L-carvone fragrance was encapsulated into the polymer-blend of EC MC and immine derivatives of chitosan by microemulsion homogenization.

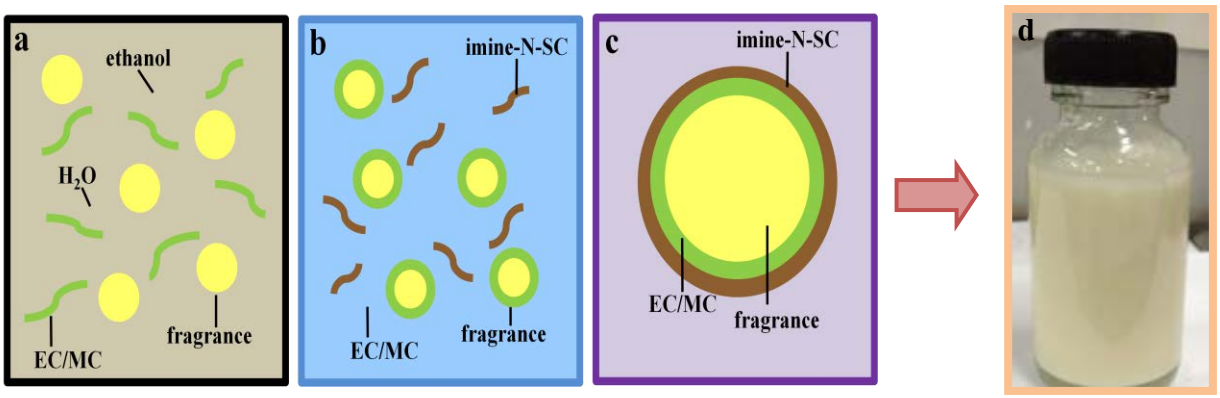
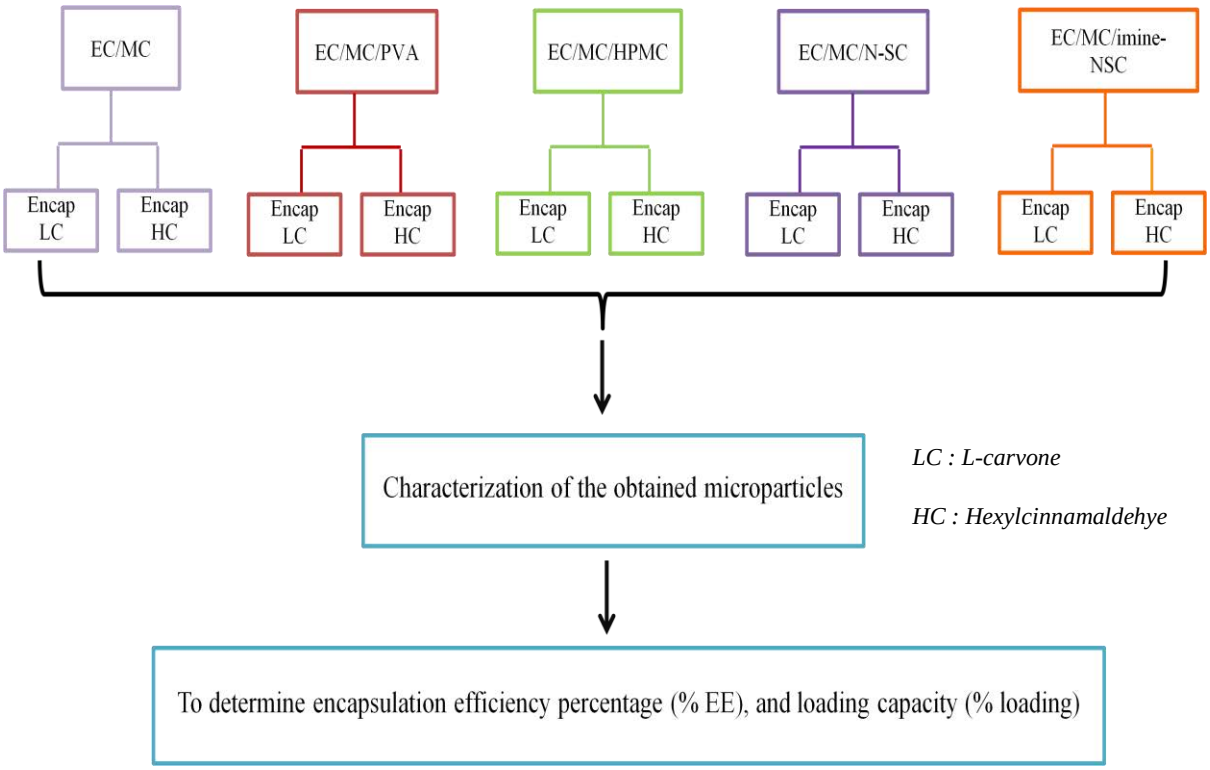


Figure 3.7 (a) Under homogenization, droplets of L-carvone were suspended in the ethanolic solution of EC and MC. (b) immine derivatives of N-SC was added slowly into polymer-blend solution. (c) microparticles were formed to encapsulate L-carvone and (d) fragrance-encapsulated microparticles



Scheme 3.4 Overview of the research

3.3.1 Encapsulation of L-carvone

3.3.1.1 Comparing the type of used polymer

Blend of EC and MC shell

L-carvone fragrance was encapsulated into the polymer-blend of EC and MC (EC: MC w/w ratio of 4:1) at weight ratio was polymer to L-carvone of 1:10 by microemulsion homogenization. The process involved slowly adding water into ethanolic solution of L-carvone and polymers under homogenizing condition. The mechanism involves the formation of L-carvone microdroplets in water with simultaneous covering of EC polymeric chains around the formed L-carvone droplets. When the polarity of the medium increases slowly during the slow addition of water into the L-carvone polymer suspension, the polymer chains precipitate on the droplets of the L-carvone. During the precipitation of EC, the MC chains were likely to be trapped along. The presence of the MC chains at the polymeric wall of the microspheres helps the spheres to be more stably dispersible in water. The encapsulation of L-carvone with EC/MC blend by microemulsion homogenization showed homogeneous colloidal suspension with no precipitation. In order to investigate the morphology of the obtained microparticles, SEM was acquired. The SEM picture indicated microspherical morphology (Figure 3.8) with the particle sizes L-carvone microparticles (mean value ± S.D.) of 5.75 ± 0.55 μm. The mean zeta potential (Table 3.1) of L-carvone microparticles was negative value (-2 ± 0.25 mV).

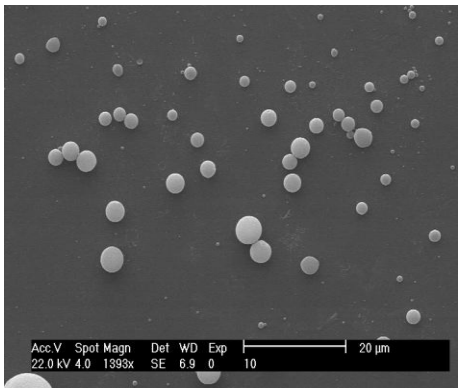


Figure 3.8 SEM photograph of L-carvone microparticles (Blend of EC and MC shell) at 22,000xmagnification

Blend of EC, MC and Polyvinyl alcohol (PVA) shell

The formation of encapsulated L-carvone into the polymer-blend of EC, MC and PVA shell was also prepared through microemulsion homogenization. Homogenization of L-carvone in the aqueous solution of PVA and EC/MC polymers was carried out. The mechanism involves the formation of L-carvone microdroplets in water with simultaneous covering of EC polymeric chains around the formed L-carvone droplets. During the deposited of EC around the L-carvone droplets, the MC chains are likely to be trapped and entangled into the polymeric shell of the microspheres. The added PVA was also expected to be part of the polymeric shell likely at the outer surface. The SEM picture indicated microspherical morphology (Figure 3.9) with the particle sizes of 8.24 ± 2.49 µm. The mean zeta potential (Table 3.1) of L-carvone microparticles was negative value (-9 ± 1.40 mV).

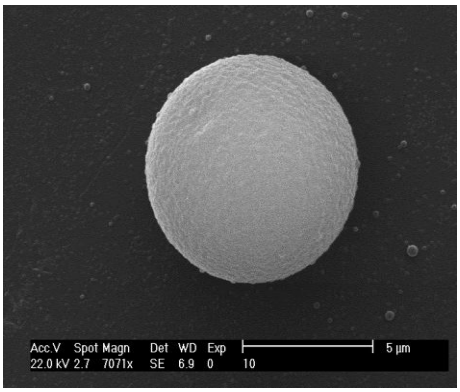


Figure 3.9 SEM photograph of L-carvone microparticles (Blend of EC MC and PVA shell) at 22,000xmagnification

Blend of EC, MC and Hydroxy Propyl Methyl Cellulose (HPMC) shell

The formation of L-carvone into the polymer-blend of EC, MC and HPMC shell was also prepared through microemulsion homogenization. Homogenization of L-carvone in the aqueous solution of HPMC and EC/MC polymers was carried out. The mechanism is similar to the encapsulation of L-carvone with EC, MC and PVA shell, except that HPMC was used in place of PVA. Also, we expected that the HPMC would be deposited at the very outer surface of the particles. The SEM picture indicated microparticles morphology (Figure 3.10) with the particle sizes of 4.40 ± 3.432 µm. The mean zeta potential (Table 3.1) of L-carvone microparticles was negative value (-1 ± 0.09 mV).

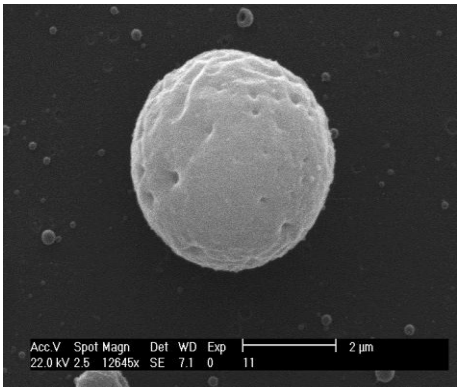


Figure 3.10 SEM photograph of L-carvone microparticles (Blend of EC MC and HPMC shell) at 22,000xmagnification

Blend of EC, MC and N-SC shell

The formation of L-carvone into the polymer-blend of EC, MC and N-SC shell was also prepared through microemulsion homogenization. Homogenization of L-carvone in the aqueous solution of N-SC and EC/MC polymers was carried out. The mechanism is similar to the encapsulation of L-carvone with EC, MC and PVA shell. However, the PVA was changed to N-SC. The SEM picture indicated microspherical morphology (Figure 3.11) with the particle sizes L-carvone microparticles of 5.75 ± 0.547 µm. The mean zeta potential (Table 3.1) of L-carvone microparticles was positive value (+49 ± 0.45 mV), confirming our hypothesis that the N-SC were deposited at the very outer surface of the particles.

