Pharmaceutical Sciences Asia

Pharm Sci Asia 2022; 49(5), 506-517

DOI:10.29090/psa.2022.05.22.110

Research Article

Controlled release of DEET and Picaridin mosquito repellents from microcapsules prepared by complex coacervation using gum Arabic and chitosan

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ABSTRACT

Microcapsules (MCs) of mosquito repellents (DEET and Picaridin) were prepared by complex coacervation using gum Arabic and chitosan as wall materials. The resulting diameters of MCs were 2.35±0.76 µm for DEET and 4.26±1.77 µm for Picaridin, analyzed by optical microscopy. Spherical mononuclear-type MCs were also observed. The mean particle size of dried DEET-MC and Picaridin-MC was 182 µm and 140 µm, respectively, indicating agglomeration of MCs. Fourier transform infrared spectroscopy confirmed the encapsulation of repellents by the appearance of carbonyl (C=O) absorption bands in the associated spectra. Moreover, the microencapsulation efficiency was 60% and 73% for DEET and Picaridin, respectively, determined by Soxhlet extraction. From Thermogravimetric analysis, delay of DEET and Picaridin losses revealed encapsulation of DEET and Picaridin inside the MCs. In addition, the higher release rate of Picaridin compared to DEET under isothermal conditions was correlated with longer protective time relative to the free repellents, as demonstrated by the "arm in cage" test. The study concludes that microencapsulated DEET and Picaridin show high promise of functional textiles for mosquito repellency.

Keywords:
Microcapsules, DEET, Picaridin, Arm in cage, Complex coacervation

1. INTRODUCTION

Mosquitoes have a long history of carrying infectious diseases, such as yellow fever, Zika fever, chikungunya, dengue fever and malaria[1-3]. Therefore, mosquito repellents have been designed so that they may, ideally, be used in many animal species, be applied to the skin without adverse effects, cause no damage to clothing or plastics, have no unpleasant odour, not leave the oil on the skin, and be chemically stable, non-toxic, and effective for significantly long enough periods of time[4-5].

DEET (N,N-diethyl-3-methylbenzamide) and Picaridin (2-[2-hydroxyethyl]-1-piperidine carboxylic acid-1-methylpropylester) are the most widely used and effective commercial mosquito repellents (Figure 1)[2-3,6]. DEET is an oily, volatile liquid, slightly yellowish in color (2) and Picaridin is a water-insoluble oil, volatile liquid, colorless and nearly odorless[2,7]. Both DEET and picaridin are formulated as a liquid, lotion or spray and applied on the skin in an indoors or outdoors environment. All formulations are intended to be used by adults (both civilian and military) or children. The active ingredients differ slightly in their effectiveness as a repellent and their chemical and biological characteristics. Nevertheless, they work similarly by producing an odour that insects find repulsive[7]. However, their use has limitations, such as unpleasant odours, skin irritation, and unacceptable toxicology[2,4-5,8-10]. DEET is the most common and most effective mosquito repellant[7,10]. However, it is not recommended for use on children up to 6 months of age and by pregnant women

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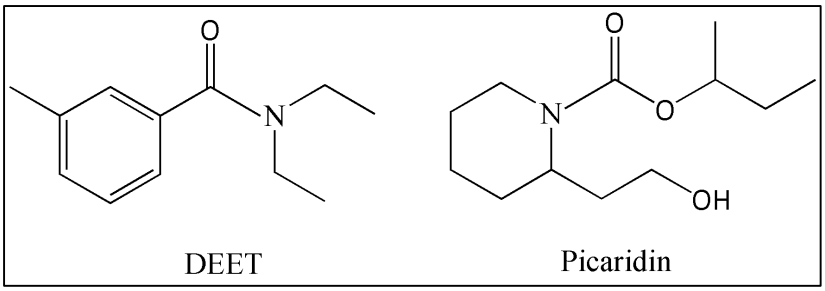

Figure 1. The chemical structure of DEET and Picaridin.

due to its high repro-toxicity. Therefore, careful handling and/or preparation are required for these populations[10]. Picaridin is an interesting choice because it possesses the same potency as DEET, but is less toxic and has the advantage of being longer acting and it does not damage the plastics and synthetic fabrics[2,10]. However, despite some data of long-term health risks for Picaridin, the data is not sufficient in quantity or quality to prove this indisputably. Even though DEET is well established and commonly used and Picaridin is a relatively new as a mosquito repellent, demonstration of their release behavior upon change in temperature together within the same article has been lacking. Therefore, in this study, we compared the efficiency of DEET and picaridin for mosquito repellency at different temperatures and demonstrated the application of mosquito repellent functional textiles working on the body, both in terms of efficiency and effectiveness.

Encapsulation technology was implemented in this work to ensure the active repellent material is protected from immediate contact with the environment or human skin. In addition, encapsulation has the potential for controlled release of the active ingredient to modulate mosquito repellency over an extended time period[11]. Microencapsulation technology has been used in various industries, including chemicals, cosmetics, food, textile, and medicine[11-13]. Microencapsulation is employed to enclose and have a time dependent release of the chemical payloads as needed, depending on the required properties. Release mechanisms involve dissolution, osmosis, diffusion, disruption, and erosion[2,14-15]. The size and shape of microencapsulated particles vary depending on the materials and methods used in the preparation[13,16].

Research articles report the development of DEET encapsulation obtained by various methods. Gomes et al.[3] successfully encapsulated DEET in the nanospheres form via miniemulsion polymerization of methyl methacrylate and n-butyl methacrylate at 70°C under nitrogen gas purge to avoid polymerization inhibition, the copolymer nanospheres with an average number diameter of 114± 37 nm and encapsulation efficiency of 29±9% were obtained. Fei and Xin[17] studied microencapsulated DEET in polymer capsules during graft copolymerization of butyl acrylate onto chitosan, and a stable aqueous emulsion was obtained. The DEET microcapsule provides a useful anti-bacterial and mosquito repellent. Kadam et al.[11] report the encapsulated DEET through interfacial polycondensation using modified cellulose nanofiber (CNF) as an emulsifier to form emulsions with a very high encapsulation efficiency of about 98%. DEET was encapsulated using interfacial precipitation chemistry technique with using carboxymethylcellulose (CMC) and benzalkonium chloride (BKC) to form the wall around the call substance and added into lotion base to form microencapsulated (ME) formulation. The ME formulation of DEET showed repellent activity against *Culex quinquefasciatus* under laboratory conditions[18]. Eyupoglu et al.[19] reported the microencapsulation of DEET via a simple coacervation method. DEET was encapsulated with gum Arabic wall material at a 1:5 ratio of wall to the core substance. The resulting microcapsules ranging from 1 to 68 μM were treated on cotton fabric as bee-repellent in beekeeping. For Picaridin, polyhexamethylene biguanide (PHMB) has been used to form the MC wall to encapsulate Picaridin by the complex coacervation method reported by Place et al.[20]. The MCs remain stable when stored in deionized water for at least six months. In addition, mosquito repellency and both antibacterial and antifungal properties were exhibited.

