International Journal of Pharmaceutics 372 (2009) 105–111

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Characterization and mosquito repellent activity of citronella oil nanoemulsion

Usawadee Sakulku[a], Onanong Nuchuchua[a], Napaporn Uawongyart[b], Satit Puttipipatkhachorn[c], Apinan Soottitantawat[d], Uracha Ruktanonchai[a,*]

[a] National Nanotechnology Center, National Science and Technology Development Agency, 111 Thailand Science Park, Paholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand
[b] Traditional Thai Medicine Development Center, The Institute of Traditional Thai Medicine, Department for Development of Traditional and Alternative Medicine, Ministry of Public Health, Thailand Science Park, Pathumthani 12120, Thailand
[c] Department of Manufacturing Pharmacy, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand
[d] Center of Excellent in Particle Technology, Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University, Bangkok 10330, Thailand

ARTICLE INFO

Article history:
Received 11 November 2008
Received in revised form 17 December 2008
Accepted 23 December 2008
Available online 31 December 2008

Keywords:
Nanoemulsion
Citronella oil
Glycerol
Mosquito repellent
High pressure homogenization

ABSTRACT

Encapsulated citronella oil nanoemulsion prepared by high pressure homogenization at varying amounts of surfactant and glycerol, was studied in terms of the droplet size, stability, release characteristics and *in vivo* mosquito protection. Transparent nanoemulsion can be obtained at optimal concentration of 2.5% surfactant and 100% glycerol. Physical appearance and the stability of the emulsion were greatly improved through an addition of glycerol, owing to its co-solvent and highly viscous property. The increasing emulsion droplet increased the oil retention. The release behavior could be attributed to the effect of droplet size and concentrations of surfactant and glycerol. By fitting to Higuchi's equation, an increase in glycerol and surfactant concentrations resulted in slow release of the oil. The release rate related well to the protection time where a decrease in release rate can prolong mosquito protection time.

1. Introduction

Mosquitoes are important vectors of several tropical diseases, including malaria, filariases, and numerous viral diseases, such as dengue, Japanese encephalitis and yellow fever (Curtis, 1992; Fradin and Day, 2002). The use of repellents is an obvious practical and economical means of preventing the transmission of these diseases to humans. The most common mosquito repellent formulations available in the market contain DEET (*N,N*-diethyl-3-methylbenzamide), which has shown excellent repellency against mosquitoes and other biting insects (Qiu et al., 1998a; Coleman et al., 1993). However, human toxicity reactions after the applications of DEET vary from mild to severe effects (Qiu et al., 1998b). To avoid these adverse effects, research on repellents that are derived from plant extracts to replace DEET has been reported (Trongtokit et al., 2005; Tawatsin et al., 2001; Chokechaijaroenporn et al., 1994; Boonyabancha et al., 1997). Citronella oil extracted from *Cymbopogon nardus* (citronella) has been widely studied as one of possible natural mosquito repellents (Trongtokit et al., 2005; Kim et al., 2005; Müller et al., 2008; Yang and Ma, 2005). Citronella oil has demonstrated good efficacy against mosquitoes in concentrations ranging from 0.05% to 15% (w/v) alone or in combination with other natural or commercial insect repellent products (Fradin, 1998).

Recently, the preparation of submicron emulsions, called nanoemulsion or mini-emulsions, has emerged as a promising alternative for both intravenous and dermal application. Nanoemulsions are fine oil-in-water dispersions, having droplet covering the size range of 100–600 nm (Solans et al., 2003). Nanoemulsions are not only kinetically stable but also long-term physically stable (with no apparent flocculation or coalescence) which makes them unique and sometimes referred to as "approaching thermodynamic stability" (Bouchemal et al., 2004). In contrast to microemulsions, nanoemulsions are metastable and can be diluted with water with no change in the droplet size distribution (Gutieĭrrez et al., 2008; Fermamdez et al., 2004). The preparation of emulsions with droplet sizes in the submicrometer range may be performed non-mechanically by phase inversion temperature (PIT) method or mechanically, which involves high-energy input that is generally achieved by high-shear stirring, high-pressure homogenizers, or ultrasonic generators (Solans et al., 2005). Apart from method of preparation, nanoemulsion is majorly affected by the systems compositions and their physicochemical characteristics. In this present work, the preparation of citronella oil loaded oil in water nanoemulsions stabilized by a non-ionic surfactant was

* Corresponding author. Tel.: +66 2564 7100x6552; fax: +66 2564 6981.
E-mail address: uracha@nanotec.or.th (U. Ruktanonchai).

