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Microencapsulation of essential oils and synthetic mosquito repellents by the complex coacervation method

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Plant essential oils can be used as effective mosquito repellents. However, they are unstable due to rapid evaporation and susceptibility to degradation when exposed to environmental stresses such as oxygen, high temperature, and light, causing loss of efficacy and shortening of the time of the repellent effect. Therefore, encapsulation technology was implemented to ensure the encapsulated material, which could be protected from immediate contact with the environment and to allow for controlled release. In this study, The EOs were extracted from 6 plants (*Cymbopogon nardus* Linn., *Litsea cubeba*, *Chrysopogon zizanioides* (L.) Roberty, *Amomum biflorum* Jack., *Eucalyptus globulus* Labill., and *Zanthoxylum limonella* Alston) by the hydrodistillation method. Microencapsulated plant essential oils (EOs) and synthetic repellents (DEET, Picaridin) microcapsules were synthesized by complex coacervation using gum Arabic and chitosan as the wall material utilizing sodium tripolyphosphate (NaTPP) as the crosslinking agent. Both natural and synthetic repellent microcapsules obtained in this study were examined by light-microscopy and found to be mononuclear with the spherical shape. The average diameter of the microcapsules ranged from 1.55 µm to 9.12 µm. The synthetic microcapsules could be dried into powder using the Freeze-drying method. Thermogravimetric analysis (TGA) found that microcapsules decomposed at 260 °C and released 58% of the encapsulating substance.

1. Introduction

Mosquito-borne diseases have a long history, such as those spread by the bite of an infected mosquito such as yellow fever, Zika fever, chikungunya, dengue, and malaria, etc. The mosquito inhibitors used for the management of mosquito-borne diseases are mostly synthetic chemicals that may have to affect people's health and environmental concern.[1]

Plant essential oils (EOs) can be used as an effective mosquito repellent.[2] However, due to its nature, which was unstable, rapidly evaporate and susceptible to degradation when exposed to oxygen, temperature, and light, causing loss of efficacy and shorten the repellent effect. Therefore, in the present work, encapsulation technology has been implemented to ensure the encapsulated material is being protected from immediate contact with the environment and offers the potential for controlled release of the active ingredients.

Microencapsulation technology has been used in a variety of industries, such as chemicals, cosmetics, food, textile, and medicine.[3] The capsule can enclose and release the chemical payloads as needed, dependent on the properties required. The size and shape of the encapsulation agent vary depending on the materials and methods used in the preparation.[4] Coacervation or phase separation is a method for preparing microcapsules consisting of two phases, and being the phase of the core

and the other being the phase of the wall, causing the substance to encapsulate and coat the core. In complex coacervation, the technique uses two polymers that have an opposite charge to form a wall wrapped around the core.[5] Generally, Gelatin and gum Arabic are used as wall materials. Natural complex polymers such as chitosan have been used to encapsulate active ingredients due to its low toxicity and the ability to mold mucous membrane films in high tensile strength.[6] Mostly crosslinking chemicals used are formaldehyde, glutaraldehyde, glyoxal, diisocyanate, and epichlorohydrin had limitations due to their biological toxicity. Butstraen and Salaün have studied the development of chitosan-gum Arabic microcapsules by using sodium tripolyphosphate (NaTPP), a non-toxic crosslinking agent.[7]

This study focuses on the preparation of microcapsule of EOs mosquito-repellent extracts from plants and synthetic repellents (DEET, Picaridin) by complex coacervation technology using gum Arabic and Chitosan as wall material and sodium tripolyphosphate (NaTPP) as the crosslinking agent. The operating process for encapsulation was conducted under room temperature conditions. The EOs microcapsules obtained in this study were characterized by light-microscopy, FTIR spectroscopy, and Thermogravimetric analysis (TGA).