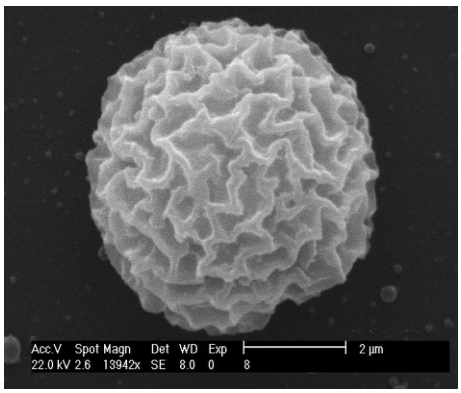


Figure 3.11 SEM photograph of L-carvone microparticles (Blend of EC MC and N-SC shell) at 22,000xmagnification

Blend of EC, MC and imine derivatives of N-SC shell

The formation of L-carvone into the polymer-blend of EC, MC and imine derivatives of N-SC shell was also prepared through microemulsion homogenization. Homogenization of L-carvone in the aqueous solution of imine derivatives of N-SC and EC/MC polymers was carried out. The mechanism is similar to the encapsulation of L-carvone with EC, MC and PVA shell. However, the final polymer was changed to imine derivative of N-SC (Scheme 3.2). The SEM picture indicated microspherical morphology (Figure 3.12) with the particle sizes of 11.4 ± 0.031 μm. The mean zeta potential (Table 3.1) of L-carvone microparticles was positive value (+40 ± 1.80). The outer surface of the particles must be covered were imine-N-SC because their surface charge were positive (when PVA and HPMC were used, the values were negative).

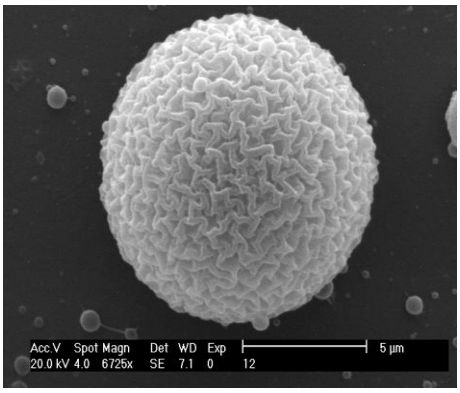


Figure 3.12 SEM photograph of L-carvone microparticles (Blend of EC MC and imine-N-SC shell) at 20,000xmagnification

The SEM photographs of each microparticles (Figure 3.8-3.12) showed similar perfect microparticles. However, using EC/MC, EC/MC/PVA and EC/MC/HPMC system as fragrance carrier presented smooth surface while using EC/MC/N-SC and EC/MC/imine-N-SC system as fragrance carrier showed rough surface. The results confirmed that the type of the dropping polymer affected morphology the particle surface.

Zeta potential technique has been used for measuring a net charge density at the particles surface. This surface charge directly affects the distribution of particle in the surrounding interfacial region and influences their aggregation behaviors. Acceptable zeta potentials of stable particles are usually more positive than +30 mV or more negative than -30 mV. In this work, the obtained zeta potentials of obtained suspensions are shown in

Table 3.1. The zeta potential values indicated that the encapsulated L-carvone with EC/MC, EC/MC/PVA and EC/MC/HPMC suspension might aggregate easily. The particles of encapsulated L-carvone with EC/MC/N-SC and EC/MC/imine-N-SC showed high zeta potential values (+49 - +40 mV). This means that encapsulated L-carvone with EC/MC/N-SC and EC/MC/Imine-N-SC colloidal were more stable than encapsulated L-carvone with EC/MC, EC/MC/PVA and EC/MC/HPMC particles (Table 3.1).

Table 3.1 The particle sizes and Zeta potential of L-carvone microparticles with various polymer.

Type of polymer blend	**Particle sizes of L-carvone microparticles (µm)**	**Zeta potential of L-carvone microparticles (mV)**
EC/MC	5.75±0.547	-2±0.25
EC/MC/PVA	8.24±2.491	-9±1.40
EC/MC/HPMC	4.40±3.432	-1±0.09
EC/MC/N-SC	5.75±0.547	+49±0.45
EC/MC/imine-N-SC	11.4±0.031	+40±1.80

As demonstrated earlier that all 5 blend polymer systems could be encapsulated into microparticles. The encapsulation efficiency of each polymer with various polymer blend was investigated. Each system was subjected to fragrance extraction and determination of recovered fragrance content by UV-Vis spectrophotometer with the aid of calibration curve. The encapsulated L-carvone with EC/MC, EC/MC/PVA, EC/MC/HPMC, EC/MC/N-SC and EC/MC/imine-N-SC gave 46.89, 35.56, 32.24, 49.80 and 96.57 % encapsulation efficiency at the loading of 39.93, 30.26, 21.65, 42.38 and 82.17 %, respectively (Table 3.2). This data indicated EC/MC/imine-N-SC system is excellent carrier for encapsulating L-carvone.

Table 3.2 %EE and %LC of L-carvone-encapsulated microparticles with various polymer.

Type of polymer blend	%EE	%Loading
EC/MC	46.89± 3.011	39.93± 0.111
EC/MC/PVA	35.56± 1.212	30.26± 0.721
EC/MC/HPMC	32.24± 0.341	21.65± 0.432
EC/MC/N-SC	49.80± 0.214	42.38± 0.171
EC/MC/imine-N-SC	96.57± 0.879	82.17± 0.431

In summary, the fabrication process of fragrance-encapsulated system could give high fragrance concentration product with no need of preconcentration step. The process involves the fabrication of micron-sized fragrance spheres that combine two release mechanisms, the barrier of Schiff base chemical linkage between fragrant ketone and succinylchitosan and the physical polymer blend barrier. That all 5 blend polymer systems could encapsulate L-carvone and form into microparticles. However, each polymer system has effects on encapsulation efficiency, loading capacity and also zeta potential. For instance, L-carvone microparticles using a blend of EC MC and imine-N-SC shell showed the highest %EE and %LC while L-carvone microparticles using only a blend of EC MC showed the lowest %EE and %LC. In addition, according to the obtained different zeta potential values from each polymer system, we can modify a net charge density at the particles surface for each application. Thus, L-carvone-loaded microparticles might be employed as a model of new fragrance-encapsulated methods for industries to inherit slow release and maintain fragrance chemical stability. The L-carvone microparticles using a blend of EC MC and imine-N-SC shell was applied in all further studies.

3.3.1.2 Optimization of polymer: L-carvone

The optimal ratio between polymer and fragrance was investigated for the microparticles proven. Five ratios 1:1, 1:3, 1:5, 1:10 and 1:20 were compared by evaluating the precipitation of EC/MC blend in the suspension. The obtained to L-carvone-loaded micro-particles at 13.56, 25.11, 51.91, 82.17 and 88.36% (w/w) loading. The process gave

38.01, 40.40, 76.08, 96.57 and 96.21% (w/w) encapsulation efficiency respectively. The best ratio between polymer and L-carvone was 1:10, since the obtained suspension showed no precipitates and gave high fragrance loading product with no need of preconcentration step. Therefore the L-carvone microcapsules prepared at the weight ratios of polymer (EC/MC/imine-N-SC) to L-carvone of 1:10 was further investigated in this research.

Table 3.3 %EE and %LC of L-carvone-encapsulated microparticles at various polymer and fragrance.

Ratio Polymer : L-carvone	**%EE**	**%Loading**
1:1	38.01± 0.430	13.56± 0.954
1:3	40.60± 2.221	25.11± 1.320
1:5	76.08± 0.154	51.91± 0.221
1:10	96.57± 0.879	82.17± 0.431
1:20	96.21± 0.133	88.36± 0.456

We can conclude that polymer: L-carvone has an effect on encapsulation efficiency and loading. The highest encapsulation efficiency (≥96%) could be obtained at 1:10 polymer to fragrance ratio (Table 3.3). However, the 1:20 polymer to fragrance ratio resulted in observable non-dispersible floating fragranced L-carvone which complicated the determination of loading capacity and encapsulation efficiency. Thus, the maximum fragrance to polymer ratio was limited to 1:10 in all further studies. Satisfyingly, fragrances could be loaded into the microparticles at a fragrance: polymer ratio up to 1:10 and the obtained microparticles possessed fragrance loading of ≥82%.

3.3.1.3 Speed of homogenization

L-carvone fragrance was encapsulated into the polymer-blend of EC, MC and imine derivatives of N-SC (EC: MC w/w ratio of 4:1) at polymer to L-carvone weight ratios of 1:10 by microemulsion homogenization**.** The homogenization speed plays an important role during

formation of encapsulated L-carvone microparticles. It is reasonable to increase homogenization speed in order to reduce particle size of the formed L-carvone microparticles. The L-carvone encapsulated microparticles obtained from the high speed homogenization gave smaller particles size than that obtained at the low speed. As seen in figures 3.13, it was found that the increase in homogenization speed from 6,000 rpm to 12,000 rpm had an effect on size of the L-carvone microparticles. However, too high speed rate of homogenization, 12,000 rpm, produced flawed morphology as seen in the figure 3.13 (g). The optimize speed rate used in microparticles preparation was 10,000 rpm. The SEM picture indicated microspherical morphology (Figure 3.13). The particle sizes of L-carvone microparticles are shown in Table 3.4.

As expected, the L-carvone encapsulated microparticles obtained from the high speed homogenization gave smaller particles size than that obtained at the low speed. Thus, the particles size of the L-carvone microparticles was dependent on speed rate used in microparticles preparation and the 10,000 rpm as homogenization speed rate was applied in all further studies.