Coacervation is a method for the preparation of MCs consisting of (a) a core phase containing the active substance and (b) a surrounding wall phase used to encapsulate and coat the core. In complex coacervation, the technique uses two polymers with an opposite charge to form the wall phases around the core using sequential steps[21].

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 their low toxicity and the ability to mold mucous membrane films with high tensile strength[22]. Many crosslinking chemicals such as formaldehyde, glutaraldehyde, glyoxal, diisocyanate, and epichlorohydrin have limitations due to their biological toxicity[23]. However, Butstraen and Salaün et al. have studied the development of chitosan-gum Arabic MCs using sodium tripolyphosphate (NaTPP), a non-toxic crosslinking agent[24]. Sharkawy et al.[25] also reported using chitosan and gum Arabic as wall material of MCs. At the same time, tannic acid was used as a hardening reagent to produce the MCs of

limonene and vanillin oils at room temperature. Goncalves et al.[26] compares wall materials (gelatin, chitosan, and gum Arabic) to produce the essential oils of the Thyme MCs. The wall materials, which combine gum Arabic and chitosan, can produce the MCs in a mononuclear structure.

We are interested in applications involving functional textiles, so we have investigated the preparation of MCs with mosquito repellents (DEET and Picaridin) using complex coacervation of polymers i.e. gum Arabic and chitosan for wall materials with sodium tripolyphosphate (NaTPP) as the crosslinking agent. The encapsulation process was carried out under room temperature conditions. The MCs obtained in this study were characterized by optical microscopy, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and thermogravimetric analysis (TGA). The release of mosquito repellents was studied under isothermal conditions. Both the free and encapsulated mosquito repellents have been tested with *Ae. Aegypti* by the "arm in cage" test according to the WHO standard method[27]. The potentials of DEET and Picaridin for mosquito repellency were compared.

2. MATERIALS AND METHODS

2.1. Materials

Chitosan (Chi) of food quality grade with degree of deacetylation of 85% was from Sinudomkasetphan limited partnership, Thailand, and gum Arabic (GA) purchased from Ajax FineChem, Australia, were selected as wall materials. The core materials, i.e. 97% N,N-Diethyl-3-methyl benzamide (DEET) was from Alfa Aesar, Thermo Fisher Scientific Inc., UK and Picaridin/Saltidin, were supplied from PS Grand Chemical co., Ltd, Thailand. Sodium tripolyphosphate (NaTPP) with 85% purity from Sigma, Aldrich, USA, was used as a crosslinking agent. Analytical grade acetic acid was purchased from RCI-Labscan, Thailand and extra virgin coconut oil was supplied by Agrilife (Thailand) Co., Ltd., Thailand.

2.2. Synthesis of the microcapsules

2.2.1. Preparation of acidic chitosan solutions

Acidic chitosan solutions of 1.25% v/v were prepared by dissolving 1.25 g of Chi powder in 100 ml of 2% v/v acetic acid under magnetic stirring (1000 rpm) at 45°C for 1h. A 5% w/v Gum Arabic solution was prepared by dispersing 5.0 g of GA powder in 100 ml of distilled water under continuous magnetic stirring of 500 rpm at room temperature for 15 min. NaTPP solution was prepared by dissolution of 2.0 g of NaTPP in 100 ml of distilled water. The NaTPP solution pH value was adjusted from pH ~8 to pH ~4 using 2% v/v acetic acid.

2.2.2. Preparation of microencapsulation of mosquito repellents

MCs preparation was carried out as described by the others[24,28] with slight modifications. The flow chart of the MC preparation is shown in Figure 2. First, 5 ml of the DEET or Picaridin were mixed with 100 ml of the gum Arabic solution. The mixture was transformed into an emulsion at 11,000 rpm for 15 min using a high-speed blender (T25 digital ULTRA-TURRAX, IKA, Germany). Next, the dropwise addition of 100 ml acidic Chi solution was carried out to obtain the GA-Chi coacervation for 15 min. The process was conducted at room temperature by homogenizing using the high-speed blender. After that, the crosslinking agent (NaTPP solution) was added to

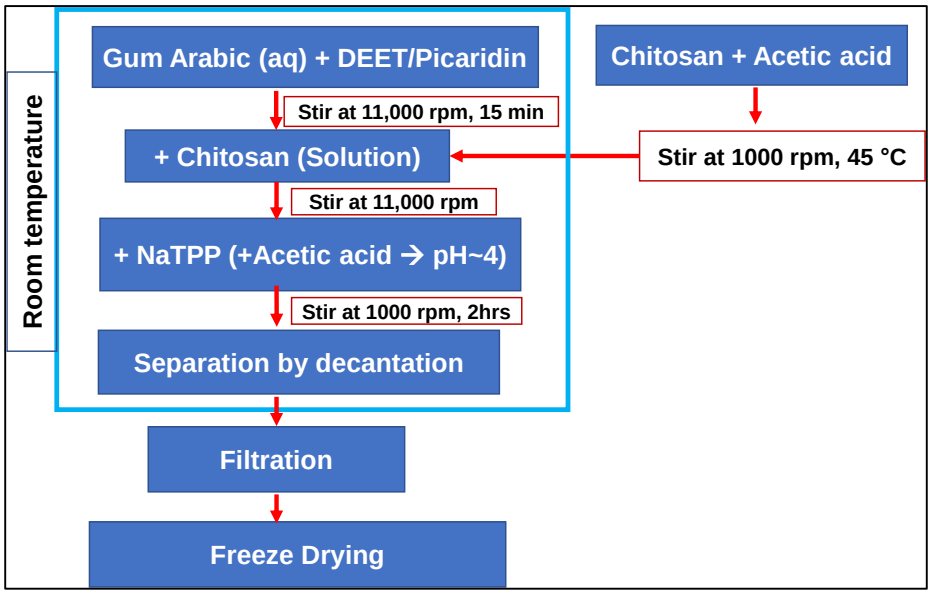

Figure 2. Diagram of DEET-MC and Picaridin-MC preparation.

form the MCs. The mixture was stirred at 1000 rpm for 2 h using a magnetic stirrer, and then microcapsules formed a sediment at room temperature for 24 h. The obtained MCs were separated by decantation, filtered by vacuum filtration (Whatman #1), and thoroughly washed with distilled water to remove acids and other residues. Finally, the resultant MCs were initially frozen at -80°C for 3 h and dried into powder using the freeze-drying method at -58°C, 4 mTorr for 10 h (Freeze Dryer, Laboratory Freeze Dryer FD 5-4, Gold Sim, Czech Republic). The dried MCs were added into plastic zip lock bags and stored at refrigerated temperature (~4°C) for further analysis.

