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Original Article

Microencapsulation of citronella oil for mosquito repellent: Preparation and evaluation of release characteristics

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Abstract

The major aims of the current study were to prepare citronella oil microcapsules using the complex coacervation technique and evaluate the *in vitro* release properties of citronella oil from the prepared microcapsules. Gelatin and acacia, which are oppositely charged polymers, were chosen as the wall materials and the core substance was citronella oil produced by steam distillation of *Cymbopogan winterianus*. Formaldehyde was employed as a hardening agent. The core-to-wall ratio was fixed at 1:2, whereas the gelatin-to-acacia ratio, concentration of citronella oil, amount of hardening agent, and hardening time were varied. Among 18 formulations studied, three formulations produced satisfactory appearances of white microcapsules. Scanning electron microscopy revealed that the microcapsules were discrete and spherical in shape. Microcapsules with the smoothest surfaces were achieved in formulation F13 containing a 1:1 gelatin: acacia ratio. The percentages of oil content in formulations F13, F14, and F15 were 25.41±1.42%, 24.94±1.31%, and 19.89±1.31%, respectively. Simple diffusion cells with a synthetic membrane were used to study the *in vitro* release of citronella oil from the microcapsules. The release kinetics of citronella oil was also examined. The release data of citronella oil followed the Higuchi model with a high coefficient of determination values (r^2>0.97). Formulation F13 had the greatest release rate of citronella oil (P<0.05), while the release rate of formulation F14 was comparable to that of formulation F15. In conclusion, the potential microcapsule formulation for mosquito repellency was formulation F14, owing to its relatively high percentage of oil content and relatively slow release rate. The rapid volatility of citronella oil would be potentially reduced with the slow release properties.

Keywords: citronella oil, coacervation, microcapsule, mosquito

1. Introduction

Up until now, both synthetic and natural mosquito repellents have been used to prevent severe transmission diseases caused by mosquito bites. In general, natural sources are considered safer than the synthetic ones (Katz *et al*., 2008). Volatile oils, derived from aromatic plants, are one of the natural origins. From the *Cymbopogon* sp., such as *Cymbopogon nardus* (Linn.) and *Cymbopogon winterianus*, citronella oil possesses the ability to repel insects, in particular mosquitoes. However, rapid evaporation of citronella oil results in poor efficacy in mosquito bite prevention. According to the National Institute of Health of Thailand, mosquito repellent products sold in the country must possess a protection time against mosquitoes of ≥2 h. In our previous studies, complexation with β-cyclodextrin and fixatives were employed in an attempt to prolong the protection time of

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citronella oil lotions (oil-in-water). The citronella oil was derived from steam distillation of *Cymbopogon winterianus* (Songkro *et al*., 2012a, b). The main components of the citronella oil were citronellal (30.59%), citronellol (19.30%), and geraniol (18.17%) (Songkro *et al*., 2012a). In the current study, microcapsules were introduced as a delivery vehicle to reduce the volatility and control the release characteristics of citronella oil. Microencapsulation is a process of enclosing small particles, solid or liquid, in polymer membranes. Microcapsules consist of two parts, namely the core and wall or shell. A variety of wall materials have been used including polysaccharides (e.g., acacia, pectin, carrageenan, and alginate) and proteins extracted from vegetables (e.g., soy and pea) or animals (e.g., gelatin and albumin) (Baken, 1994; Xiao *et al*., 2014). The sizes of the microcapsules can vary from 1 to 5,000 μm (AppaRao *et al*., 2010). For the production of microcapsules, several techniques have been developed to encapsulate particles of solid or liquid within a continuous film of a polymer membrane. These include coacervation or phase separation, pan coating, solvent evaporation, spray drying, and congealing (Baken, 1994). The coacervation process is used extensively (Maji *et al*., 2007; Xiao *et al*., 2014). There are two types of coacervation, namely simple coacervation (one polymer) and complex coacervation (two oppositely charged polymers). Previously, Solomon and co-workers (2012) used the simple coacervation technique to prepare microcapsules of citronella oil (*Cymbopogon nardus*). Gelatin was used as the wall material. In the current work, the gelatin-acacia coacervation process was selected since it can encapsulate either a solid core (e.g., ketoprofen) or a liquid core (e.g., vitamin A palmitate) (Huang *et al*., 2007; Junyaprasert *et al*., 2001; Marquest *et al*., 2013; Nixon & Wang, 1989; Palmieri *et al*., 1996). Furthermore, the gelatin-acacia coacervation system was investigated using several essential oils and flavoring agents (Xiao *et al*., 2014). A stronger wall is generally obtained from the complex coacervation method compared with simple coacervation. Both gelatin and acacia (gum) are nontoxic naturally occurring polymers. They are biodegradable and inexpensive.