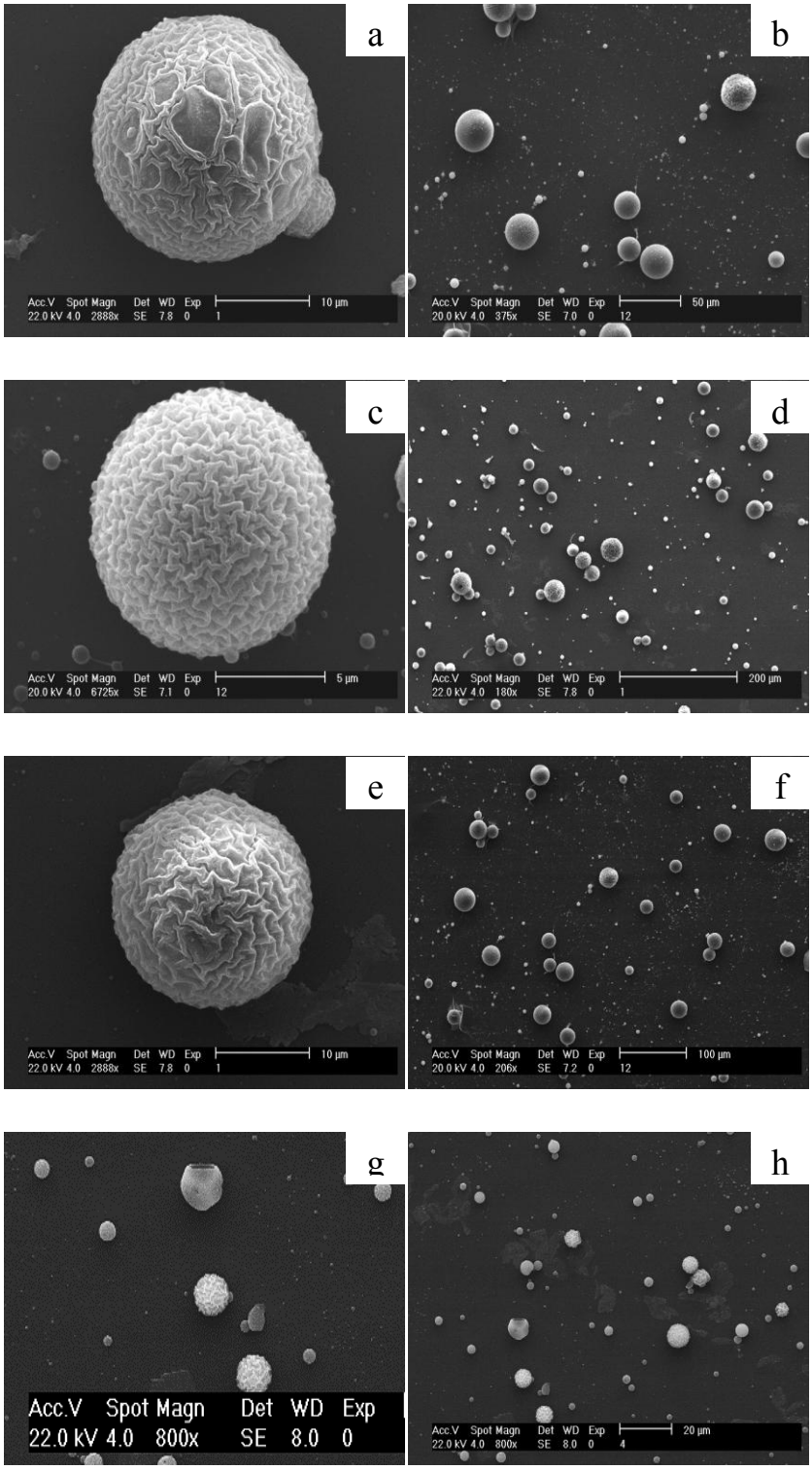


Figure 3.13 SEM photograph of L-carvone microparticles by homogenizer at various speed rate (a, b) 6000, (c, d) 8000, (e, f) 10000, (g, h) 12000 rpm respectively at 22,000xmagnification

Table 3.4 The particle sizes of L-carvone microparticles at various speed rate.

Speed rate (rpm)	Particle sizes of L-carvone microparticles (μm)
6000	22.1±0.247
8000	11.4±4.316
10000	9.82±0.409
12000	3.97±6.952

3.3.2 Encapsulation of Hexylcinnamaldehyde

3.3.2.1 Comparing the type of used polymer

Blend of EC and MC shell

Hexylcinnamaldehyde fragrance was encapsulated into the polymer-blend of EC and MC (EC: MC w/w ratio of 4:1) at weight ratio was polymer to hexylcinnamaldehyde of 10:1 by microemulsion homogenization. The process involved slowly adding water into ethanolic solution of hexylcinnamaldehyde and polymers under homogenizing condition. The mechanism involves the formation of hexylcinnamaldehyde microdroplets in water with simultaneous covering of EC polymeric chains around the formed hexylcinnamaldehyde droplets. When the polarity of the medium increases slowly during the slow addition of water into the hexylcinnamaldehyde polymer suspension, the polymer chains precipitate on the droplets of the hexylcinnamaldehyde. During the precipitation of EC, the MC chains were likely to be trapped along. The presence of the MC chains at the polymeric wall of the microspheres helps the spheres to be more stably dispersible in water. The encapsulation of hexylcinnamaldehyde with EC/MC blend by microemulsion homogenization showed homogeneous colloidal suspension with no precipitation. In order to investigate the morphology of the obtained microparticles, SEM was acquired. The SEM picture indicated microparticles morphology (Figure 3.14) with the particle sizes hexylcinnamaldehyde microparticles (mean value ± S.D.) of 14.9 ± 0.228 μm. The mean zeta potential (Table 3.5) of hexylcinnamaldehyde microparticles was negative value (-1 ± 0.43 mV).

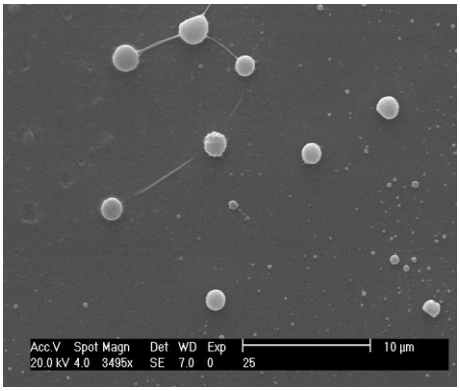


Figure 3.14 SEM photograph of hexylcinnamaldehyde microparticles (Blend of EC and MC shell) at 22,000xmagnification

Blend of EC, MC and Polyvinyl alcohol (PVA) shell

The formation of encapsulated hexylcinnamaldehyde into the polymer-blend of EC, MC and PVA shell was also prepared through microemulsion homogenization. Homogenization of hexylcinnamaldehyde in the aqueous solution of PVA and EC/MC polymers was carried out. The mechanism involves the formation of hexylcinnamaldehyde microdroplets in water with simultaneous covering of EC polymeric chains around the formed hexylcinnamaldehyde droplets. During the deposited at EC around the hexylcinnamaldehyde droplets, the MC chains are likely to be trapped and entangled into the polymeric shell of the microspheres. The added PVA was also expected to be part of the polymeric shell likely at the outer surface. The SEM picture indicated microparticles morphology (Figure 3.15) with the particle sizes of 11.5 ± 1.098 µm. The mean zeta potential (Table 3.5) of hexylcinnamaldehyde microparticles was negative value (-6 ± 1.35 mV).

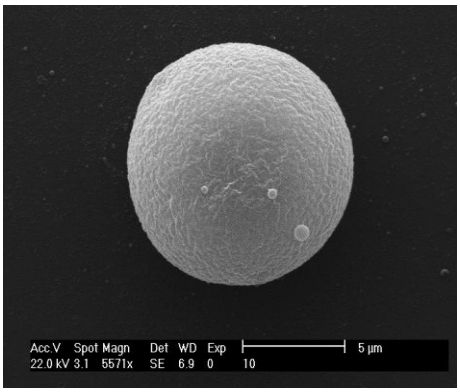


Figure 3.15 SEM photograph of hexylcinnamaldehyde microparticles (Blend of EC MC and PVA shell) at 22,000xmagnification

Blend of EC, MC and Hydroxy Propyl Methyl Cellulose (HPMC) shell

The formation of hexylcinnamaldehyde into the polymer-blend of EC, MC and HPMC shell was also prepared through microemulsion homogenization. Homogenization of hexylcinnamaldehyde in the aqueous solution of HPMC and EC/MC polymers was carried out. The mechanism is similar to the encapsulation of hexylcinnamaldehyde with EC, MC and PVA shell, except that HPMC was used in place of PVA. Also, we expected that the HPMC would be deposited at the very outer surface of the particles. The SEM picture indicated microparticles morphology (Figure 3.16) with the particle sizes of 4.3 ± 0.088 µm. The mean zeta potential (Table 3.5) of hexylcinnamaldehyde microparticles was negative value (-1 ± 0.68 mV).

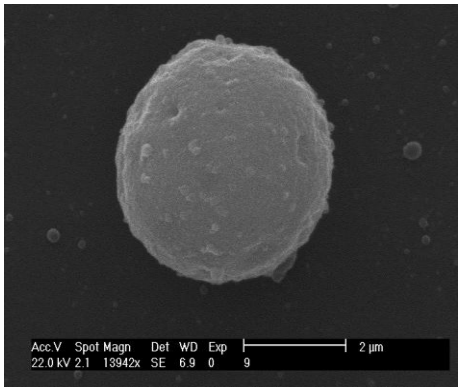


Figure 3.16 SEM photograph of hexylcinnamaldehyde microparticles (Blend of EC MC and HPMC shell) at 22,000xmagnification

Blend of EC, MC and N-SC shell

The formation of hexylcinnamaldehyde into the polymer-blend of EC, MC and N-SC shell was also prepared through microemulsion homogenization. Homogenization of hexylcinnamaldehyde in the aqueous solution of N-SC and EC/MC polymers was carried out. The mechanism is similar to the encapsulation of hexylcinnamaldehyde with EC, MC and PVA shell. However, the PVA was changed to N-SC. The SEM picture indicated microparticles morphology (Figure 3.17) with the particle sizes hexylcinnamaldehyde microparticles of 14.9 ± 0.196 µm. The mean zeta potential (Table 3.5) of hexylcinnamaldehyde microparticles was positive value (+46 ± 1.22 mV), confirming our hypothesis that the N-SC were deposited at the very outer surface of the particles.