2.3. Characterization of microcapsules

The morphology of the MCs was examined using optical microscopy (light microscope standard CX43, Olympus, Japan) equipped with a Canon DS126571 camera (Canon Inc, Japan). The mean diameter of the wet MCs was measured using at least 100 cells in the same picture and averaged using Image Frame Work software version 3.6.0.067 (Tarosoft®; Nonthaburi, Thailand).

MCs particle size and size distribution were determined by laser diffraction with a particle size analyzer, Mastersizer S (Malvern Instruments, Malvern, UK). Particle size distribution measurements were obtained after the dried MCs being dispersed in distilled water by ultrasonic treatment for 2 min. The particle size range analysis was 0.05-900 µm.

The field emission scanning electron microscope (FESEM, JEOL, JSM-7610F Plus, Japan) equipped with secondary electron (SE) and back scattered electron (BSE) image was used to investigated the morphology of the MCs under high vacuum mode with accelerating voltage of 15.0 kV. The MC sample was sputter-coated with a thin layer of gold, and the final micrographs were taken at x2000 magnification.

Fourier transform infrared spectroscopy (FTIR) was used to identify the chemical structures. The wall materials (Chitosan and gum Arabic), MCs of mosquito repellent, and free MCs were pressurized using high-pressure compression to form a pellet. Nujol technique was used to prepare samples of DEET and Picaridin in their liquid forms. Their FTIR spectra were recorded in the range of 600 cm^{-1} to 4,000 cm^{-1} using Spectrum Two, FTIR Spectrometer, PerkinElmer Inc., USA.

To determine the microencapsulation efficiency (ME) of the synthesized MCs. the total mosquito repellent in the MCs and the free mosquito repellent (non-encapsulated mosquito repellent) was analyzed by a modified method of Liu et al.[29], Karaca et al.[30], and Rubilar et al.[31] The total mosquito repellent of the MCs was analyzed by Soxhlet extraction using a cellulose thimble containing 1 g of dry MCs. Extraction was performed using 150 ml of ethanol for 3 h. Then the extracted MCs were dried in a hot air oven at 60°C for 3 h and kept in a desiccator. The free mosquito repellent was determined by adding 1 g of MC to 100 ml of ethanol and stirring at room temperature for 15 min. This was filtered (Whatman #1) and dried at 60°C for 3 h and kept in a desiccator. The free mosquito repellent was determined by the weight loss of the extracted MCs. All experiments were carried out in triplicate. The microencapsulation efficiency was determined by the ratio between the free mosquito repellent and total mosquito repellent with the equation;

$$\%ME = \left(\frac{\text{Total mosquito repellent-Free mosquio repellent}}{\text{Total mosquito repellent}}\right) \times 100$$

DEET-MCs and Picaridin-MCs will be further incorporated into a fabric for the functional textile purpose in future studies so it is important to understand release characteristics of DEET and Picaridin from MCs at various temperatures. Thermal characteristics of the wall materials, mosquito repellents, repellent MCs, and free MCs were analyzed using thermogravimetric analysis (TGA, TG8120, Rigaku, Japan) with a heating rate of 10°C/min from 30 to 650°C in a nitrogen atmosphere[32]. The MCs were kept in a desiccator for 3 days to remove adsorbed moisture on the MC surface prior to analysis.

2.4. Study of release characteristics of mosquito repellents from MCs

To investigate release amount and rate of DEET and Picaridin for the further application in textiles worn on the human body at temperature of 37°C, we studies the release characteristics of DEET and Picaridin from MCs under isothermal conditions (36.5±0.5°C) using a thermogravimetric analyzer (TGA) (TG8120, Rigaku, Japan). The weight loss (%) of MCs was determined at 1 min intervals for 180 min with a 40 mL/min of nitrogen gas flow. The DEET-MC and Picaridin-MC samples were kept in a desiccator for 3 days to remove adsorbed moisture on the MC surface prior to analysis.

2.5. Mosquito repellent testing

The mosquito repellent testing was conducted in the Center of Insect Vector Study, Department of Parasitology, Faculty of Medicine, Chiang Mai University (CMU), Chiang Mai, Thailand. Mosquito repellents and MCs were tested for repellent protection time against *Ae. aegypti* using modified methods[33] which followed the standard "arm-in-cage" test by the World Health Organization[27].

2.5.1. Human volunteers

Four adult human volunteers of both sex (2 females and 2 males), 25-45 years old, who had no record of allergic reaction to arthropods were selected were recruited

Figure 3. Mosquito repellency testing with *Ae. aegypti* by "arm in cage" test according to the WHO standard method.

from graduated student population at Chiang Mai University. The volunteers were interviewed and advised on the objectives, methodology, and probable discomfort of this research before signing an informed consent form. The volunteers were also asked to refrain from drinking alcoholic beverages and avoid using scented products such as perfumes, colognes, deodorants, and lotions for the trial duration.

2.5.2. Mosquito repellent activity

Mosquito repellent activity was performed against *Ae. aegypti* according to the "arm-in-cage" method as shown in Figure 3, the repellent testing was conducted between 06.00 to 18.00 h at 25-30°C, and 60-68% RH. A total of 250 blood-starved female mosquitoes, 5-7 days-old, were selected randomly, placed in a standard mosquito cage (30×30×30 cm), and left to acclimatize for 1 hr. Before the test, each volunteer's arms were rinsed with distilled water, air-dried; rubber gloves protected their hands. At the beginning of the test, the mosquito's biting activity was checked by placing bare hands (no gloves) into the mosquito cage tested for up to 30 seconds. When ten or more mosquitoes had come onto their hands, the forearms were immediately pulled from the cage. This indicated that the mosquitoes were ready for testing.

The Each volunteer wore rubber gloves that contained a 30 cm^2 (3 cm × 10 cm) hole in a test area of the forearm. Approximately 0.1 ml of 10% pure DEET, pure Picaridin, DEET-MC, Picaridin-MC, and Free-MCs in coconut oil was gently applied to the test area of one forearm of each volunteer, while the other forearm applied with only coconut oil served as control. After air drying for 1 min, the test and control arms were then placed in the mosquito cage for 3 min. To start the experiment, the control arm was placed inside the test cage. Complete protection time was recorded after exposing the test forearm for 3 min at 30 min intervals up until two bites occurred in a single period of exposure or one bite occurred in each of two successive intervals. Each test was replicated on each of the four volunteers on different days, and no volunteer tested with more than one sample per day. Therefore, 8 replicates for each sample were made with each volunteer being tested twice.