Gelatin is a solid substance produced from partial hydrolysis of collagen. The sources of collagen are the by-products of animals that include skin, connective tissue, organs, and bone tissue. Gelatin is a mixture of polypeptide chains with different chain lengths. When gelatin dissolves in hot water, a viscous liquid is formed. On cooling, the liquid sets to jelly. Gelatin does not dissolve in cold water but it can swell by absorbing approximately 5-10 times its weight in water. It is insoluble in alcohol, chloroform, ether, fixed oils, and volatile oils. The charge of gelatin depends on the pH. Two commercial products of gelatin are available: type A (acid treatment) and type B (alkali treatment). Gelatin type A has a positive charge at pH 4-4.5 and gelatin type B exhibits a negative charge at pH 8 (Allen *et al*., 2005; Xiao *et al*., 2014).

In the case of acacia gum (gum Arabic), it is prepared from exudates of *Acacia senegal* (Linn.) and other African acacia species belonging to the Fabaceae family. Acacia gum is tasteless, odorless, and nontoxic. Acacia gum has been used since ancient times. The gum is a polysaccharide with a complex structure. Acacia gum is composed mainly of a mixture of arabic acid ($C_{12}H_{22}O_{11}$) and salts of magnesium, calcium, and potassium. The gum also contains other substances like moisture and sugar. Generally, the molecular weight of the gum is in the range of 240,000-580,000 daltons. Acacia has a negative charge. It is soluble in hot water and cold water and it forms a slightly acidic mucilaginous solution. Acacia mucilage has a low viscosity. The gum is insoluble in oils, most organic solvents, and alcohol. Nevertheless, the gum is soluble in aqueous ethanol (up to 60%) (Allen *et al*., 2005; Panda, 2002; Rowe *et al*., 2012).

The aims of the current study were to prepare microcapsules of citronella oil using the gelatin-acacia coacervation technique. Scanning electron microscopy (SEM) was used to study the morphology of the prepared microcapsules. Simple diffusion cells (double-jacket beakers) and synthetic membranes were used to investigate the *in vitro* release of the citronella oil from the microcapsules.

2. Experimental

2.1 Materials

Citronella oil (Java type) was purchased from Thai-China Flavours and Fragrances Industry Co., Ltd (Ayutthaya, Thailand). The oil was obtained by steam distillation of *Cymbopogan winterianus*. Acacia (pharmaceutical grade) was available from P.C. Drug Center Co., Ltd. (Bangkok, Thailand). Gelatin type B was supplied by Sigma-Aldrich Co., Ltd. (USA). Absolute ethanol, sodium hydroxide, and propan-2-ol (isopropyl alcohol) were purchased from RCI Labscan Ltd. (Samut Sakhon, Thailand). Virgin coconut oil, prepared from centrifugation, was supplied by Tropical Nutrition Co. Ltd. (Prachuabkirikhan, Thailand). Formaldehyde was obtained from VWR International Ltd. (England). Acetic acid was purchased from Merck KGaA (Germany). Cellulose acetate membrane (Spectra/Por 3 dialysis tubing, MWCO 3500) was supplied by Spectrum Laboratories Inc. (USA & Canada). Distilled water was used as a solvent for the preparation of acacia solution (10% w/w). Acetic acid solution (10% v/v) and sodium hydroxide solution (20% w/v) were used as described in section 2.2. All chemicals were analytical grade except where specified.

2. 2 Microcapsule preparation

Citronella oil microcapsules were produced by the gelatin-acacia coacervation technique according to the method published by Junyaprasert *et al*. (2001). The method is considered suitable for an oily liquid core. A combination of gelatin and acacia gum was used as the wall material while the volatile oil was the core material. In the present work, the core-to-wall ratio was fixed at 1:2 based on a study done by Junyaprasert and co-workers (2001) where liquid vitamin A palmitate was employed as the core material. In our case, 25-60% w/w citronella oil in virgin coconut oil or 100% w/w citronella oil was used. Virgin coconut oil was chosen in the present work since it is able to dissolve substantial amounts of citronella oil. Furthermore, it becomes a solid when the temperature is below its melting point. This unique property may assist in hardening of the microcapsule shells, increasing stability, and diminishing the release of citronella oil from microcapsules. The standard procedure of gelatin-acacia coacervation process is shown in Figure 1. Four parameters were varied in the current study: i) amount of citronella oil