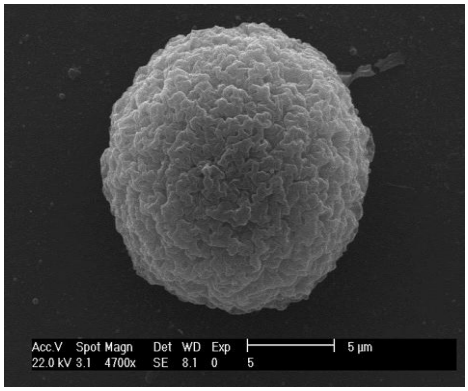


Figure 3.17 SEM photograph of hexylcinnamaldehyde microparticles (Blend of EC MC and N-SC shell) at 22,000xmagnification

Blend of EC, MC and immine derivatives of N-SC shell

The formation of hexylcinnamaldehyde into the polymer-blend of EC, MC and imine derivatives of N-SC shell was also prepared through microemulsion homogenization. Homogenization of hexylcinnamaldehyde in the aqueous solution of imine derivatives of N-SC and EC/MC polymers was carried out. The mechanism is similar to the encapsulation of hexylcinnamaldehyde with EC, MC and PVA shell. However, the final polymer was changed to imine derivative of N-SC (Scheme 3.3). The SEM picture indicated microparticles morphology (Figure 3.17) with the particle sizes of 17.6 ± 0.388 µm. The mean zeta potential (Table 3.5) of hexylcinnamaldehyde microparticles was positive value (+23 ± 0.55). The outer surface of the particles must be covered will imine-N-SC because their surface charge were positive (when PVA and HPMC were used, the values were negative).

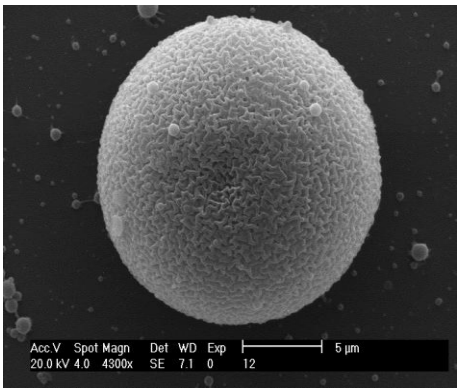


Figure 3.18 SEM photograph of hexylcinnamaldehyde microparticles (Blend of EC MC and imine N-SC shell) at 22,000xmagnification

The SEM photographs of each microparticles (Figure3.14-3.17) showed similar perfect microparticles. However using EC/MC, EC/MC/PVA and EC/MC/HPMC system as fragrance carrier presented smooth surface while using EC/MC/N-SC and EC/MC/imine-N-SC system as fragrance carrier showed rough surface. The result confirmed that the type of the dropping polymer affected morphology the particle surface.

Zeta potential technique has been used for measuring a net charge density at the particles surface. This surface charge directly affects the distribution of particle in the surrounding interfacial region and influences their aggregation behaviors. Acceptable zeta potentials of stable particles are usually more positive than +30 mV or more negative than -30 mV. In this work, the obtained zeta potentials of obtained suspensions are shown in Table 3.5. The zeta potential values indicated that the encapsulated hexylcinnamaldehye with EC/MC, EC/MC/PVA and EC/MC/HPMC suspension might aggregate easily. The particles of encapsulated hexylcinnamaldehyde with EC/MC/N-SC and EC/MC/imine-N-SC showed high zeta potential values (+49 - +23 mV). This means that encapsulated hexylcinnamaldehyde with EC/MC/N-SC and EC/MC/imine-N-SC colloidal were more stable than encapsulated hexylcinnamaldehyde with EC/MC, EC/MC/PVA and EC/MC/HPMC particles (Table 3.5).

Table 3.5 The particle sizes and Zeta potential of hexylcinnamaldehyde microparticles with various polymer

Type of polymer blend	**Particle sizes distribution of hexylcinnamaldehye microparticles (µm)**	**Zeta potential of hexyl cinnamaldehye microparticle (mV)**
EC/MC	14.9±0.228	-1±0.43
EC/MC/PVA	11.5±1.098	-6±1.35
EC/MC/HPMC	4.3±0.088	-1±0.68
EC/MC/N-SC	14.9±0.196	+46±1.22
EC/MC/imine-N-SC	17.6±0.388	+23±0.556

As demonstrated earlier that all 5 blend polymer systems could be encapsulated into microparticles. Here encapsulation efficiency of each polymer was investigated. Each system was subjected to fragrance extraction and determination of recovered fragrance content by UV-Vis spectrophotometer with the aid of calibration curve. The encapsulated hexylcinnamaldehyde with EC/MC, EC/MC/PVA, EC/MC/HPMC, EC/MC/N-SC and EC/MC/imine-N-SC gave 48.01, 21.81, 16.54, 52.11 and 81.43% encapsulation efficiency at the loading of 40.85, 18.55, 14.08, 69.30 and 56.92%, respectively (Table 3.6). This data indicated EC/MC/imine-N-SC system is excellent carrier for encapsulating hexylcinnamaldehyde.

Table 3.6 %EE and %LC of hexylcinnamaldehyde-encapsulated microparticles with various polymer.

Type of polymer blend	**%EE**	**%Loading**
EC/MC	48.01± 0.231	40.85± 0.674
EC/MC/PVA	21.81± 0.613	18.55± 1.012
EC/MC/HPMC	16.54± 1.027	14.08± 0.567
EC/MC/N-SC	52.11± 0.987	69.30± 0.653
EC/MC/Imine-N-SC	81.43± 0.677	56.92± 0.932

In summary, the fabrication process of fragrance-encapsulated system could give high fragrance concentration product with no need of preconcentration step. The process involves the fabrication of micron-sized fragrance spheres that combine two release mechanisms, the barrier of Schiff base chemical linkage between fragrant ketone and succinylchitosan and the physical polymer blend barrier. That all 5 blend polymer systems could encapsulate hexylcinnamaldehyde and form into microparticles. However, each polymer system has effects on encapsulation efficiency, loading capacity and also zeta potential. For instance, hexylcinnamaldehyde microparticles using a blend of EC MC and imine-N-SC shell showed the highest %EE and %LC while hexylcinnamaldehyde microparticles using only a blend of EC MC showed the lowest %EE and %LC. In addition, according to the obtained different

zeta potential values from each polymer system, we can modify a net charge density at the particles surface for each application. Thus, hexylcinnamaldehyde-loaded microparticles might be employed as a model of new fragrance-encapsulated methods for industries to inherit slow release and maintain fragrance chemical stability. The hexylcinnamaldehyde microparticles using a blend of EC MC and imine-N-SC shell was applied in all further studies.

3.3.2.2 Optimization of polymer: Hexylcinnamaldehyde

The optimal ratio between polymer and fragrance was investigated for the microparticles proven. Five ratios 1:1, 1:3, 1:5, 1:10 and 1:20 were compared by evaluating the precipitation of EC/MC blend in the suspension. The obtained to hexylcinnamaldehyde -loaded microparticles at 9.21, 19.68, 51.14, 69.30 and 56.92% (w/w) loading. The process gave 25.32, 31.17, 69.04, 81.43 and 61.18% (w/w) encapsulation efficiency respectively. The best ratio between polymer and hexylcinnamaldehyde was 1:10, since the obtained suspension showed no precipitates and gave high fragrance loading product with no need of preconcentration step. Therefore the hexylcinnamaldehyde microcapsules prepared at the weight ratios of polymer (EC/MC/imine-N-SC) to hexylcinnamaldehyde of 1:10 was further investigated in this research.

Table 3.7 Amounts of materials used during the encapsulation of hexylcinnamaldehye

Ratio Polymer : Fragrance	**%EE**	**%Loading**
1:1	25.32± 0.141	9.21± 0.122
1:3	31.17± 0.321	19.68± 0.145
1:5	69.04± 0.256	51.14± 2.011
1:10	81.43± 0.677	69.30± 0.653
1:20	61.18± 1.100	56.92± 0.896

We can conclude that polymer: hexylcinnamaldehyde has an effect on encapsulation efficiency and loading. The highest encapsulation efficiency (≥81%) could be obtained at 1:10 polymer to fragrance ratio (Table 3.7). However, the 1:20 polymer to fragrance ratio resulted in observable non-dispersible floating fragranced hexylcinnamaldehyde which complicated the determination of loading capacity and encapsulation efficiency. Thus, the maximum fragrance to polymer ratio was limited to 1:10 in all further studies. Satisfyingly, fragrances could be loaded into the microparticles at a fragrance: polymer ratio up to 1:10 and the obtained microparticles possessed fragrance loading of ≥69%.

3.3.2.3 Speed of homogenization

Hexylcinnamaldehyde fragrance was encapsulated into the polymer-blend of EC, MC and imine derivatives of N-SC (EC: MC w/w ratio of 4:1) at polymer to hexylcinnamaldehyde weight ratios of 1:10 by microemulsion homogenization**.** The homogenization speed plays an important role during formation of encapsulated hexylcinnamaldehyde microparticles. It is reasonable to increase homogenization speed in order to reduce particle size of the formed hexylcinnamaldehyde microparticles. The hexylcinnamaldehyde encapsulated microparticles obtained from the high speed homogenization gave smaller particles size than the suspension obtained with the low speed homogenization. As seen in figures 3.19, it was found that the increase in homogenization speed from 6,000 rpm to 12,000 rpm had an effect on size of formed hexylcinnamaldehyde microparticles. The particle size was reduced for all formulas. In contrast, too high speed rate of homogenization, 12000 rpm, produced flawed morphology as seen in the figure 3.13 (g). The optimize speed rate used in microparticles preparation was 10,000 rpm. The SEM picture indicated microparticles morphology (Figure 3.19). The particle sizes of hexylcinnamaldehyde microparticles are shown in Table 3.8.