2.5.3. Statistical analysis

The median complete-protection time was used as a standard repellency of the tested samples against *Ae. aegypti.* The Kruskal-Wallis one-way ANOVA (IBM SPSS Statistics, version 22.0, Armonk, NY, USA*)* was used to compare the repellent activity between pure DEET and pure Picaridin with coconut oil, MC-DEET and MC-Picaridin, with Free-MCs in coconut oil. The acceptance level of statistical significance was $p<0.05$ in all instances.

3. RESULTS AND DISCUSSION

3.1. Preparation of the microcapsules

MCs of mosquito repellents were successfully prepared by complex coacervation using gum Arabic and chitosan as wall material. DEET and Picaridin form an emulsion with gum Arabic using a homogenizer (Figure 4 (a)). The resulting mixture is a colloidal dispersion or oil-in-water emulsion. The addition of chitosan solution generated coacervation complex of GA-Chi by interactions between oppositely charged, carboxylic groups ($-COO^-$) of gum Arabic and amino groups ($-NH_3^+$) of

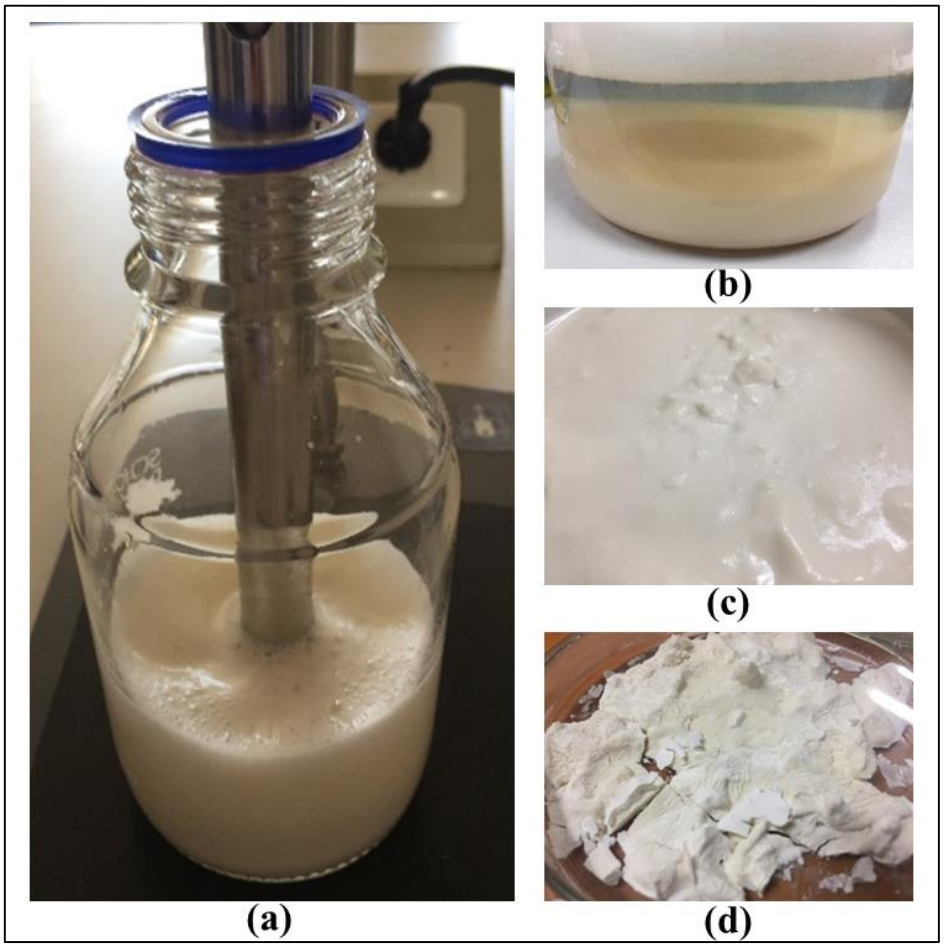

Figure 4. Microcapsule preparation using a homogenizer(a), sedimention of MCs after left for 24 h (b), microcapsules paste (c), and freeze-dried microcapsules powder (d).

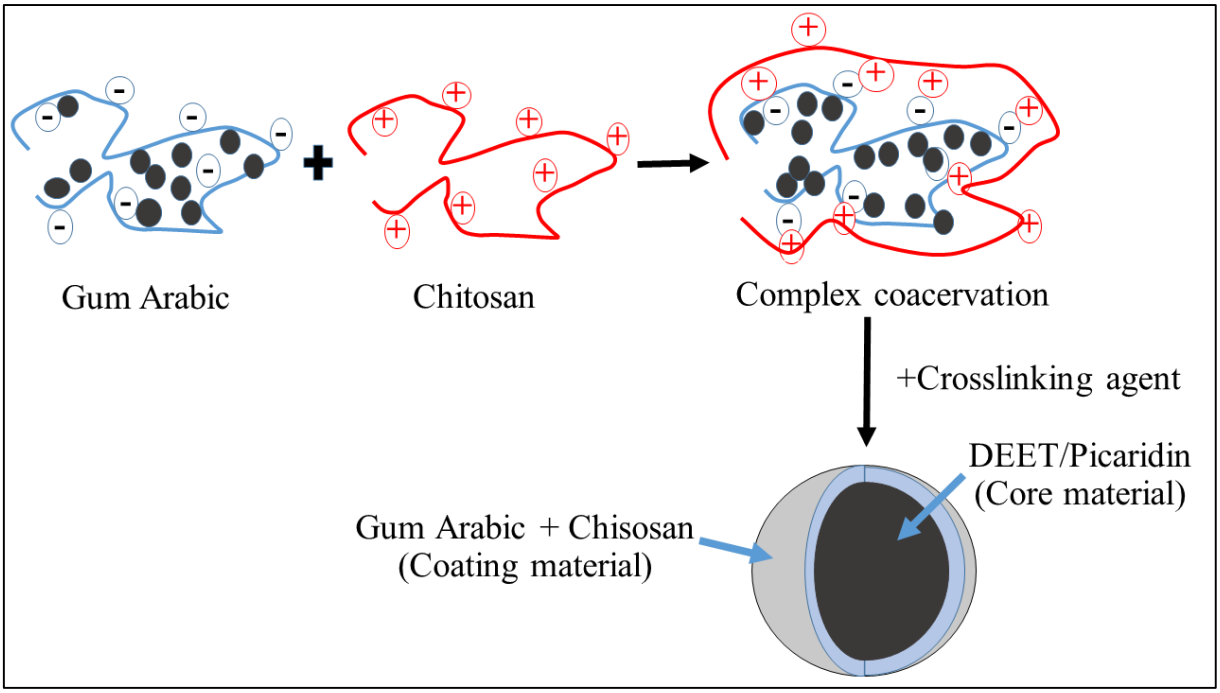

Figure 5. Encapsulation of DEET and Picaridin by complex coacervation of gum Arabic and chitosan.