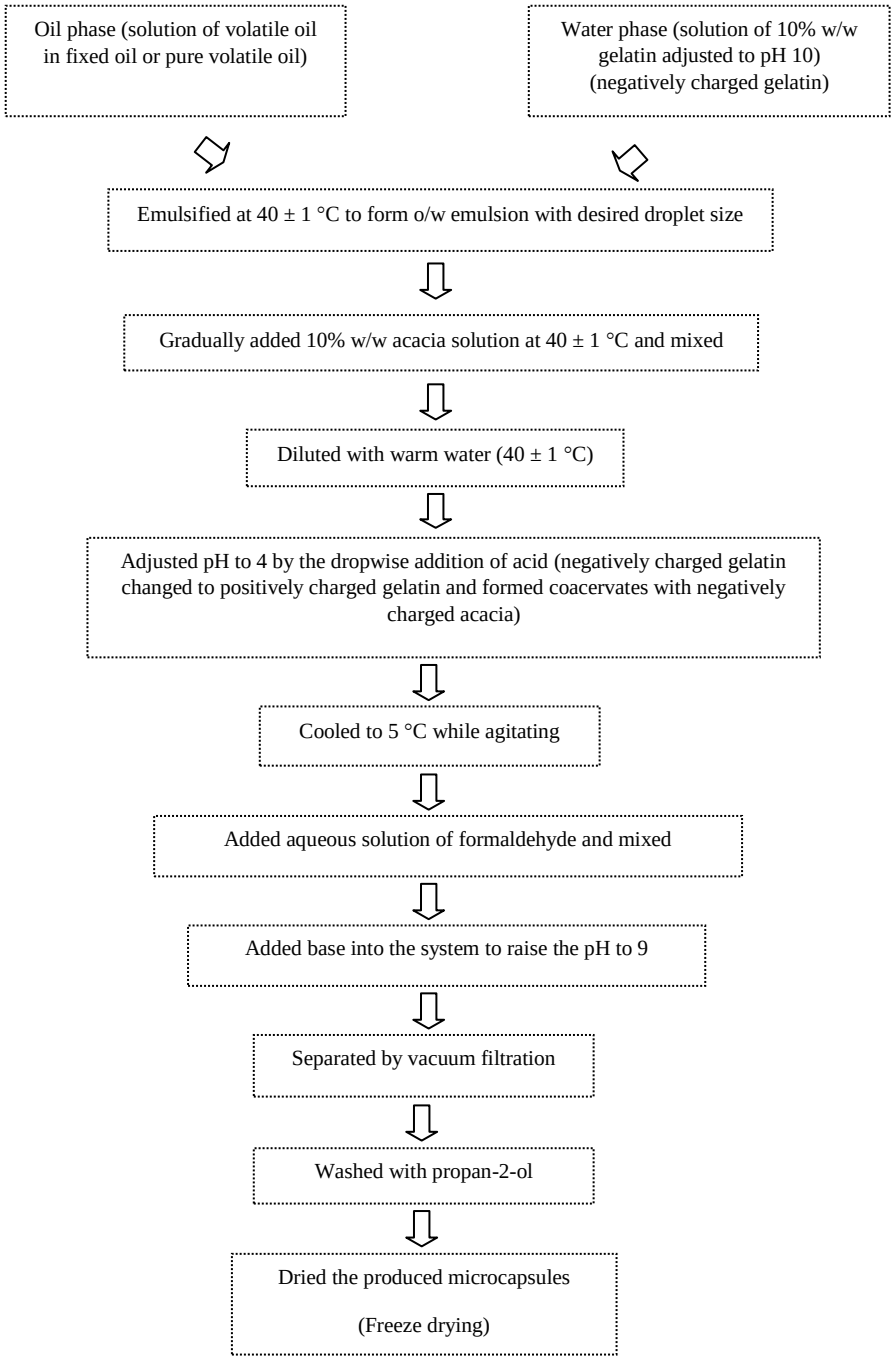

Figure 1. Schematic diagram of complex coacervation process used to produce citronella oil microcapsules.

used, ii) ratio between gelatin and acacia, iii) amount of hardening agent (formaldehyde), and iv) hardening time. The compositions of each formulation of citronella oil microcapsules are summarized in Table 1. The citronella oil or solution of the citronella oil in virgin coconut oil was emulsified in gelatin solution (10% w/w) at 40±1 °C. The gelatin solution was previously adjusted to pH 10 to produce a negative charge. With the aid of a magnetic stirrer (Framo-Geratetechnik, Germany), the obtained emulsion was stirred at 200 rpm for 30 min. Then, the acacia solution (10% w/w) was gradually added into the emulsion under gentle stirring for 30 min. A dilution of the colloidal concentration (<3%) was achieved by adding warm water at 40±1 °C (100 g of water per 30 g of colloidal solution). To achieve coacervation, the pH of the mixture was adjusted to 4 by dropwise addition of an acetic acid solution (10% v/v). The temperature of the mixture was then reduced to 5 °C under continuous agitation. Formaldehyde was slowly added to harden the shell. The amounts of formaldehyde used were from 2 ml per 280 g of colloidal solution to 25 ml per 280 g of colloidal solution. The

Table 1. Formulations of citronella oil microcapsules.