2. Materials and Methods

2.1 Materials

Chitosan (Chi) (deacetylation degree = 85%) and gum Arabic (GA) (Ajax FineChem, Australia) were selected as wall materials. Sodium tripolyphosphate (NaTPP) (Sigma, Aldrich, USA) and acetic acid (99.8 %, RCI Labscan, Thailand) were of the analytical grade used. The core materials, the EOs were extracted from 6 plants by the hydrodistillation method, including *Cymbopogon nardus* Linn. (leaf), *Amomum biflorum* Jack. (all parts), *Eucalyptus globulus* Labill. (leaf), and *Zanthoxylum limonella* Alston)(fruit) purchased from N&B Organizer, Thailand. *Litsea cubeba* (Aromahub Group Co., Ltd., Thailand) and *Chrysopogon zizanioides* (L.) Roberty (The Royal Project Foundation, Thailand). The synthetic repellents were obtained from N, N-Diethyl-3-methyl benza mide (DEET) (Alfa Aesar, Thermo Fisher Scientific Inc.), and Picaridin/Saltidin (PS Grand Chemical, USA).

2.2 Preparation of microcapsules

Microencapsulated EOs and synthetic repellents (DEET, Picaridin) microcapsule were synthesized by complex coacervation techniques[7] using gum Arabic and chitosan as wall materials and NaTPP as a crosslinking agent at room temperature.

2.2.1 Solutions preparation

Acidic chitosan solutions (1.25% v/v) were prepared by dissolving Chi powder (1.25 g) in a 100 ml acetic acid (2% v/v) under magnetic stirring condition (1000 rpm) at 45 °C. Gum Arabic solutions (5% w/v) was prepared by dispersing 5.0 g of gum Arabic powder in 100 ml of water. NaTPP solution was prepared by dissolution of 2.0 g of NaTPP in 100 ml of water, and the pH value of solutions was adjusted from pH ~8 to pH ~4 using acetic acid (2% v/v).

2.2.2 Microencapsulation of essential oils and synthetic mosquito repellents

The EOs or synthetic repellents of 5 ml were mixed with 100 ml of an aqueous solution of gum Arabic and formed an emulsion. The mixture was homogenized using Homogenizer/high-speed blender (T25 digital ULTRA-TURRAX, IKA, Germany) at 11,000 rpm, followed by room temperature for 15 min. Then the dropwise addition of acidic Chi solution of 100 ml was added to create GA-Chi coacervation. After that, the crosslinking agent (NaTPP solution) was added to form a microcapsule. The mixture was stirred at 1000 rpm for 2 hr. Finally, the microcapsule was

separated by decantation, filtered, and washed with water.

The synthetic microcapsules were dried into powder using the Freeze-drying method (Freeze Dryer, Laboratory Freeze Dryer -58 °C, FD 5-4, Gold Sim, Czech Republic).

2.3 Characterization of microcapsules

To obtain the size and morphology, both natural and synthetic mosquitoes repellent microcapsules were examined using the optical microscopy (light-microscope CX43 standard, Olympus, Japan) with Canon DS126571 camera (Canon Inc., Japan), Fourier-transform infrared spectroscopy, FTIR (Spectrum Two, FTIR Spectrometer, PerkinElmer Inc., USA) was used to identify the chemical structure of microcapsules. The samples were mixed with KBr in the pellet form using high-pressure compression. The test samples were the wall materials (Chitosan, gum Arabic), microcapsule of mosquito repellent, and microcapsule without mosquito repellent.

The wall materials, mosquitoes repellent, microcapsule of repellent, and microcapsule without mosquitoes repellent were analyzed using thermogravimetric analysis (TGA, TG8120, Rigaku, Japan) at a heating rate of 10 °C/min from 30-600 °C in a nitrogen atmosphere.