As expected, the hexylcinnamaldehyde encapsulated microparticles obtained from the high speed homogenization gave smaller particles size than that obtained at the low speed. Thus, the particles size of the hexylcinnamaldehyde microparticles was dependent on speed rate used in microparticles preparation and the 10,000 rpm as homogenization speed rate was applied in all further studies.

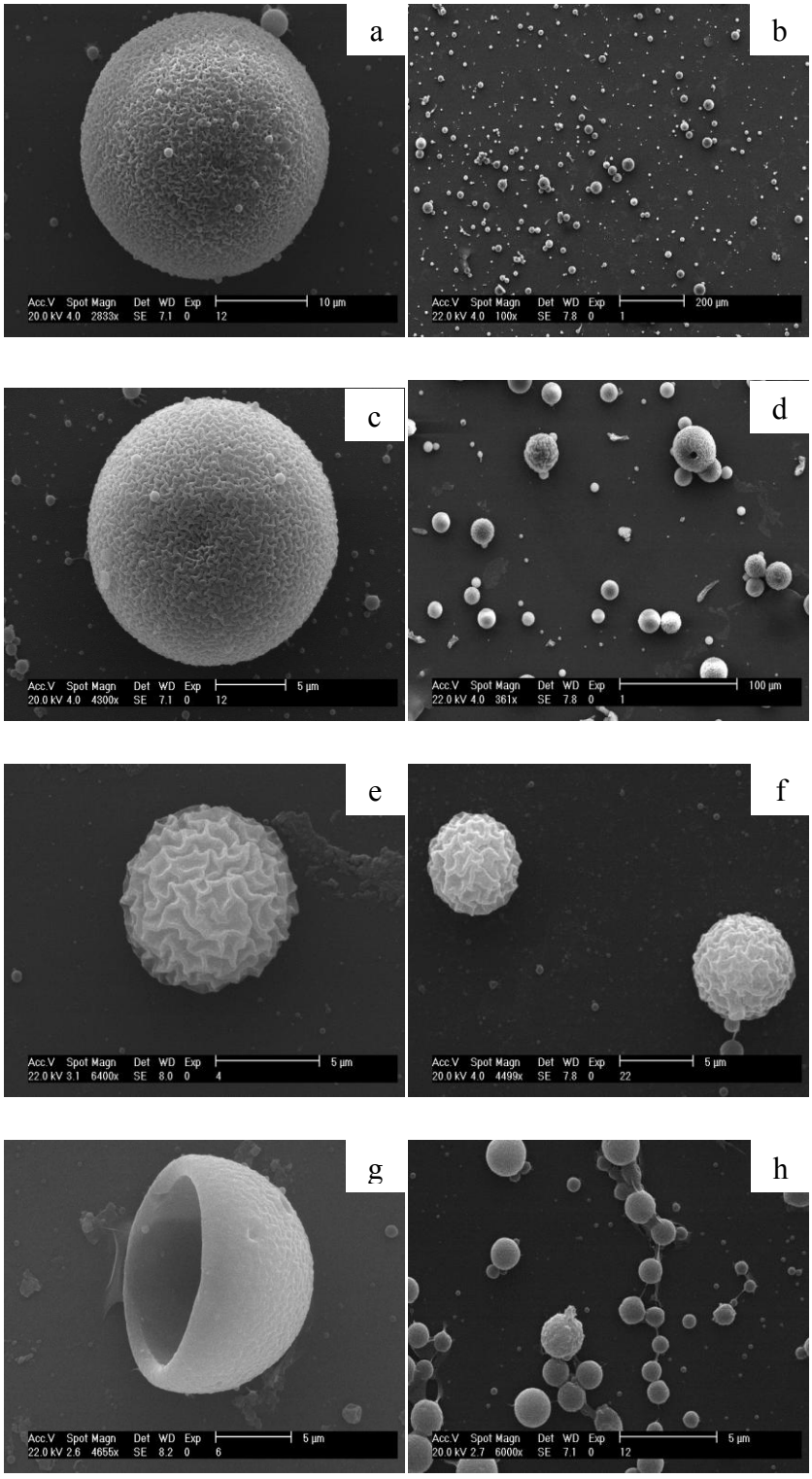


Figure 3.19 SEM photograph of hexylcinnamaldehyde loaded microparticles by homogenizer at various speed rate (a, b) 6000, (c, d) 8000, (e, f) 10000, (g, h) 12000 rpm respectively at 22,000xmagnification

Table 3.8 The particle sizes of hexylcinnamaldehyde-encapsulated microparticles at various speed rate

Speed rate (rpm)	Particle sizes distribution of hexyl cinnamaldehye microparticles (µm)
6000	24.7±7.338
8000	17.6±0.388
10000	8.31±0.128
12000	2.27±0.389

2.6 Determination of release profiles

2.6.1 The release of L-carvone from microparticles

An aqueous suspension of the L-carvone- loaded microparticles was freshly prepared using EC/MC/imine-N-SC and its L-carvone-release behavior was monitored by UV-Vis spectrophotometer. A controlled release system in this work should be able to retard the L-carvone volatility and should therefore show slow rate of L-carvone release. We examined the release of L-carvone by quantitating the remaining L-carvone molecules in the system by subjective the septum to hexane extraction under acidic condition. In other words, hexane extraction coupled with acid hydrolysis was used to recover the remaining L-carvone. The extract was subjected to UV-Vis analysis with the aid of calibration curve. As shown in Figures 3.20, the remained amount of L-carvone decreased with passing times. All release profiles indicated sustained L-carvone release for the L-carvone encapsulation microparticles comparing to free L-carvone.

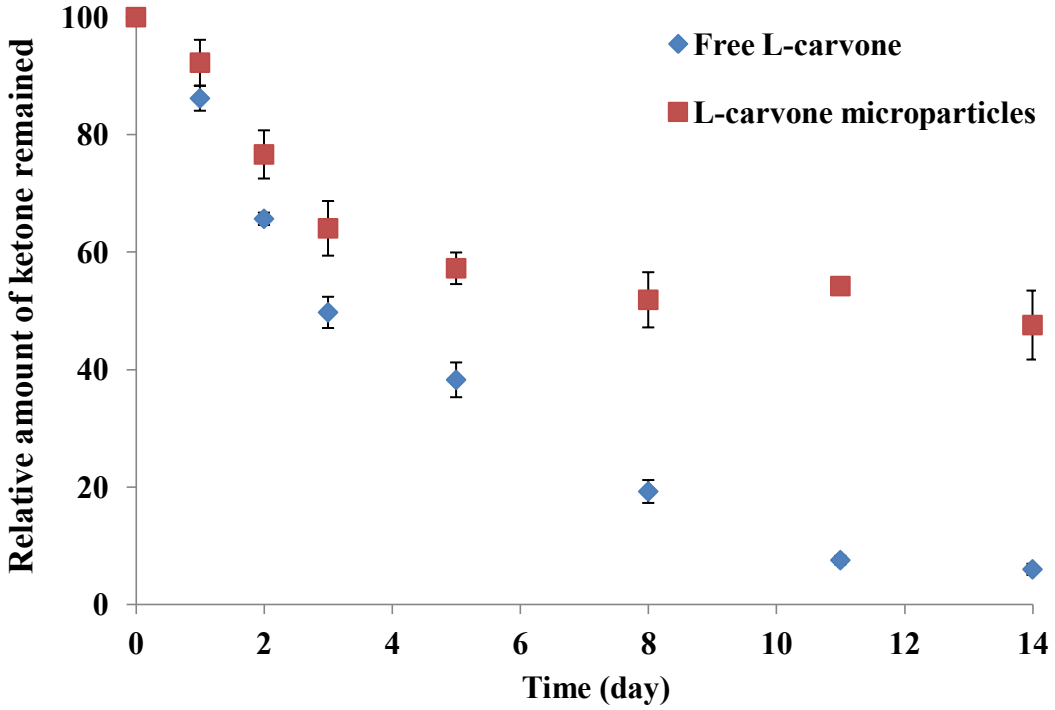


Figure 3.20 Release profile of L-carvone remained in encapsulation microspheres compared with free L-carvone.

2.6.2 The release of hexylcinnamaldehye from microparticles

An aqueous suspension of the hexylcinnamaldehyde-loaded microparticles was freshly prepared using EC/MC/imine-N-SC and its hexylcinnamaldehyde-release behavior was monitored by UV-Vis spectrophotometer. A controlled release system in this work should be able to retard the hexylcinnamaldehyde volatility and should therefore show slow rate of hexylcinnamaldehyde release. We examined the release of hexylcinnamaldehyde by quantitating the remaining hexylcinnamaldehyde molecules in the system by subjective the septum to hexane extraction under acidic condition. In other words, hexane extraction coupled with acid hydrolysis was used to recover the remaining hexylcinnamaldehyde. The extract was subjected to UV-Vis analysis with the aid of calibration curve. As shown in Figures 3.20, the remained amount of hexylcinnamaldehyde decreased with passing times. All release profiles indicated sustained hexylcinnamaldehyde release for the hexylcinnamaldehye encapsulation microparticles comparing to free hexylcinnamaldehyde.