Formulation code	Core: wall ratio	Gelatin: acacia ratio	Formaldehyde (ml/280 g of colloidal sol.)	Concentration of citronella oil (%)	Hardening time (min)	Problems found	Remarks
F1	1:2	1:1	2	100	30	Separated by vacuum filtration was not possible.	Separated by centrifugation before Freeze-drying
F2	1:2	1:1	10	100	30	Hardening time of 30 min was not enough.	
F3	1:2	1:1	15	100	30		
F4	1:2	1:1	20	100	30		
F5	1:2	1:1	10	100	60	Citronella oil leaked from microcapsules after freeze drying.	Concentration of citronella oil was too high.
F6	1:2	1:1	15	100	60		
F7	1:2	1:1	15	50	60	Aggregation of very dry and hard microcapsules after freeze drying.	Sieving was used to separate aggregated microcapsules
F8	1:2	1:1	25	50	60	Filtration was not possible. Microcapsules agglomerated similar to coconut jelly.	Amount of formaldehyde was too high.
F9	1:2	3:2	10	60	60	Microcapsules stuck together forming a thick sheet after filtration and freeze drying.	High ratio of gelatin to acacia and high amount of coconut oil.
F10	1:2	3:2	10	40	60	Filtration was difficult because of solidification of coconut oil.	Amount of coconut oil was too high.
F11	1:2	3:2	15	60	60	Aggregation of microcapsules	High ratio of gelatin to acacia and a large amount of coconut oil.
F12	1:2	3:2	10	100	60	Citronella oil leaked from microcapsules after freeze drying.	Concentration of citronella oil was too high.
F13	1:2	1:1	10	50	60	No	Rather dry and hard shell microcapsules were obtained.
F14	1:2	2:3	10	50	60		
F15	1:2	2:3	10	40	60		
F16	1:2	1:1	10	25	60	Filtration was difficult. Microcapsules were similar to shredded coconut.	Concentration of coconut oil was extremely high.
F17	1:2	2:3	10	25	60	Filtration was not possible.	
F18	1:2	3:2	10	25	60	Filtration was difficult. After freeze drying, citronella oil leaked slightly from microcapsules.	

hardening times were either 30 or 60 min. The pH of the solution was brought up to 9 by adding sodium hydroxide solution (20% w/v). The microcapsules were separated by vacuum filtration, washed with propan-2-ol to remove excess volatile oil, and dried by a freeze dryer (Kinetics Flexi-Dry, Kinetic Thermal System, USA). The freeze drying technique is suitable for volatile components. A dry white powder was kept in a glass bottle and a cool place until use.

2. 3 Morphology of microcapsules

Morphology of the microcapsules was examined using a scanning electron microscope (Quanta 400, FEI Companny, Czech Republic) equipped with a Everhart-Thornley detector. The specimens were subjected to gold sputter coating (SPI Supplies, USA) and evaluated under a high vacuum condition ($<1.3x10^{-2}$ Pa) at 20.00 kV.

2. 4 Determination of encapsulated oil (oil content)

An accurately known weight of microcapsules (0.5 g) was placed into a mortar and crushed with 3 ml of absolute ethanol (n=3). The obtained suspension was filtered through filter paper (Whatman No. 1) to separate the shell fragments from the filtrate. The filtrate flowed into a 10-ml volumetric flask. The remaining shell fragments were then crushed with absolute ethanol (3 ml) and the filtrate was collected in the same volumetric flask. The same procedures (crushing with absolute ethanol and filtration) were performed again to collect the final filtrate. The filtrate was added up to volume with absolute ethanol. The amount of citronella oil was measured at 229 nm by UV visible spectrophotometer (Spectronic Genesys 5, Thermo Fisher Scientific, USA). An appropriate dilution was required when the absorbance value was greater than 1. The calibration curve of citronella oil was

prepared. A stock solution was made by weighing an accurate weight of citronella oil (0.0403 g), transferring it into a 50-ml volumetric flask, dissolving with absolute ethanol, and adding up to volume with the same solvent. Standard solutions were then prepared from the stock solution by diluting with absolute ethanol. The absorbance of each standard solution was measured at 229 nm (Songkro *et al*., 2012a). Absolute ethanol was used as a blank. The calibration curve was constructed by plotting five standard concentrations (81, 162, 243, 324, and 405 µg/ml) against the recorded absorbance. The percentage of the citronella oil content was calculated using the following equation:

$$\% \textbf{ Content } = \frac{amount of\ active \times \mathbf{100}}{amount\ of\ microcapsules} \quad (1)$$