3. Results & Discussion

3.1 Preparation of microcapsules

Microencapsulation of EOs and synthetic mosquitoes repellents were successfully synthesized by complex coacervation using gum Arabic and Chitosan as the wall material. Firstly, the oils (EOs or synthetic mosquitoes repellents) were mixed with gum Arabic solution to form emulsion by using a homogenizer. The mixture is a colloid of oil spread in water (oil-in-water emulsion). Then to form the polymeric wall around the core, the dropwise of chitosan solution was added to create the GA-Chi coacervation complex by the interactions between carboxylic groups ($–COO^-$) of gum Arabic and the amino groups ($–NH_3^+$) of chitosan(Figure 1a). Finally, the crosslinking agent solution (NaTPP) was added to form the particle. The mixture was stirred at 1000 rpm for 2 hr. Finally, the obtained white paste microcapsules were separated by decantation (Figure 1b), filtered under vacuum, and lastly, washed with distilled water to get rid of acids and other residues. It was observed that the final product is stable, white powder. The resulting microcapsules were divided into two portions; the first part was dried in a hot air oven at 45 °C, 12 hr and the second part was freezed at -80°C for 3 hr, and dried by Freeze Dryer at -58 °C, 4 mTorr, 20 hr. The microcapsules that were dried with a hot oven turned a yellow/brown solid film (Figure 1c). There was some oil on the outside, which was caused by the surface being evaporated quickly at high temperatures. Therefore, there were a part of the oil evaporated from the capsule, and the capsules are flat, tight, not stable in the same sphere. For the freeze-dried microcapsules, it was a solid, yellowish-white powder (Figure 1d).

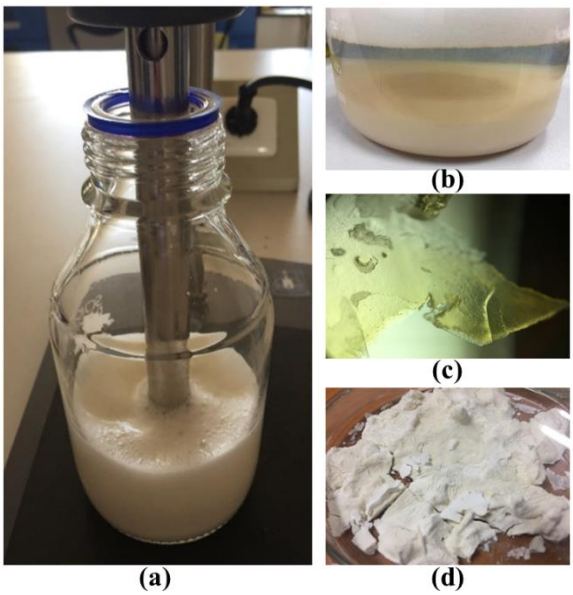

Figure 1. Microcapsule preparation (a), decantation (b) microcapsules dried by the hot oven (c) and freeze dryer (d)

Figure 1 shows the microcapsule preparation and illustrates the separation by decantation and the white powder microcapsule product after dried using the Freeze-drying method.

3.2 Characterization of microcapsules

Morphology of resulting microcapsules was investigated by optical microscopy. The capturing optical micrographs of MCs obtained in this study (Figure 2) show that the microencapsulated EOs and synthetic mosquitoes repellent fabricated in the same type of mononuclear with a spherical shape and their apparent diameter which varied from 1.55 to 9.12 µm.

Table 1 shows the mean diameters of the produced microcapsules; Litsea cubeba (Ø 1.55 µm), DEET (Ø 2.79 µm), Amomum biflorum Jack. (Ø 2.93 µm), Eucalyptus globulus Labill. (Ø 3.35 µm), Cymbopogon nardus Linn. (Ø 3.82 µm), Chrysopogon zizanioides (L.) Roberty (Ø 3.95 µm), Picaridin (Ø 5.13 µm) and Zanthoxylum limonella Alston (Ø 9.12 µm) respectively.