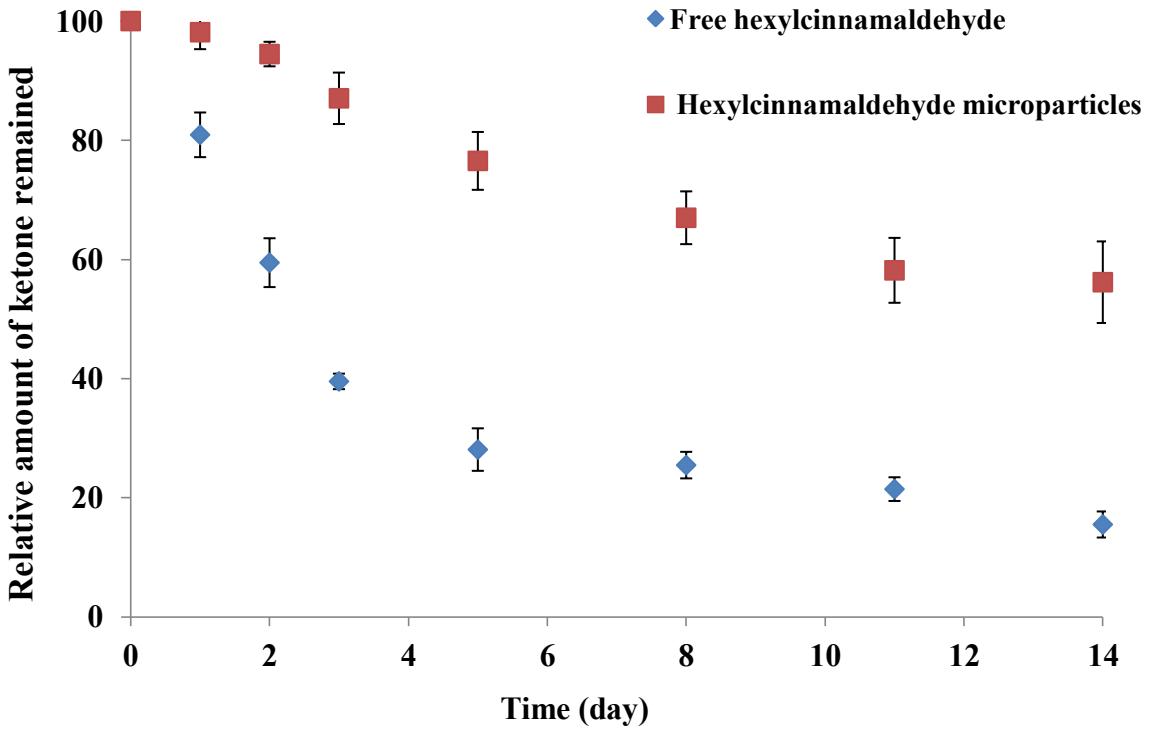


Figure 3.21 Release profile of hexylcinnamaldehye remained in encapsulation microspheres compared with free hexylcinnamaldehye.

CHAPTER IV

CONCLUSION

Here a novel high loading fragrance encapsulated microparticles using imine derivatives of N-succinylchitosan (N-SC), ethylcellulose (EC) and methylcellulose (MC) was successfully created in a form of microparticles. The system contained a chemical derivatization in which the fragrances (L-carvone and hexylcinnamaldehyde) were linked to the N-SC via imine linkages, and a physical encapsulation in which active molecules were surrounded by a layer of EC/MC polymers. The fabrication involves the synthesis of N-succinylchitosan through the reaction between chitosan and succinic anhydride with degree succinyl substitution of 0.19 to obtain transparent colloidal suspension, and a chemical barrier involviny imine formation from the grafting reaction of representative perfumery ketone and aldehydes (L-carvone and hexylcinnamaldehyde) onto amino group of N-SC derivative. All two Schiff base products, L-carvonenesuccinylchitosan and hexylcinnamalidenesuccinylchitosan were used in combination with polymer blend (EC/MC) to encapsulate fragrance by homogenization. The best weight ratio between polymer blend and fragrance (L-carvone and hexylcinnamaldehyde) was 1:10 for the formation of fragrance microparticles. The obtained L-carvone-loaded micro-particles and hexylcinnamaldehyde-loaded micro-particles at 96.57 ± 0.87% and 81.43 ± 0.67% (w/w) loading. The process gave 82.17 ± 0.41% and 69.30 ± 0.6% (w/w) encapsulation efficiency respectively. The obtained microparticles from homogenization speed at 10,000 rpm showed perfect microparticles and gave the agreeable particle sizes of 9.82 ± 0.41 and 8.31 ± 0.12 μm respectively. The microparticles showed 14 days slower release of the L-carvone and hexylcinnamaldehyde comparing to free L-carvone and hexylcinnamaldehyde. We have successfully fabricated a long-lasting release material for fragranced delivery system. This reorganization possibility should be applicable to the design of other novel encapsulated fragrance materials.

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APPENDICES

APPENDIX A

1. Determination of degree of succinyl substitution

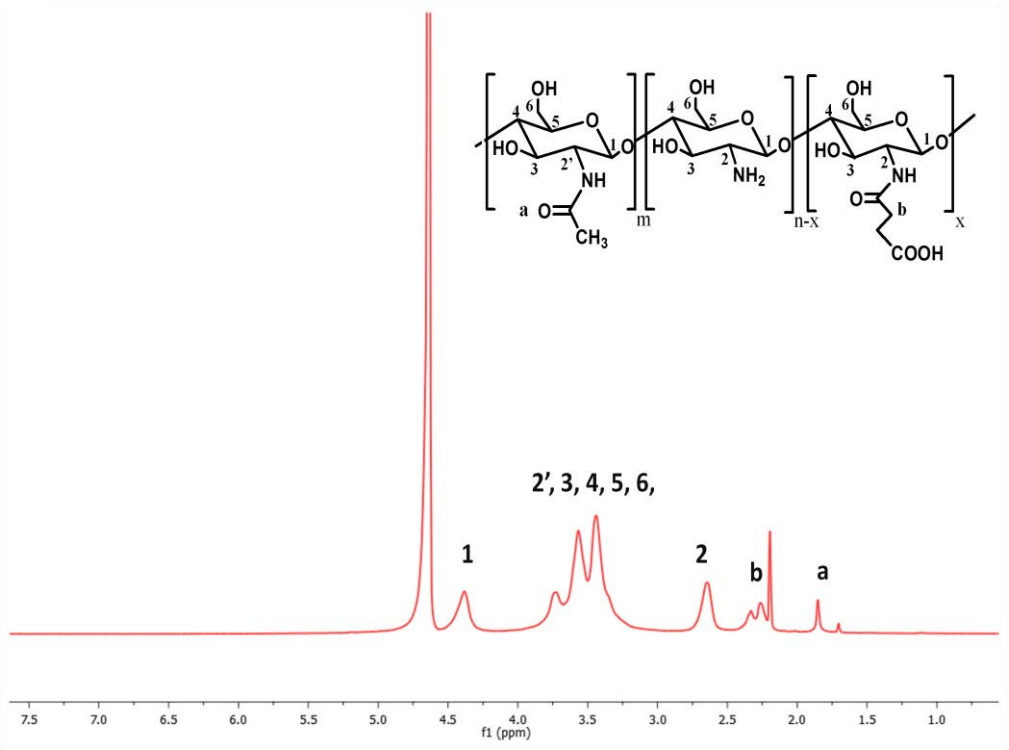

Figure A1. ^{1}H NMR spectrum of N-succinylchitosan (N-SC)

The degree of grafting could be determined using equation (1):

(1)

I_{graft} = the intensity of grafted moiety
DD = the amount of deacetylation degree
n = number of protons of grafted moiety
I_{cs} = the intensity of hydrogen om o hi o ' lu o mi e u i

From the ^{1}H NMR spectrum (Figure A1), using the integral ratio between 4H from ethyl group of succinyl (2.28-2.36 ppm) and 1H from C2 of glucosamine unit (at 2.7 ppm) with 85% deacetylation degree:

DG = 0.15

The degree of succinyl substitution could be estimated as 0.15.

APPENDIX B

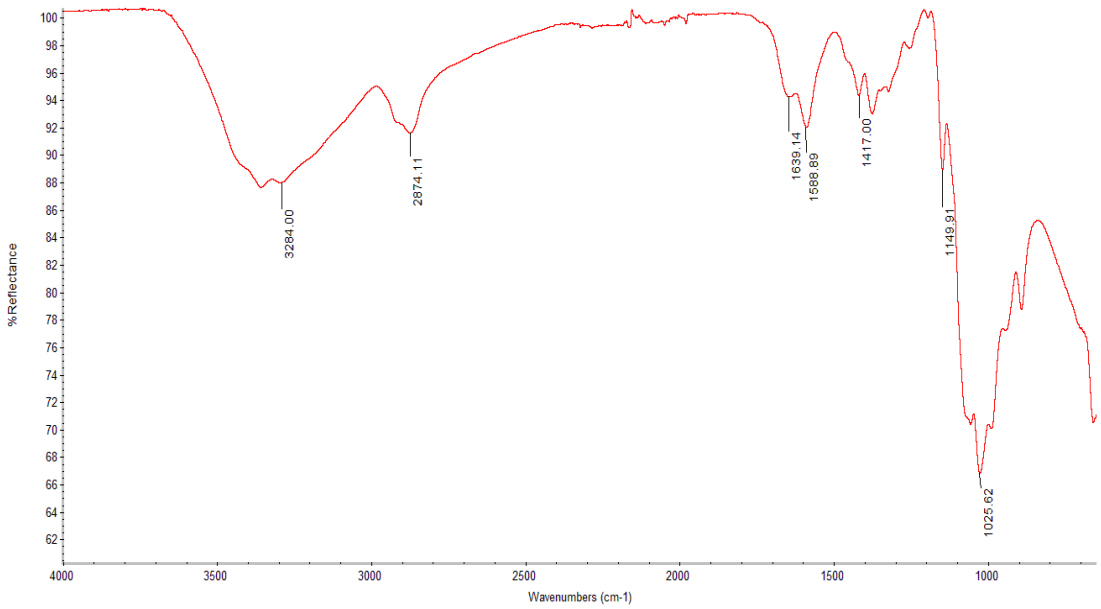

Figure B1. ATR-FTIR spectrum of chitosan

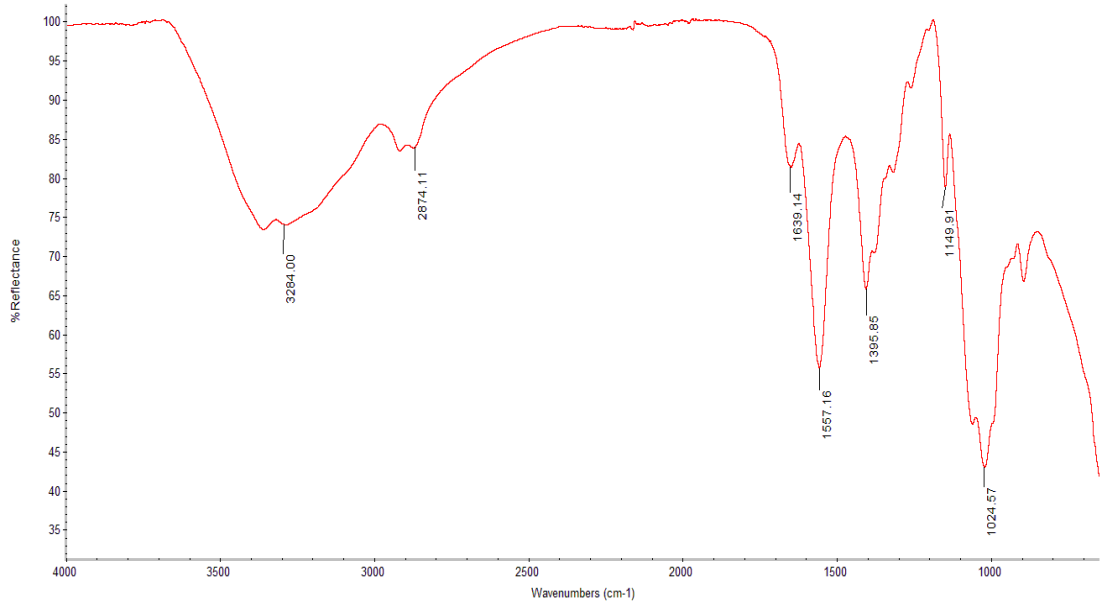

Figure B2. ATR-FTIR spectrum of N-succinylchitosan (N-SC)