2.5 *In vitro* release study of citronella oil microcapsules

The release of citronella oil from microcapsules was performed using a synthetic membrane (Spectra/Por 3) and simple diffusion cells. The diffusion apparatus was composed of a receptor phase (i.e. a double-jacket beaker: internal diameter, 4.5 cm; height, 6.0 cm) and a donor phase (i.e. a cylindrical glass donor cell: internal diameter, 1.7 cm; height, 1.0 cm; diffusion area, 2.27 cm^2). The membrane separating the donor phase from the receptor phase was prepared by boiling a suitable size of dialysis tubing sheet in distilled water twice, cleaning with distilled water, immersing in distilled water, and storing in a cool place (refrigerator). A mixture of water and absolute ethanol (1:1 v/v) was degassed and used as the receptor medium (80 ml) (Songkro *et al*., 2012a). The temperature of the receptor fluid (32 °C) was controlled by a thermostat circulating water bath. To achieve a homogenous system, a magnetic bar was used to stir the receptor fluid. Citronella oil microcapsules (formulations F13, F14, and F15) were accurately weighed (1 g) into glass donors. The donors were covered with the hydrated membrane pieces, wrapped around with rubber bands and carefully immersed in the receptor fluid. At the appropriate time intervals (0.5, 1, 2, 3, 4, 6, 8, 10, 24, 36, and 48 h), 5-ml aliquots were taken from the receptor compartment and replaced immediately in the degassed receptor fluid. The withdrawn sample was assayed for citronella oil content at 229 nm using a UV visible spectrophotometer. Blank microcapsules were run at the same time to check for any interference. The concentrations of citronella oil were calculated from the calibration curve. The cumulative amount of citronella oil (Q_t) released from the microcapsules in time (t) was calculated from Equation 2:

$$Q_t = V_r C_t + \sum_{i=0}^{t-1} V_s C_i \quad (2)$$

where C_t is the citronella oil concentration of the receptor fluid at each sampling time, C_i is the citronella oil concentration of the i^{th} sample, and V_r and V_s are the volumes of the receptor fluid and the sampling solution, respectively. The percentages of the cumulative release of each formulation were plotted against time. The release profiles were tested for the best fit to three kinetic models: zero order ($Q_t = Q_0 + K_0 t$), first order ($lnQ_t = lnQ_0 + K_1\ t$) and Higuchi's model ($Q_t = K_H t^{1/2}$) (Varshosaz *et al*., 2006), where Q_0 is the initial amount of citronella oil in the receptor fluid and K_0, K_1, and K_H are the rate constants of zero order, first order, and the Higuchi model, respectively. The linear regression analysis was used to analyze the data.

2.6 Statistical analysis

The statistical analysis of citronella oil content and release data were determined by means of one-way analysis of variance (ANOVA). The SPSS statistics software, version 22, was used to analyze the data. A significant difference was considered when a P-value was less than 0.05.

3. Results and Discussion

3.1 Preparation of citronella oil microcapsules

The characteristics of the 18 formulations of citronella oil microcapsules prepared are given here in detail and summarized in Table 1.

3.1.1 Formulations F1 through F4

All parameters for formulations F1 through F4 were the same except the amounts of formaldehyde which varied from 2 to 20 ml per 280 g of colloidal solution. It was found that the microcapsules of F1 through F4 did not separate from the solutions by vacuum filtration. Therefore, centrifugation was used to separate the abundant microcapsules at the top of the solutions. After freeze drying, the microcapsules of F1 through F4 were applied on a smooth surface to check the strength of the shells. It was found that 2 ml of formaldehyde gave the microcapsules very thin shells which broke easily after the smooth surface application, whereas 20 ml of formaldehyde provided microcapsules with shells that were too tough. The most suitable amount of formaldehyde was 10 ml (F2) followed by 15 ml per 280 g of colloidal solution (F3). In conclusion, a hardening time of 30 min was not enough to produce hard microcapsules which resulted in difficult filtration. Thus, the hardening time was increased to 60 min for F5 and F6.

3.1.2 Formulations F5 and F6

A hardening time of 60 min was found most favorable for the encapsulation process when we compared F5 to F2 and F6 to F3. The separation of microcapsules of F5 and F6 was achieved by vacuum filtration. However, there was leakage of citronella oil from the microcapsules of both formulations after freeze drying which resulted in an aggregation of the microcapsules. This was probably due to a substantial amount of citronella oil or the shells of the microcapsules were too thin.

3.1.3 Formulations F7 and F8

Since it was speculated that the leakage of citronella oil from microcapsules was, in part, caused by a high amount of citronella oil, a certain fixed oil was introduced in order to reduce the amount of citronella oil. As previously mentioned, virgin coconut oil was selected because of its several benefits. Virgin coconut oil at 50% was mixed with citronella oil at

50% and the amount of formaldehyde for formulations F7 and F8 were 15 ml and 25 ml per 280 g of colloidal solution, respectively. After vacuum filtration, small microcapsules of formulation F7 were obtained. In addition, the dry microcapsules of F7 had a glittery appearance similar to sugar crystals. After freeze drying, an aggregation of very dry and hard microcapsules was observed. Sieving was used to produce individual microcapsules. Vacuum filtration could not separate the microcapsules of F8. Therefore, centrifugation was applied in an attempt to separate the microcapsules from the solutions. However, the microcapsules of F8 agglomerated in a similar way to coconut jelly. This was possible because of the very high amount of formaldehyde (25 ml). The viscosity of the vehicle at the high amount of formaldehyde increased since the excess formaldehyde affected the cross linking between the gelatin in the equilibrium colloid-poor layer (Deasy, 1984).