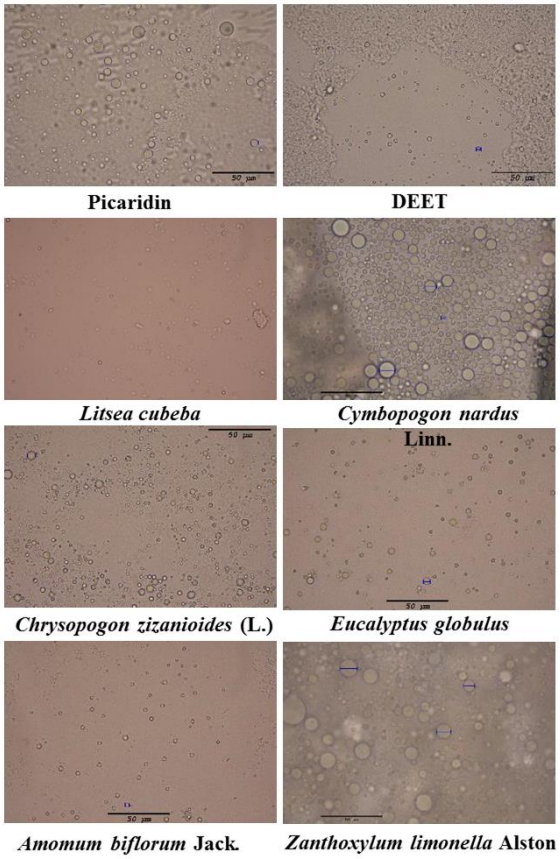

Figure 2. Optical microscope images of microcapsules of EOs and synthetic repellents (100x)**.**

Table 1 Mean diameter and morphology of the microcapsules.

No.	Core materials	Mean diameter (µm)	Morphology
1	Picaridin	5.13	mononuclear
2	DEET	2.79	mononuclear
3	*Litsea cubeba oils*	1.55	mononuclear
4	*Cymbopogon nardus* Linn. oils	3.82	mononuclear
5	*Chrysopogon zizanioides* (L.) Roberty oils	3.95	mononuclear
6	*Eucalyptus globulus* Labill. oils	3.35	mononuclear
7	*Amomum biflorum* Jack. oils	2.93	mononuclear
8	*Zanthoxylum limonella* Alston oils	9.12	mononuclear

FTIR spectra of gum Arabic, chitosan, and MCs without core substance (Figure 3) were analyzed in their solid-state in order for the characteristic of their structure. The FTIR spectrum of Chi showed the strong absorption band in the region 3290-2350cm^{-1} corresponds to N–H and O–H stretching, a typical band at 3350 cm^{-1} stretching band of hydroxyl groups (–OH). This band is broad due to inter and intramolecular hydrogen bonding. The absorption bands at approximately 2910 and 2870 cm^{-1} can be attributed to C–H symmetric and asymmetric stretching vibration of >CH_2, group. The strong bands at 1590 cm^{-1} and 1400 cm^{-1} are due to the asymmetric and symmetric stretching vibration of the carboxylic acid salt –COO^- and the absorption bands at 1275 cm^{-1} and 1050 cm^{-1} is the stretching of the C–O bond. GA–Chi microcapsule showed a band at 3265 cm^{-1} of -NH_2 and -OH groups stretching vibration. The FTIR of the GA–Chi coacervate changed significantly in the carbonyl-amide region. The –NH_3^+ groups (band at 1530 cm^{-1}) and asymmetric and symmetric –COO^- stretching vibration at 1615 cm^{-1} and 1410 cm^{-1}, respectively. The FTIR spectra show characteristic bands at 1200 cm^{-1} assigned to P–O stretching, 1155 cm^{-1} for the stretching of the PO_2 groups, 1055 and 885 cm^{-1} attributed to the stretching vibration of the PO_3 groups and P–O–P asymmetric stretching, respectively.[7]