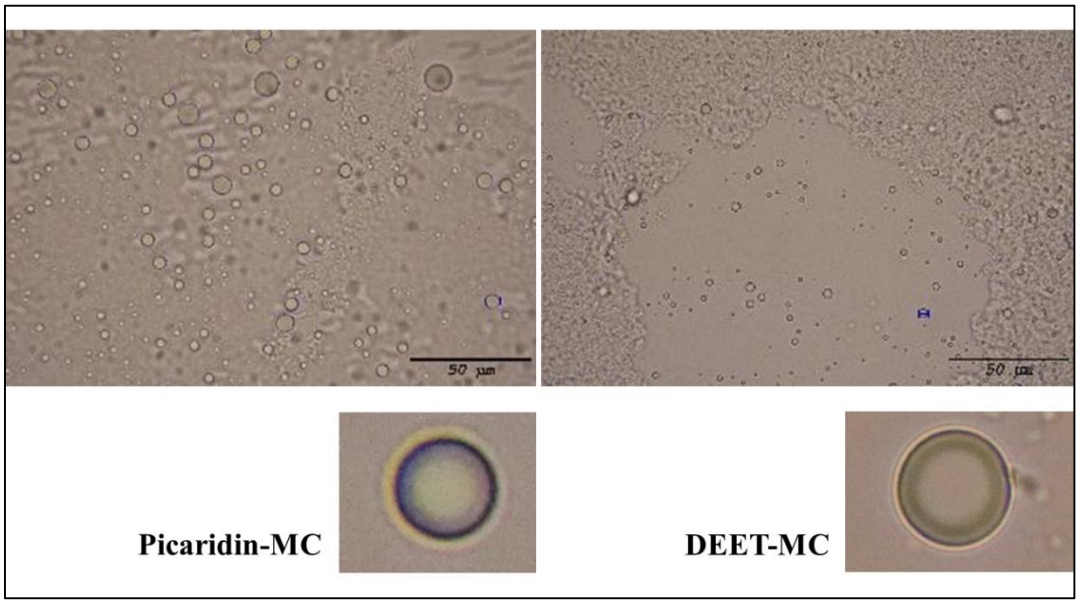

Figure 6. Optical microscope images of MCs containing DEET and Picaridin.

chitosan[24]. After that the GA-Chi complex deposited onto the mosquito repellents as microcapsule wall/coating materials (graphically illustrated in Figure 5), triggered by adjusting the pH to 4 which maximizes the coacervate yield, forming homogenous particles as reported elsewhere[28]. Finally, the crosslinking agent (NaTPP) solution was added to form the particles by ionic gelation in the hardening stage. The sedimentation of MCs occurred after leaving for 24 hrs (Figure 4 (b)). Thereafter, the microcapsule paste was extracted from the mixture by decantantion (Figure 4 (c)) and resulting in the formation of DEET or Picaridin MCs. For long-term storage, freeze-drying of DEET-MCs or Picaridin-MCs was carried out to produce microcapsules in powdered form (Figure 4 (d)).

3.2. Characterization of the microcapsules

The morphology of the mosquito-repellent-loaded MCs was investigated by optical microscopy. The optical micrographs of the MCs obtained in this study (Figure 6) show that the MCs were characterized by the same spherically shaped mononuclear structure as found by Goncalves et al.[26], who studied the encapsulation of thyme oil using chitosan/gum Arabic as wall materials. Furthermore, by our optical microscopy investigations, the diameters of the DEET-MC and Picaridin-MC complexes were determined to be from 1-4 µm and 1-12 µm with the mean diameters of 2.35±0.76 µm and 4.26±1.77 µm, respectively. The size of Picaridin-MCs was larger than DEET-MCs probably due to differences in physicochemical properties. The Picaridin molecule is bigger and bulkier and has a higher polarity. Therefore, it may have more interaction force between Picaridin and the polymeric walls than DEET during formation of microcapsules in the hardening stage after addition of NaTPP. In contrast, DEET has a higher hydrophobicity, less ionic interaction force with polymeric walls resulting in the relatively smaller size of microcapsules. DEET-MCs and Picaridin-MCs synthesized by complex coacervation in the present work are smaller to those found with MCs loaded with DEET prepared from simple coacervation of solely gum Arabic, which resulted in a wide variety of microcapsule sizes observed (ranging from 1-68 µm)[19].

The size distribution and mean particle size of the freeze-dried MCs were analyzed using the particle size analyzer, as shown in Figure 7. The particle sizes of 182 µm and 140 µm for DEET-MC and Picaridin-MC, respectively, were in agreement with the range for particles produced by complex coacervation as mentioned in other literature[16].

SEM micrographs of freeze-dried MCs (DEET-MC, Picaridin-MC) (Figure 8) show deformed microcapsules during water evaporation in the drying stage, giving agglomerated microcapsules with a highly porous structure. The SEM micrograph showed an amorphous film with sphere-like structures (MCs) inside. The nature of the external surface of the particles in the film may be related to particle-particle cohesion because of the physical interactions between the particles. In contrast, the irregular shapes of the particles in the sample without the cores added (no mosquito repellent added in the synthesis process) were examined by SEM. Without any binding of the polymeric complexes onto the core particles, none of sphere-like particles were observed[16].

FTIR spectra of gum Arabic, chitosan, and MCs without core substance (Figure 9) were analyzed in their solid-state to characterize their structures. Chi's FTIR spectrum (Figure 9 (a)) showed a strong absorption band in the region of 3,500-3,000 cm^{-1}, corresponding to the stretching of N-H and O-H bond; a typical band at 3,355 cm^{-1} [34] is a stretching band of hydroxyl groups (-OH).