3.1.4 Formulations F9 through F12

Apart from the extensive amount of citronella oil used, leakage of citronella oil from the microcapsules may be, in part, due to the very thin shells of the microcapsules. Therefore, we changed the gelatin: acacia ratio from 1:1 to 3:2. To check the effect of citronella oil concentration, three concentrations of citronella oil were tested: 60% (F9 and F11), 40% (F10), and 100% (F12). All formulations except F11 contained formaldehyde at 10 ml per 280 g of colloidal solution, whereas F11 had 15 ml of formaldehyde. For F9 and F11 which contained the same amounts of citronella oil (60%) and coconut oil (40%), thick sheets of microcapsules formed after vacuum filtration and freeze drying. Microcapsules of F11 possessed harder shells than the microcapsules of F9 because of the higher amount of formaldehyde which is the hardening agent. For F10, which contained a high amount of coconut oil (60%), solid white flakes were obtained after freeze drying. Formulation F12 (100% citronella oil, no coconut oil) produced microcapsules with wet surfaces, probably due to leaking citronella oil. Notably, hard sheets of microcapsules were produced from F9, F10, and F11. This was due to the high amounts of virgin coconut oil and the high ratio of wall materials (3:2 gelatin: acacia ratio).

3.1.5 Formulations F13 through F15

Formulations F13, F14, and F15 contained the same 1:2 oil: wall ratio and the same amount of formaldehyde (10 ml per 280 g of colloidal solution). The differences were the gelatin: acacia ratio and the concentration of citronella oil. F13 had a 1:1 gelatin: acacia ratio, 50% citronella oil, and 50% virgin coconut oil. After vacuum filtration, beautiful spherical, white microcapsules of F13 were obtained. Although they were slightly wet, the appearance of the F13 microcapsules was better than the previous products. Among the three formulations studied, F13 produced the highest quantity of microcapsules. After freeze drying, the microcapsules displayed slight leakage of citronella oil. Additionally, the microcapsules became yellow on exposure to air. F14 contained the same ingredients as F13 except the ratio of wall materials was a gelatin: acacia ratio of 2:3. The wall material was known to influence the release of the active compound. After filtration, the obtained microcapsules were slightly wet but much drier after freeze drying. F15 contained the same ingredients as F14 except for a lower concentration of citronella oil (40%) and a higher concentration of coconut oil (60%). Vacuum filtration of the microcapsules was quite difficult and took time possibly due to solidification of the coconut oil. However, dry microcapsules were obtained after freeze drying.

3.1.6 Formulations F16 through F18 and F10

Formulations F16, F17, F18, and F10 were composed of the same oil: wall ratio (1:2) and the same amount of formaldehyde (10 mL per 280 g of colloidal solution). F16 was modified from F13 by decreasing the concentration of citronella oil while increasing the concentration of coconut oil. Thus, F16 contained 25% citronella oil and 75% coconut oil. Formulation F16 had a gelatin: acacia ratio of 1:1. Vacuum filtration of F16 was very difficult because of the exceedingly high amount of coconut oil. The microcapsules obtained from F16 were similar to shredded coconut. F17 also contained 25% citronella oil and 75% coconut oil but the ratio of wall materials was 2:3 (gelatin: acacia) for F17, whereas it was 1:1 (gelatin: acacia) for F16. Vacuum filtration of F17 microcapsules was not accomplished because of the very high amount of coconut oil and high ratio of wall materials. F18 had the same concentrations of citronella oil and coconut oil as F16 and F17 but the ratio of wall materials (gelatin: acacia) of F18 was 3:2. Even though vacuum filtration was difficult, beautiful microcapsules were obtained for F18 and the microcapsules were dry and glittery. After freeze drying, there was slight leakage of citronella oil from the microcapsules. F10 contained the same ingredients as F18 except for the higher amount of citronella oil (40% citronella oil). As previously mentioned, there was difficulty in the filtration process. Solid white flakes/hard sheets of microcapsules occurred after freeze drying. We did not obtain intact microcapsules after washing with propan-2-ol.

Among the 18 formulations studied, the optimum formulations were F13 (1:1 gelatin: acacia ratio and 50% citronella oil), F14 (2:3 gelatin: acacia ratio and 50% citronella oil), and F15 (2:3 gelatin: acacia ratio and 40% citronella oil). These formulations produced stable, rather dry microcapsules with hard shells and all microcapsules were white. Further studies were performed on these three formulations.