0378-5173/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2008.12.029

studied by using high-energy emulsification method. Since the energy input by this method is high and coalescence of newly formed droplets is inevitable, there should be an optimization of emulsification process along with appropriate selection of disperse phase and surfactant composition in order to obtain stable submicron emulsion. The present study investigated nanoemulsion containing 20% (w/w) of citronella oil. Effect of high pressure process, the ratio of water to glycerol and surfactant concentration on physical properties (ternary phase behavior, droplet size, stability, release kinetics) and *in vivo* bioactivity on mosquito repellency of citronella oil loaded-nanoemulsion were determined.

2. Materials and methods

2.1. Materials

Citronella oil extracted from *C. nardus* and terpinene-4-ol were obtained from Thai-China Flavours and Fragrances Industry (TCFF) Co., Ltd. (Bangkok, Thailand). D-Limonene and citronellal were obtained by Sigma–Aldrich (UK). Glycerol (AnalaR®) was obtained from BDH (Poole, England). Montanov®82 (a mixture of cetearyl alcohol and cocoyl glucoside) was obtained from Adinop Co., Ltd. (Bangkok, Thailand). The water used for all experiments was deionized water obtained from a MilliQ Plus (Millipore, Schwalbach, Germany). All other reagents used were commercially available and were of analytical grade.

2.2. Headspace/GC–MS analysis

Chemical compositions of citronella oil were analyzed by Headspace/GC–MS analysis in which citronella oil was incubated at 80 °C for 10 min. GC–MS analyses were carried out using Thermo Electron Corporation gas chromatograph (FOCUS PolarisQ, Thermo Fisher Scientific Inc. USA) equipped with a capillary column ZB-5ms (Phenomenex®, Torrance, USA) with 30 m × 0.25 mm i.d. × 0.25 mm film thickness coated with 5% phenyl and 95% dimethylpolysiloxane. Helium gas was used as the carrier at a flow rate of 1 mL/min. The oven temperature program was 60 °C (5 min) then 3 °C/min to 170 °C (40 min). The 1.5 mL of samples was injected with 1:10 split ratio. Injector and detector temperatures were 80 and 275 °C, respectively. Mass spectra were recorded over 35–650 amu range at 1 scan/sec with ionization energy of 70 eV and ion source temperature of 240 °C. Component identification was carried out by comparing the obtained MS data with library on Wiley by NIST MS search version 2.0.

For standard curve preparation, D-limonene and citronellal used as the markers were diluted with methanol to varying concentrations of 0.1, 0.5, 1, 5 and 10 ppm. Standard curve was performed by peak area ratio of marker to terpinene-4-ol as internal standard versus each marker concentration.

2.3. Preparation of nanoemulsions

Aqueous dispersions of nanoemulsion were composed of 20% (w/w) citronella oil, glycerol at varying concentrations of 0%, 50%, 75% and 100% (w/w) and surfactant (Montanov®82) at varying concentrations of 2.5%, 5% and 10% (w/w) as shown in Table 1. The 20% (w/w) of citronella oil was kept constant and added to the melted surfactant at 45 °C. To obtain nanoemulsion, oil phase was dispersed in the hot aqueous glycerol solution under stirring condition at 200 rpm, 50 °C for 5 min. The mixture was emulsified by high-speed homogenizer (Ultra-Turrax T25, IKA-WERKE, Germany) at 16,500 rpm for 3 min. Subsequently, this pre-emulsion was passed through the high pressure homogenizer (EmulsiFlex-C3, Avestin, Canada) for five cycles at pressure of 1500 bars. After high pressure homogenization the produced O/W nanoemulsion was cooled down to room temperature and stored at 25 °C. Samples were taken from the formulation one day after the production day and subsequently on months 1 and 2 to study their stability. The samples were then evaluated in terms of droplet size and polydispersity index (PI).

2.4. Measurement of emulsion droplet size

Measurement of droplet size and PI of nanoemulsion was performed by using photon correlation spectroscopy (PCS) (NanoZS4700 nanoseries, Malvern Instruments, UK). Nanoemulsions were diluted with 1 mL of deionized water to eliminate the effect of viscosity caused by the ingredients. The refractive index of nanoemulsion and water were set at 1.46 and 1.33, respectively. Droplet size and PI were obtained as the average of three measurements at 25 °C.