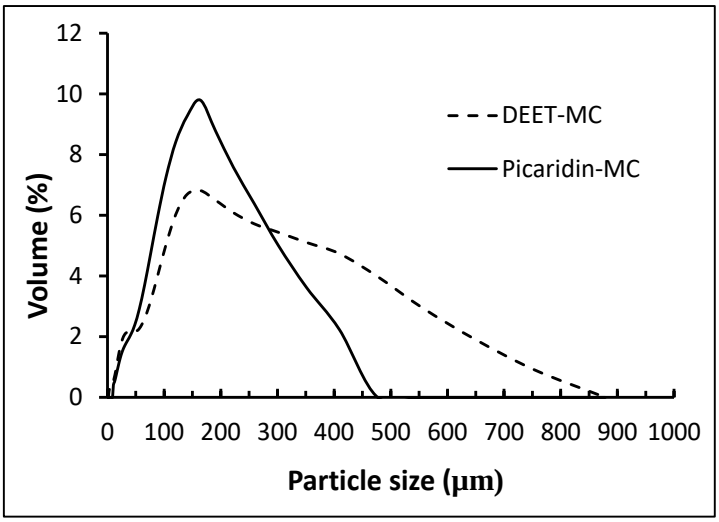

Figure 7. Size distribution of MCs containing DEET and Picaridin analysed by particle size analyzer.

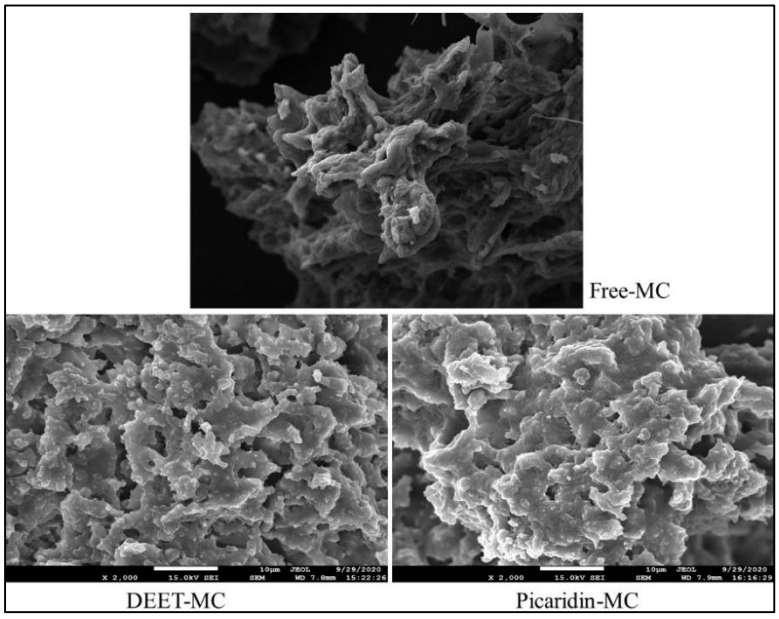

Figure 8. SEM micrographs of freeze-dried free-MC, microcapsules containing DEET and Picaridin (DEET-MC, Picaridin-MC).

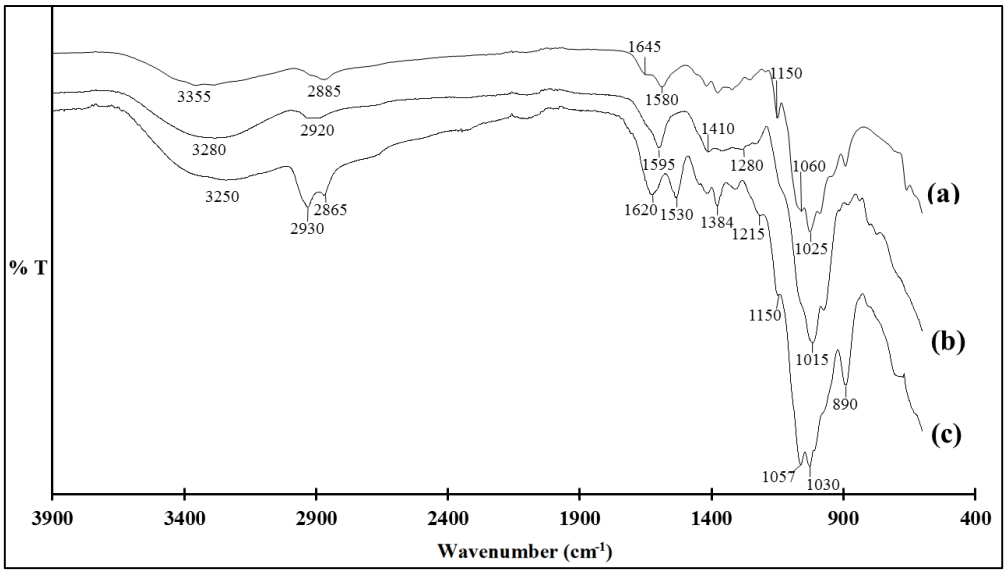

Figure 9. FTIR spectra of chitosan (a), gum Arabic (b), and MCs without core substance (C).

This band is broad due to inter and intramolecular hydrogen bonding. The absorption bands at approximately 2,885 cm^{-1} [34] can be attributed to the C-H stretching vibration of $>CH_2$. The band at 1,645 cm^{-1} [35] is attributed to the C=O vibration of the acetylated units ($-CONH_2$) groups. An absorption band at 1,580 cm^{-1} [36] corresponds to the N-H bending of the primary amine. The absorption band at 1,150 cm^{-1} [34] corresponds to the symmetric stretching of C-O-C; the absorption bands at 1,060 cm^{-1} [34] and 1,025 cm^{-1} are associated with the C-O stretching vibration.

GA (Figure 9 (b)) shows typical bands of the O-H bond stretching at 3,280 cm^{-1} [34]. The band at 2,920 cm^{-1} [35] is characteristic of the carboxylic group. The strong bands at 1,595 cm^{-1} and 1,410 cm^{-1} [34] are due to the asymmetric and symmetric stretching vibration of the carboxylic acid salt $-COO^-$ and the absorption bands at 1,015 cm^{-1} [34] are the stretching of the C-O bond.

The GA-Chi MC (Figure 9 (c)) showed a band at 3,250 cm^{-1} of $-NH_2$ and -OH groups that stretched vibration[35]. The FTIR of the GA-Chi coacervate changed significantly in the carbonyl-amide region. The $-NH_3^+$ groups shows the band at 1,530 cm^{-1})[36] and asymmetric and symmetric $-COO^-$ stretching vibration at 1,620 cm^{-1} [35] and 1,410 cm^{-1} [36]. The FTIR spectra show bands at 1,215 cm^{-1} [24] attributed to P-O stretching, 1,150 cm^{-1} [24] for the stretching of the PO_2 groups, 1,057 cm^{-1} [24] for the stretching vibration of the PO_3 groups, and 890 cm^{-1} [24] for P-O-P asymmetric stretching, respectively. This is attributed to the interaction between the anionic TPP and cationic polyelectrolyte (Chitosan) forming an intermolecular complex as the MC wall[24].