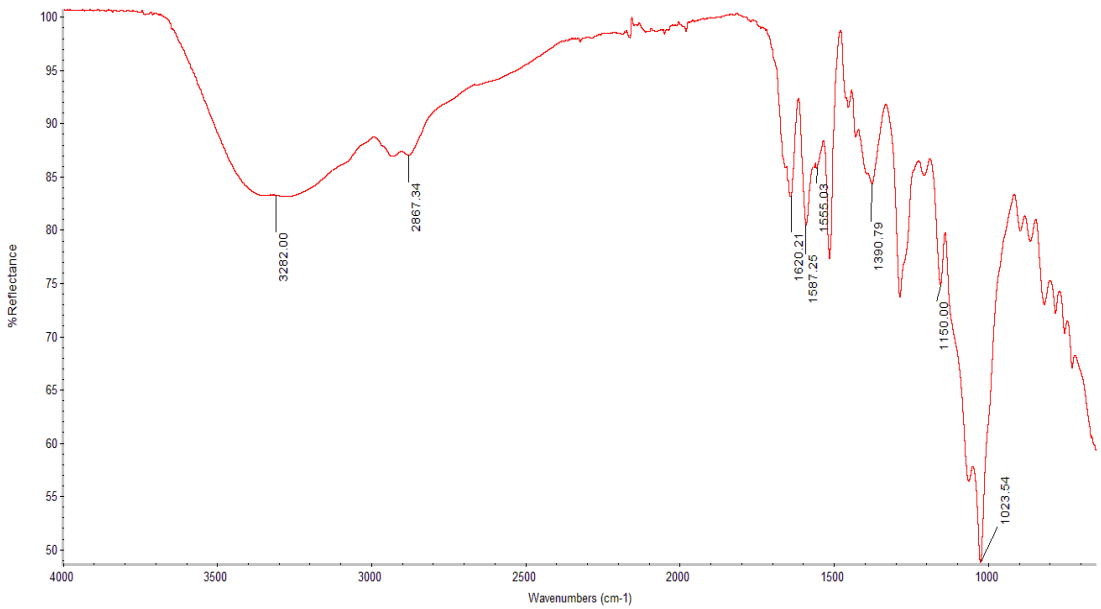

Figure B3. ATR-FTIR spectrum of L-carvonenesuccinylchitosan

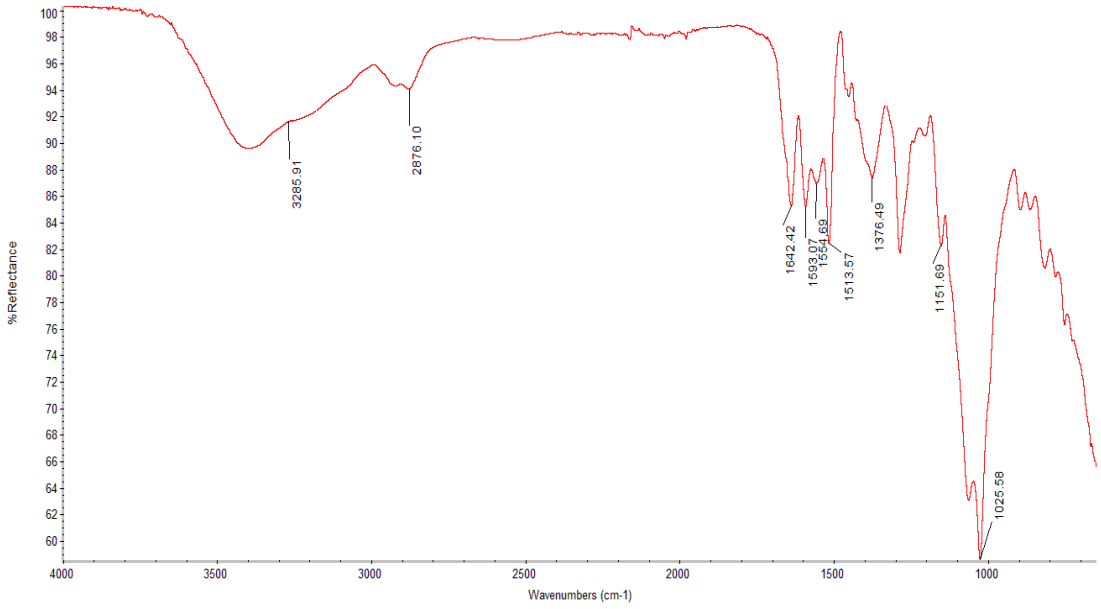

Figure B4. ATR-FTIR spectrum of Hexylcinnamalidenesuccinylchitosan

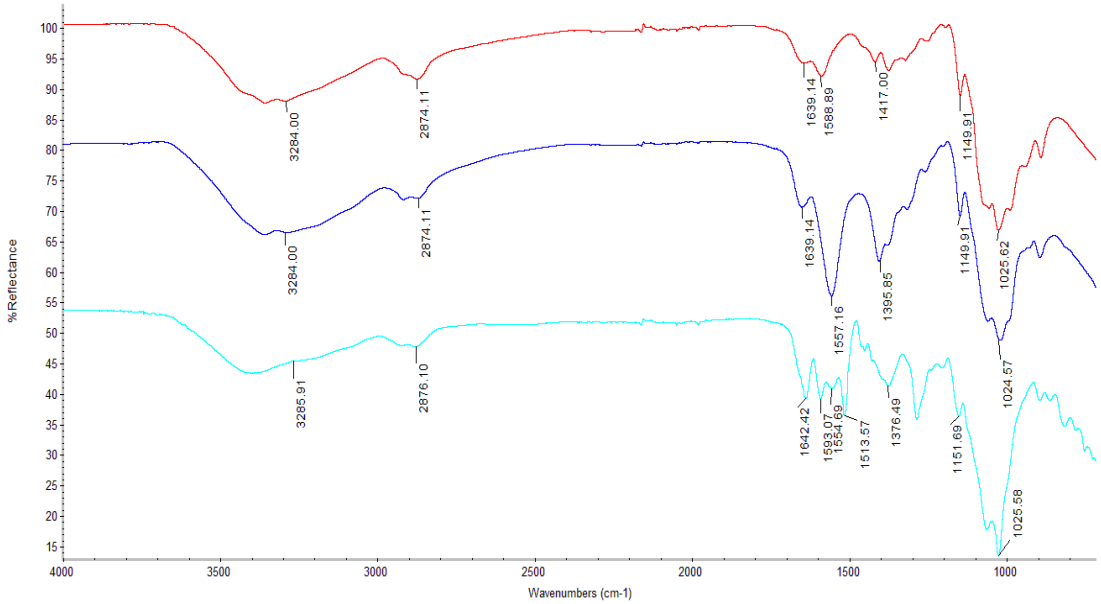

Figure B5. ATR-FTIR spectra of (a) chitosan (b) N-SC and (c) L-carvonenesuccinylchit.

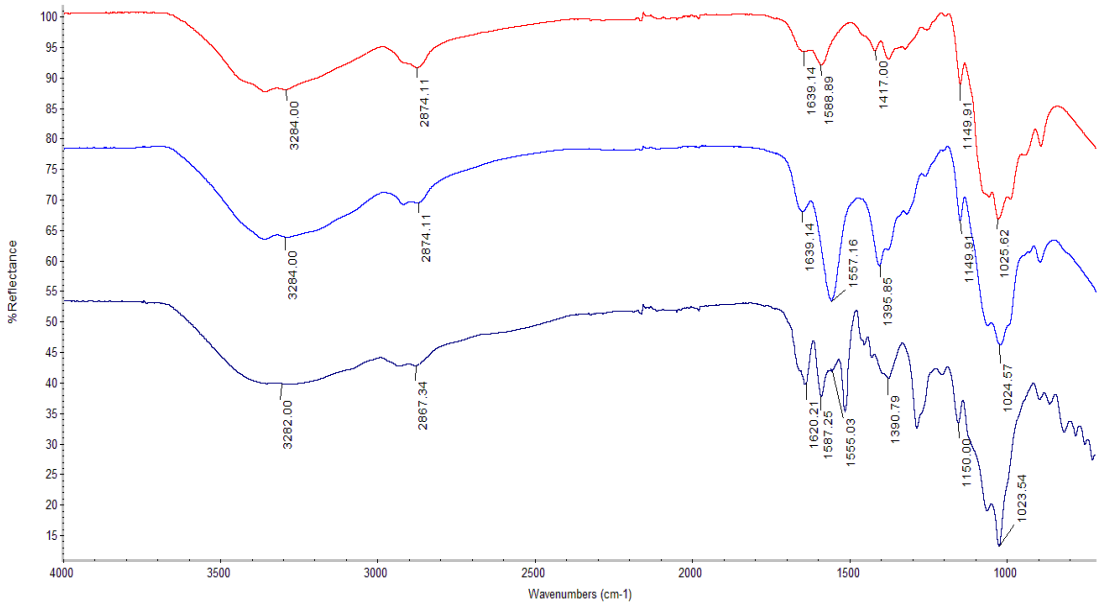

Figure B6. ATR-FTIR spectra of (a) chitosan (b) N-SC and (c) Hexylcinnamalidenesuccinylchitosan