2.5. Viscosity measurement

Viscosity of nanoemulsions was measured using small sample adapter of Brookfield digital rheometer (Model RV-DVII, Brookfield Engineering Labs., Inc., Stoughton, MA, USA) at 25 ± 1 °C. Average and standard deviation of three data of the single point viscosity at a shear rate of 120.0 s^{-1} were reported.

2.6. Release study

The experiment was performed to evaluate the amount of essential oil release from each formulation. The nanoemulsion was loaded at 1 ml per cell in separated closed container, which was accurately controlled at 32 °C to mimic human skin. At fixed time intervals, the containers were removed and added with terpinene-4-ol as internal standard. The sample amount was analyzed by headspace GC–MS technique as mentioned above. The relative amounts of individual components are based on peak areas obtained. D-Limonene was selected as the major marker of citronella oil. The data presented are average values of two separate experiments.

The citronella oil release kinetics from nanoemulsion in various formulations were investigated by fitting the release data into Higuchi's model, which can be expressed using Eq. (1) as followed:

$$Q_t = kt^{0.5} \quad (1)$$

where Q_t is the percent of essential oil released at a given time (t) and k is the release rate.

2.7. Mosquitoes repellent test

Mosquito repellent effect of citronella oil formulations was evaluated using the human-bait technique based on standard test of World Health Organization (WHO) (WHO, 1996; Tawatsin et al., 2001). All chemicals used in the formulations are generally regarded as safe materials for human use. This study was approved and conducted by Department of Medical Sciences, Ministry of Public Health, Thailand. The tests were carried out in a 6 m × 6 m × 3 m room, at 25–29 °C and relative humidity of 60–80%. An area 3 cm × 10 cm on each forearm of three human volunteers (n = 3) was marked out with a permanent marker. Approximately 0.1 ml of nanoemulsion was applied to the marked area of one forearm of each volunteer. During the test, the forearm was covered by a paper sleeve with a hole corresponding to the marked area. Each volunteer put the tested forearm in a mosquito cage (40 cm × 40 cm × 40 cm), containing 250 female *Aedes aegypti* mosquitoes (3–5 days old), for the first 3 min of every half-hour exposure. If at least two mosquitoes landed on or bite the hand, the repellency test was then continued. The test continued until at least two bites occurred in a

Table 1
Composition of citronella oil loaded-nanoemulsion formulations.

Ingredients (%, w/w)	Glycerol concentration (%, w/w)											
	0			50			75			100		
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
DI water	77.5	77.5	77.5	38.8	38.8	38.8	19.4	18.8	17.5	0	0	0
Glycerol	0	0	0	38.8	38.8	38.8	58.1	56.3	52.5	77.5	77.5	77.5
Surfactant	2.5	5	10	2.5	5	10	2.5	5	10	2.5	5	10
Citronella oil	20											

3 min period, or until a bite occurred and was followed by a confirmatory bite (second bite) in the following exposure period. The time between application of the repellents and the second successive bite was recorded as the protection time.

2.8. Statistical analysis

Values were expressed as mean ± standard deviation (SD). Statistical significance of differences was examined using one-way analysis of variance (ANOVA) by LSD post hoc test. A probability value (p) of less than 0.05 was considered to be significantly different.

3. Results and discussion

3.1. Citronella oil analysis

Citronella oil was extracted by steam distillation and was analyzed by using head space technique. Qualitative analysis was performed by using GC–MS. Chromatogram of citronella oil is shown in Fig. 1. Two major components, D-limonene and citronellal, were found at 11.68 and 17.52 min, respectively, and were selected as markers due to their high content at 40.85% and 40.04% of total peak area, respectively.