FTIR was used to confirm the presence of both repellents in the MCs, as shown in the FTIR spectra of free MCs, DEET-MC and Picaridin-MC (Figure 10 (A)). The FTIR spectrum of the free MCs (Figure 10 (a)) has a broad band at 3,000-3,500 cm^{-1} due to the O-H [35] and N-H stretching, at 2,850-3,000 cm^{-1} due to C-H stretching[35], and at the region near 1625 cm^{-1} due to C=O stretching[34]. Given the molecular structures of DEET and Picaridin (Figure 1), the FTIR spectra of both DEET and Picaridin show characteristic absorption bands of C-H stretched vibration at 2,940-2,870 cm^{-1} [35], C=O stretched at 1,870-1,540 cm^{-1} [34]. In addition, Picaridin also shows the O-H stretching band.

The MC spectrum loaded with DEET (Figure 10 (b)) showed characteristic bands at 2,931 and 2,871 cm^{-1} due to the symmetric and asymmetric stretch of CH_3 and the stretching of C=O at 1,635-1,530 cm^{-1} (6). On the other hand, the MC FTIR spectra of Picaridin (Figure 10 (c)) showed characteristic broad absorbance at 3,300 cm^{-1} [20] from the O-H group, as well as sharp peaks at 2,940 cm^{-1} [20] and 2,878 cm^{-1} corresponding to asymmetric and symmetric CH_2, respectively. The peak of C=O ester is confirmed at 1,655 cm^{-1} and 1,690 cm^{-1} [20] (Figure 10 (B)). It can be concluded that both repellents were encapsulated.

The Soxhlet extraction was utilized to determine the microencapsulation efficiency. The microencapsulation efficiencies of DEET and Picaridin MCs are 60% and 73%, respectively. The value of DEET is slightly lower than Thyme essential oil in MCs of GA-Chi which was 67% while that of Picaridin is found higher[26].

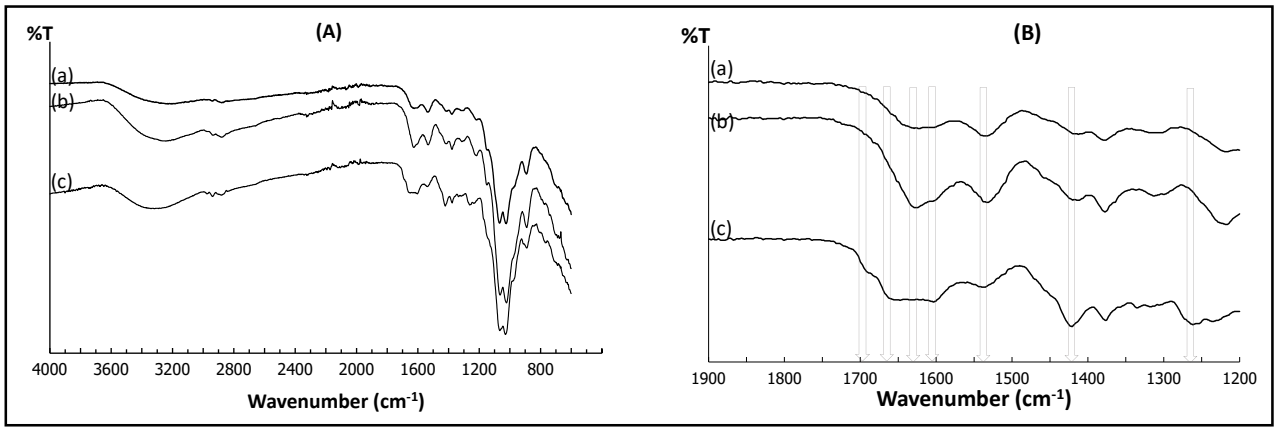

Figure 10. FTIR spectra of MCs without core substance (a), MCs containing DEET (b), and MCs containing Picaridin (c). (A) in the range of 400-4,000 cm^{-1}, (B) in the range of 1,200-1,900 cm^{-1}.

Figure 11 shows the thermograms of DEET, Picaridin, Chitosan, gum Arabic, MCs without core substance, and MCs containing mosquito repellents (DEET-MC, Picaridin-MC). The TGA analysis of DEET and Picaridin demonstrates a rapid loss of both repellents depending upon temperature. The weight loss begins at 100°C, DEET is almost entirely lost at 205°C, and Picaridin is completely lost below 225°C. These results differ from those of Fei and Xin[17] in which the pure DEET begins and completely loses weight at higher temperatures (130-260°C).

MCs without core substances show a weight loss between 25°C and 200°C, corresponding to the evaporation of the water adsorbed in the capsules. The weight loss between 260°C and 650°C corresponds to the decomposition of the wall.

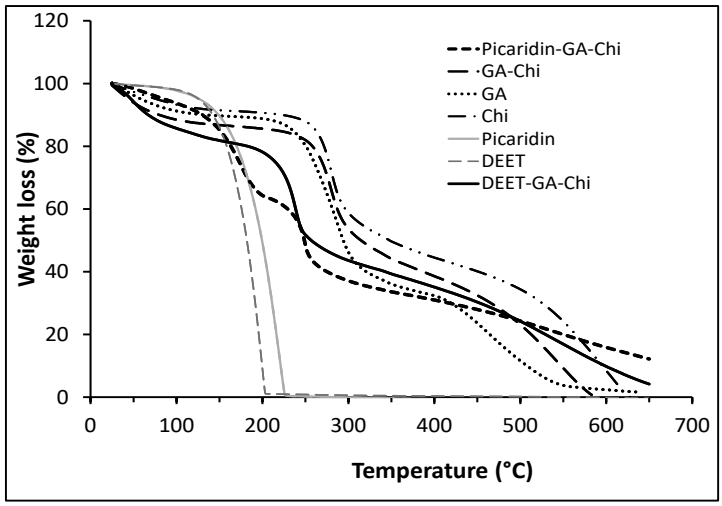

Figure 11. Thermogram of Picaridin, DEET, Chitosan (Chi), Gum Arabic (GA), MCs without Picaridin (GA-Chi), MCs containing Picaridin (Picaridin-GA-Chi) and MCs containing DEET (DEET-GA-Chi).

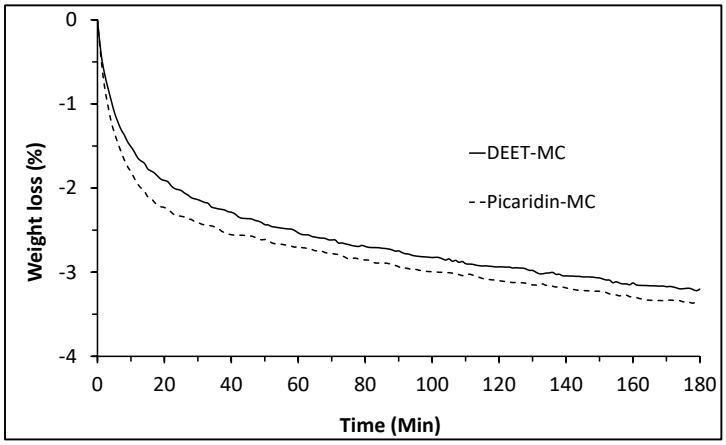

Figure 12. Weight loss of MCs containing DEET (DEET-MC) and Picaridin (Picaridin-MC) determined under isothermal condition at 36.5°C for 180 min.