APPENDIX C

1. Calculation of % encapsulation efficiency and loading content of fragrance - encapsulated into the blend of polymer microparticles

1.) Calibration curve of L-carvone

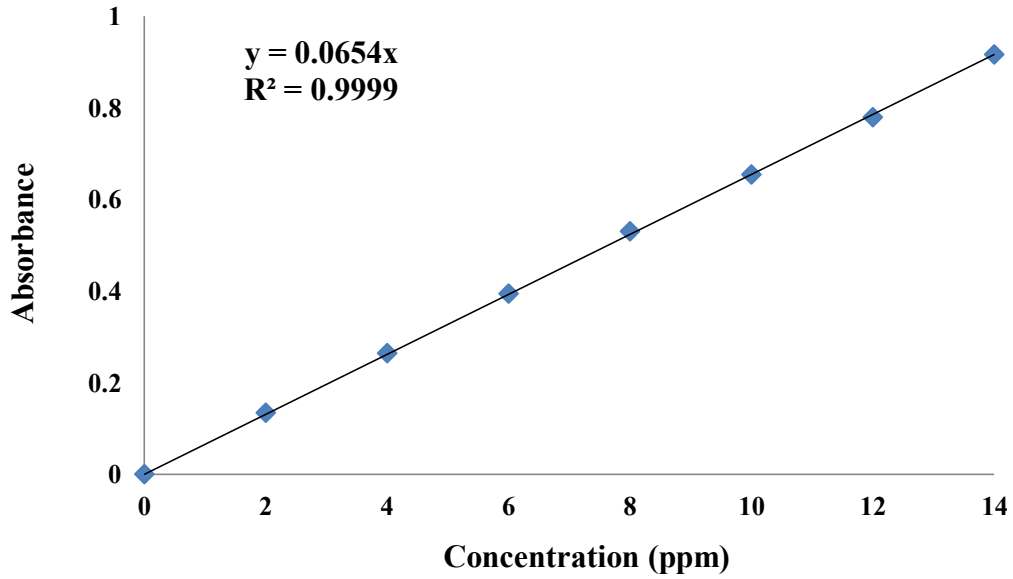

Figure C1. Calibration curve of L-carvone

By plotting a graph between absorbance and concentrations of L-carvone solutions, a linear relationship was obtained and used for calculation of concentration of fragrance.

Polymer : L-carvone at 1:1 (5,000 ppm : 5,000 ppm)

From the equation of calibration curve;

$$Y = 0.0654X \text{ , } R^2 = 0.999 \qquad (1)$$

The amount of L-carvone at the outside of particles was calculated by equation (1);

$$0.310 = 0.0654X$$

$$X = 4.75 \text{ ppm} = 4.75 \text{ mg/L}$$

The L-carvone diluted One thousand -fold, so the amount of L-carvone was

$$4.75 \times 1000 = 4{,}750 \text{ mg/L}$$

In final volume of 6 mL had L-carvone of $(4{,}750 \times 6)/1000 = 28.5$ mg

Weight of employed L-carvone and the blend of polymer were 1,500 mg and 500 mg
Weight of encapsulated L-carvone = 1,500– 46.14 = 1,453 .86 mg

$$\% \text{ EE} = \frac{\text{weight of encapsulated fragrance}}{\text{weight of fragrance used initially}} \times 100$$

$$= (1{,}453.86 / 1{,}500) \times 100$$

$$= 96.92\%$$

$$\% \text{ LC} = \frac{\text{weight of encapsulated fragrance}}{\text{weight of encapsulated fragrance} + \text{weight of polymer}} \times$$

$$= (1{,}453.86 / (1{,}500 + 500) \times 100$$

$$= 72.69\ \%$$

Controlled release study

The amount of remained L-carvone

- L-carvone from hydrolysis of microparticles suspension at 0 day

From equation (1) $Y = 0.0654X$

$Y = 0.065X$

$$0.654 = 0.0653X$$

$$X = 10 \text{ ppm}$$

Dilution factor = 100,000

$$X = 10 \times 100{,}000$$

$$X = 1{,}000{,}000 \text{ ppm}$$

Weight of encapsulated L-carvone in 65 mL = 1,000,000 x 0.065

= 65,000 mg

∴ Relative amount of L-carvone remained at 0 day = (65,000 /65,000) x 100

= 100%

- L-carvone from hydrolysis of microparticles suspension at 14 day

From equation (1) $Y = 0.0654X$

$$0.310 = 0.0654X$$

X = 4.74

Dilution factor = 100,000

X = 4.74 x 100,000

X = 474,000 ppm

Weight of encapsulated L-carvone in 65 mL = 474,000 x 0.065

= 30,810 mg

∴ Relative amount of L-carvone remained at 14 day = (30,810 /65,000) x 100

= 47.40%

2.) Calibration curve of hexylcinnamaldehyde

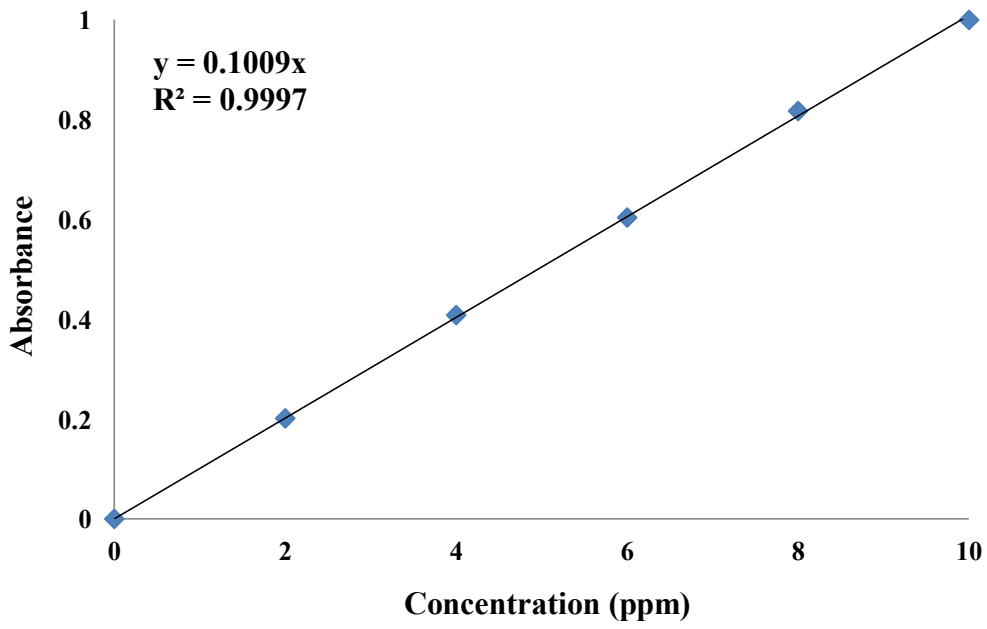

Figure C2. Calibration curve of hexylcinnamaldehyde

By plotting a graph between absorbance and concentrations of L-carvone solutions, a linear relationship was obtained and used for calculation of concentration of fragrance.

Polymer : hexylcinnamaldehyde at 1:1 (5,000 ppm : 5,000 ppm)

From the equation of calibration curve;

$$Y = 0.1009X \text{ , } R^2 = 0.999 \qquad (1)$$

The amount of hexylcinnamaldehyde at the outside of particles was calculated by equation (1);

$$0.527 = 0.1009X$$

$$X = 5.22 \text{ ppm} = 5.22 \text{ mg/L}$$

The hexylcinnamaldehyde diluted One thousand, so the amount of hexylcinnamaldehyde was 5.22 x 1000 = 5,220 mg/L

In final volume of 70 mL had hexylcinnamaldehyde of (5,220 x 70)/1000 = 365.4 mg

Weight of employed hexylcinnamaldehyde and the blend of polymer were 5000 mg and 500 mg

Weight of encapsulated hexylcinnamaldehyde = 5000– 365.4 = 4,634.6 mg

$$\% \text{ EE} = \frac{\text{weight of encapsulated fragrance}}{\text{weight of fragrance used initially}} \times 100$$

$$= (4{,}634/5000) \times 100$$

$$= 92.68 \%$$

$$\% \text{ LC} = \frac{\text{weight of encapsulated fragrance}}{\text{weight of encapsulated fragrance} + \text{weight of polymer}} \times$$

$$= (4{,}634/ (5000+ 500) \times 100$$

$$= 84.25 \%$$

Controlled release study

The amount of remained hexylcinnamaldehyde

- *Hexylcinnamaldehyde from hydrolysis of microparticles suspension at 0 day*

From equation (1) $Y = 0.1009X$

$$0.527 = 0.1009X$$

$$X = 5.22 \text{ ppm}$$

Dilution factor = 100,000

X = 5.22 x 100,000

X = 522,000 ppm

Weight of encapsulated hexylcinnamaldehyde in 70 mL = 522,000 x 0.070

= 36,540 mg

∴ Relative amount of hexylcinnamaldehyde remained at 0 day

= (36,540 /36,540) x 100

= 100%

- *Hexylcinnamaldehyde from hydrolysis of microparticles suspension at 14 day*

From equation (1) Y = 0.1009X

0.295 = 0.1009X

X = 2.931 ppm

Dilution factor = 100,000

X = 2.931 x 100,000

X = 293,100 ppm

Weight of encapsulated hexylcinnamaldehyde in 70 mL = 293,100 x 0.070

= 20,517mg

∴ Relative amount of hexylcinnamaldehyde remained at 0 day

= (20,517 /36,540) x 100

= 56.15%

VITAE

Miss Ratree Phookchaub was born on May 15, 1987 in Bangkok, Thailand. She received a Bachelor's Degree of Science in Biotechnology from Thammasat University in 2008. Then she started her graduate study on Master's degree in the Program of Biotechnology, Faculty of Science, Chulalongkorn University. During master study, she had the great opportunity to present her work in poster session in the topic of "Microparticles of fragrance: preparation, characterization and release property" at the Pure and Applied Chemistry International Conference 2013 (PACCON 2013), Chonburi, Thailand.

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