3.2 Morphology of citronella oil microcapsules

The surface morphologies of the microcapsules of formulations F13, F14, and F15 were further examined by SEM (Figure 2). It was observed that the microcapsules of all formulations were separable and spherical in shape. The microcapsules of F13, which contained a 1:1 ratio of gelatin: acacia, displayed smooth surfaces, whereas the microcapsules of F14 and F15, which were composed of a 2:3 ratio of gelatin: acacia, showed surfaces that were quite rough. It appeared that equal amounts of gelatin and acacia generated a smooth surface morphology. The sizes of microcapsules in each formulation appeared to vary. Overall, the ranking order of the sizes was F15>F13>F14. The particle sizes of the prepared microcapsules were smaller than 8 μm.

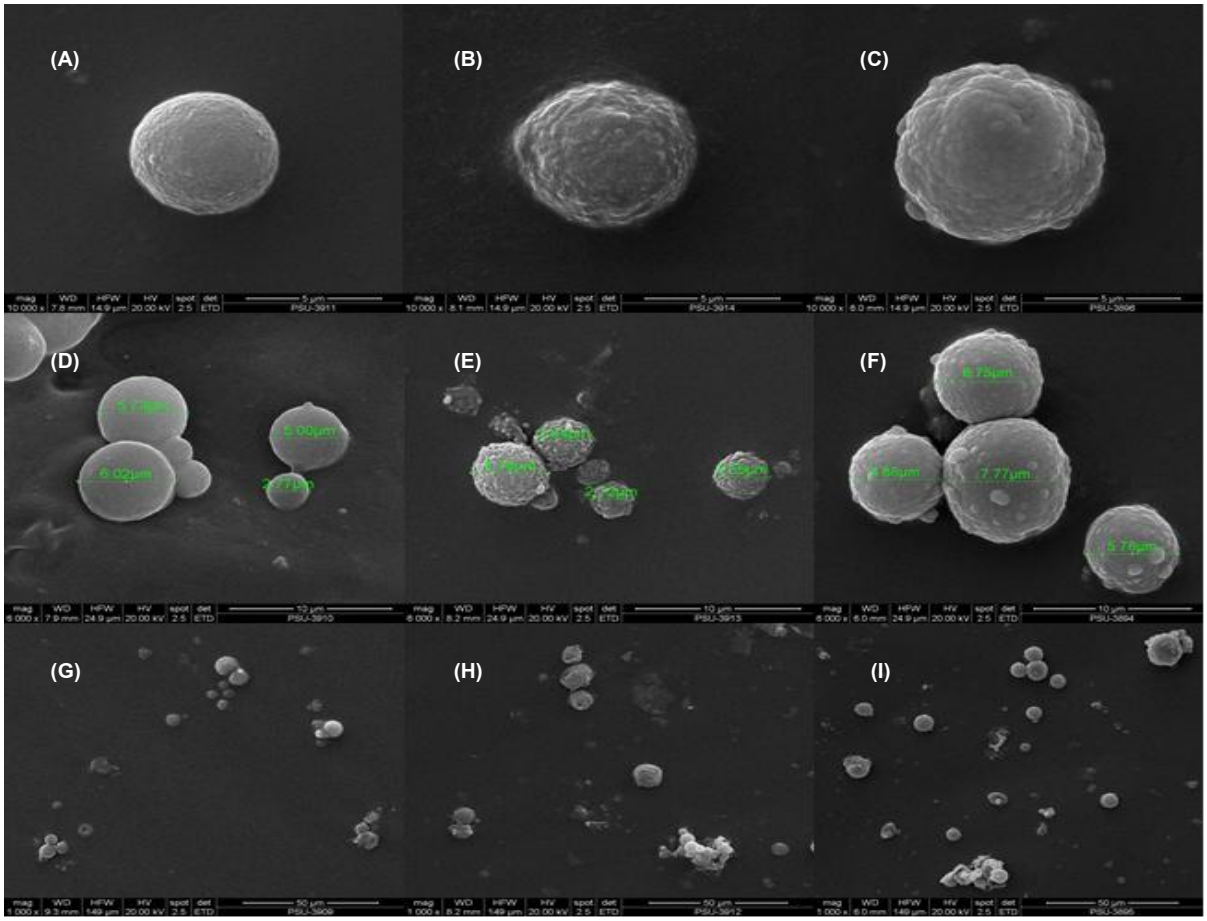

Figure 2. SEM photographs of citronella oil microcapsules: A, D and G showing formulation F13 (1: 1 gelatin acacia ratio, 50% citronella oil); B, E and H showing formulation F14 (2: 3 gelatin acacia ratio, 50% citronella oil); C, F and I showing formulation F15 (2: 3 gelatin acacia ratio, 40% citronella oil), magnification x 10000 (A, B and C); magnification x 6000 (D, E and F) and magnification x 1000 (G, H and I).

3.3 Citronella oil content

The mean±SD percentages of citronella oil content of microcapsules F13, F14, and F15 were 25.41±1.42%, 24.94±1.31%, and 19.89±2.04%, respectively. Overall, the oil content was quite low (<30%). The percentages of oil content of F13 and F14 were significantly higher than F15 ($P<0.05$, one-way ANOVA). The oil content of F13 did not differ statistically from F14.