Thermograms of only DEET and Picaridin show slow decomposition that is complete by 230°C (Figure 11). However, the wall materials (Chitosan, gum Arabic) and MC without repellents decompose at almost 300°C, compared to the repellent MCs (Picaridin-GA-Chi, DEET-GA-Chi), which slowly decompose. Thus, the repellent MCs show three steps of weight loss. For Picaridin; (1) the Picaridin-load MCs exhibit progressive weight loss until 200°C corresponds to the moisture and unencapsulated Picaridin; (2) weight loss after 250°C corresponds to the release of encapsulated Picaridin; (3) and after that weight loss in the range of 260-650°C corresponds to the thermal degradation of the wall materials. The DEET-MCs show a similar set of steps in the TGA curves. Likewise, the thermal behavior of DEET-copolymers prepared by miniemulsion polymerization is similar to that observed by others (3). In addition, faster weight loss in DEET-MC compared to Picaridin-MC is observed. The encapsulated repellents are slowly released when the temperature increases indicating that they exhibited higher thermal stability than the free repellents. Others have observed that repellents have dipolar interaction with polar groups of polymers in the walls of MCs, thus improving the repellents' thermal stabilities (3).

3.3. Release rate of mosquito repellents from MCs

The thermogram of DEET and Picaridin MCs under an isothermal program at 36.5°C for 180 min is shown in Figure 12, showing that the release rate of DEET and Picaridin from MCs are changing with time. A high release rate due to high weight loss of both DEET and Picaridin MCs is observed in the initial period of approximately at 10 min. After that, the rate of DEET and Picaridin release decreases over time, up to 180 min; however, MC-Picaridin showed faster release than MC-DEET until the end of the test.

These results suggest that DEET-MCs and Picaridin-MCs incorporated in fabrics would show considerable thermal stability. Further studies will be needed to evaluate the controlled-release behavior of those materials.

3.4. Mosquito repellent testing

Using the WHO "arm in cage" standard test[27], the protection time or mosquito repellency of repellents and MCs is shown in Table 1. There are many factors affect the efficiency of mosquito repellents, such as carbon dioxide released, sweat, etc. as noted in other studies[7]. With the control conditions described in experimental section, DEET shows longer protection time than Picaridin with the median values of 4.00 and 3.50 hrs, respectively and no protection time for free MCs. With the encapsulation, increased median repellency/protection times are observed at 3.50 hrs and 5.00 hrs for DEET-MCs and Picaridin-MCs, respectively.

These results were consistent with the release analysis by TGA with the isothermal condition and microencapsulation efficiency of the Picaridin-MCs, which was higher than that of the DEET-MCs. Furthermore, when considering the release of MCs by TGA in isothermal mode and the mosquito repellency, it was found that Picaridin MCs could release substances much faster than the DEET-MCs, which is consistent with the mosquito repellency tested from both MCs.

The results agree with those of other studies in which Picaridin and DEET were trapped in composites of clay-LLDPE (linear low-density polyethylene) and -EVA [poly (ethylene-co-vinyl acetate)] based strands. However, Picaridin release rate was less than DEET and Picaridin was more effective in mosquito repellency than the DEET[37].

Table 1. Mosquito repellency test of mosquito repellents and MCs with *Ae. aegypti*

Samples	Median Complete Protection time (Range, hrs)*
Coconut oil	0.00[a,]
Picaridin	3.50 (2.00-4.00)[b]
DEET	4.00 (2.00-5.00)[b]
Free-MC	0.00[A]
Picaridin-MC	5.00 (3.00-6.50)[B]
DEET-MC	3.50 (2.00-4.00)[B]

There were 8 replicates of each test.
*Values followed by different letters in a column were significantly different (Kruskal-Wallis one-way ANOVA, $p<0.05$)

4. CONCLUSION

This work successfully prepared repellent-containing MCs (DEET and Picaridin) using a complex coacervation method. An optical microscope examined the resulting MCs morphology and showed spherical mononuclear structures with an average diameter was 2.35±0.76 µm and 4.26±1.77 µm, respectively. A particle size analyzer examined the resulting MCs dried by the freeze-drying method. The mean particle size of the DEET-MC and Picaridin-MC were 182 µm and 140 µm, respectively. The SEM micrographs showed that the morphology of dried MCs was amorphous but sphere-like. FTIR analysis confirmed the bonding between the functional groups of the wall material and the repellents encapsulated within the MCs. The thermogravimetric analysis showed the decomposition temperature of MCs at 260°C. Repellents were encapsulated in MCs and this delayed the decomposition of the particles. The Soxhlet extraction method determined that the microencapsulation efficiency of DEET and Picaridin MCs were 60% and 73%. The thermograms of the repellent MCs under the isothermal program revealed that Picaridin-MC had higher release rate than DEET-MC. Thus, the retention efficiency within the MCs of Picaridin-MC may be higher than that of DEET-MC. Additionally, the protection time of the repellent MCs or mosquito repellency showed that the encapsulated Picaridin was longer than DEET. Therefore, the results obtained from this work suggest that chitosan/gum Arabic MCs improve the repellent's stability and release. DEET and Picaridin-encapsulated MCs are promising materials to be further applied in functional textiles for mosquito repellency in our ongoing in our investigations.

5. ACKNOWLEDGEMENT

The instruments for this work were supported by the Applied Chemistry Program of the Faculty of Science of Maejo University. Furthermore, the Department of Parasitology of the Faculty of Medicine of Chiang Mai University supported the mosquito repellency testing. Finally, we thank Prof. Richard L. Deming, and Dr. Peter L. Aldred for their helpful suggestions and guidance.

Conflict of interest

The authors declare that they have no conflict of interest**.**

Funding

This research was supported by The Northern Science Park in Thailand, funded through the Industrial Research and Technology Capacity Development Program, IRTC.

Ethics approval

Institutional Review Board Statement: The study was carried out according to the Declaration of Helsinki guidelines and was approved by the Research Ethics Committee of the Faculty of Medicine of the CMU (protocol code PAR-2558-03391/Research ID: 3391).

Article info:
Received March 31, 2022
Received in revised form June 11, 2022
Accepted July 26, 2022

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