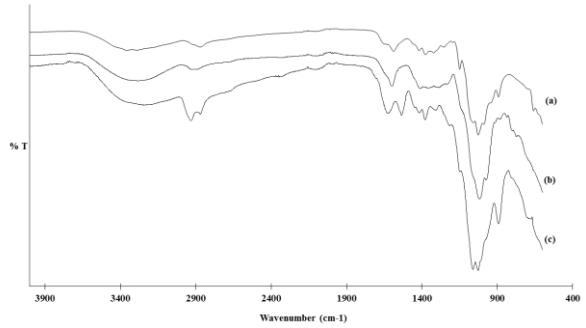

Figure 3. FTIR spectra of chitosan (a), gum Arabic (b) and MCs without core substance (c).

respectively. At 1635 cm^{-1} characteristic amide I band attributed to C=O vibration of the acetylated units (–$CONH_2$ groups. An absorption band at 1580 cm^{-1} corresponds to the N–H bending of the primary amine. The absorption band at 1150 cm^{-1} corresponds to the symmetric stretching of C–O–C; the absorption bands at 1065 cm^{-1} and 1025 cm^{-1} are associated with the C–O stretching vibration[8]. GA showed typical bands of the –OH bond stretching at 3330 cm^{-1}. The band at 2925 cm^{-1} is characteristic of the carboxylic

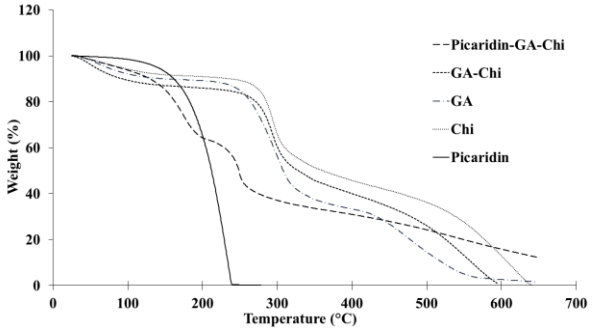

Figure 4. TGA diagram of Picaridin, Chitosan (Chi), Gum Arabic (GA), MCs without Picaridin (GA-Chi) and MCs containing Picaridin(Picaridin-GA-Chi).

Percent encapsulation of microcapsule was examined by Thermogravimetric analysis. Figure 4 shows TGA diagrams of Picaridin, Chitosan, Gum Arabic, MCs without Picaridin, and MCs containing Picaridin. TGA analysis of the MCs without Picaridin shows the mass loss between 25 ºC and 200 ºC, corresponded to the evaporation of the water adsorbed in the capsules. The mass loss between 260 ºC and 650 ºC corresponds to the decomposition of the wall. The mass loss of around 58% shows the release of the encapsulating substance. Confirmation by a thermogram of materials used in the preparation, solely thermogram of the Picaridin, starts to decompose at the beginning until completely decompose quickly before 200 °C. However, the wall materials (Chitosan, Gum Arabic) and MCs without Picaridin decompose at almost 300 °C, as

compared with the microcapsule of Picaridin, which slowly decomposes.

4. Conclusion

The microencapsulation of EOs (*Cymbopogon nardus* Linn., *Litsea cubeba*, *Chrysopogon zizanioides* (L.) Roberty, *Amomum biflorum* Jack., *Eucalyptus globulus* Labill., and *Zanthoxylum limonella* Alston), DEET and Picaridin were undertaken by complex coacervation using gum Arabic and chitosan as the wall material, utilizing sodium tripolyphosphate (NaTPP), a non-toxic cross-linking agent. An optical microscope examined the resulting microcapsules obtained in this study; the morphology of MCs was mononuclear with a spherical shape. The average diameter of the microcapsules ranged from 1.55 µm to 9.12 µm. The synthetic microcapsules could be dried into powder using the Freeze-drying method. FTIR was employed for the composition of MCs. Moreover, the spectra confirmed the encapsulation of materials. The thermogravimetric analysis found that microcapsules decomposed at 260 °C and released 58% of the encapsulating substance. In the present work, it was found that the encapsulation of the EOs and synthetic mosquito repellent by complex coacervation was a success. In a further study, MCs will be applied to textile materials as the mosquito repellent agent continues to understand the additional properties of MCs.

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