3.4 *In vitro* release study of citronella oil microcapsules

The *in vitro* release profiles of citronella oil microcapsules of formulations F13, F14, and F15 are given in Figure 3. The amounts of citronella oil released from F13, which contained 1:1 gelatin: acacia ratio, appeared to be higher than F14 and F15, which had gelatin acacia ratios of 2:3. The release profiles of citronella oil obtained from 0.5 to 48 h were also fit into different order kinetic models: zero order, first order, and the Higuchi model. Data analysis was achieved by linear regression. The coefficient of determination (r^2) and the rate constants of each model are summarized in Table 2. For all tested microcapsules, the data fit the Higuchi equation best with r^2 from 0.9749-0.9932. This result indicated that the synthetic membrane that separated the donor

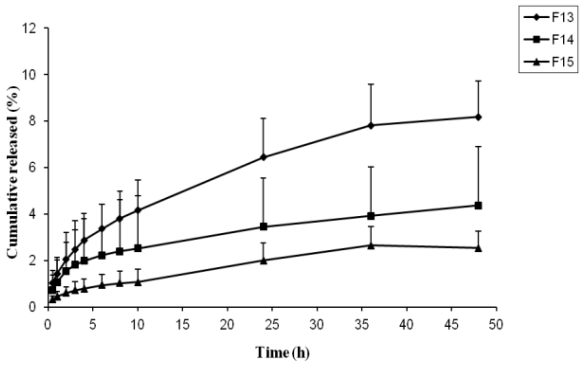

Figure 3. *In vitro* release profiles of citronella oil from microcapsules of formulation F13 (1: 1 gelatin acacia ratio, 50% citronella oil), formulation F14 (2: 3 gelatin acacia ratio, 50% citronella oil) and formulation F15 (2: 3 gelatin acacia ratio, 40% citronella oil). Each point represents mean ± SD, n = 3 where n = numbers of samples.

Table 2. Release kinetic parameters of citronella oil microcapsules.

Formulation code	Zero order		First order		Higuchi equation	
	r^2	K_0 (%/h)	r^2	K_1(1/h)	r^2	K_H (%/$h^{1/2}$)
F13	0.9238	0.1487±0.0299	0.7220	0.0370±0.0081	0.9932	1.1931±0.2529
F14	0.8816	0.0672±0.0206	0.7573	0.0333±0.0110	0.9763	0.5472±0.1926
F15	0.9369	0.0495±0.0124	0.8214	0.0395±0.0066	0.9749	0.3905±0.1053

K_0, K_1, K_H are rate constants of zero order, first order, and Higuchi model, respectively (mean±SD, n=3)

and receptor compartments did not affect the release of citronella oil from the microcapsules. The ingredients of microcapsules themselves controlled the release of the active oil (Guy & Hadgraft, 1990).

Therefore, only the rate constants or release rates of the Higuchi model were taken into account in the current work. The average release rates of F13, F14, and F15 were 1.1931 %/$h^{1/2}$, 0.5472 %/$h^{1/2}$, and 0.3904 %/$h^{1/2}$, respectively (Table 2). F13 showed a significantly faster release rate of citronella oil than F14 and F15 ($P<0.05$, one-way ANOVA). This may be due to the 1:1 gelatin: acacia ratio of wall materials of F13. There was no significant difference in the release rates of F14 and F15. Among the three formulations studied, the most suitable formulation for mosquito repellent was F14 since it provided a slow release rate of citronella oil and possessed a relatively high percentage of oil content.

4. Conclusions and Future Work

It can be concluded that the gelatin-acacia coacervation technique is a practical method to prepare citronella oil microcapsules for the prevention of mosquito bites. The microcapsules obtained from the experiment were white and spherical. Several parameters influenced the formation of the microcapsules. Citronella oil at 100% produced unstable microcapsules. A very high amount of virgin coconut oil made vacuum filtration very difficult. The gelatin: acacia ratio of the wall materials seemed to affect the smoothness of the microcapsule surfaces and release characteristics of citronella oil from the microcapsules. In the current study, the strength of the microcapsule shells needs improvement. In future work, we plan to strengthen the microcapsule shells by the following strategies: (1) employ other hardening agents such as glutaraldehyde, (2) increase the amount of hardening agent, and (3) increase the hardening time or use all three techniques together. Nevertheless, the microcapsules that were obtained were able to contain 26% citronella oil. In addition, the prepared microcapsules could somewhat control the release of citronella oil. Thus, it is possible to apply the citronella oil microcapsules as a mosquito repellent. By incorporating citronella oil microcapsules with different release rates into cream bases, we can develop mosquito repellent products which possess rapid and long repellent actions against mosquitoes. Again, an improvement of the shell strength as previously mentioned will help control the release of the active oil.

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