3.2. Phase behavior of water/nonionic surfactant/glycerol

To investigate phase behavior of three components, which were water, surfactant and glycerol, an effect of glycerol concentration of 0%, 50%, 75% and 100% (w/w) and surfactant concentration of 2.5%, 5% and 10% (w/w), were investigated with the fixed content of citronella oil at 20% (w/w). An increase in both surfactant and glycerol concentrations led to increasing of the sample viscosity. Samples were classified according to their appearance into three areas: Phase I, Phase II and Phase III as shown in Fig. 2. Sample with glycerol concentration of 0%, 50% and 75% (w/w) in aqueous phase (F1–F9) appeared as milky solution (Phase I). The samples with glycerol concentration of 100% (w/w) in aqueous phase (F10, F11 and F12) located in Phase II and Phase III, respectively. A clear solution of nanoemulsion can only be obtained in Phase II (F10), which was a formulation with the lowest surfactant concentration (2.5%). It was obvious that glycerol played a major role on the appearance as well as the stability of the emulsion. Glycerol behaves as a co-solvent in this case. It not only causes transparency of the nanoemulsion (F10) but also increases the viscosity of the continuous phase and will therefore decrease the droplet collision frequency (Chanasattru et al., 2009; Chanasattru et al., 2007). This influence was more pronounced at higher concentration until glycerol concentration of 100%. It has been reported that co-solvents can alter the bulk physicochemical properties of the continuous phase (e.g., dielectric constant, refractive index, density, interfacial tension), which will alter the magnitude and range of the colloidal interactions operating between the droplets (e.g., van der Waals, electrostatic, hydrophobic, depletion) (Chanasattru et al., 2007). However, in a presence of 100% (w/w) glycerol in aqueous phase, phase separation was still found in Phase III (F11 and F12), which contain high amount of surfactant at 5% and 10%, respectively.

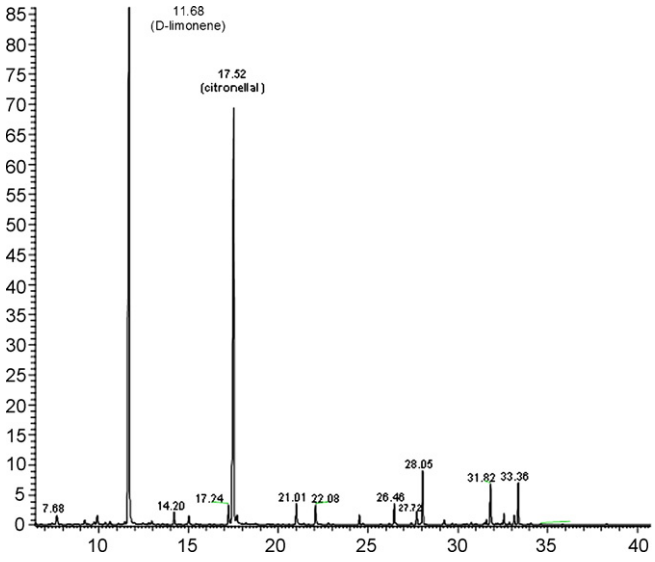

Fig. 1. Chromatogram of citronella oil analyzed by using GC–MS.

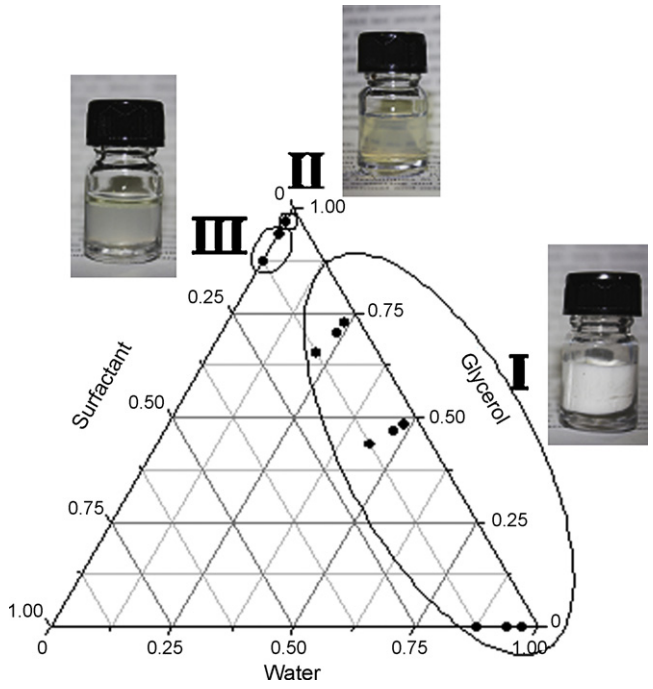

Fig. 2. Phase diagram of 20% citronella oil loaded-nanoemulsion with varying contents of water/surfactant/glycerol. The appearance of samples is as follows: milky solution (Phase I), clear solution (Phase II) and oil-aqueous phase separation (Phase III).

3.3. Emulsion droplet size

All formulations were produced using high pressure homogenization process. The droplet size after the production was shown in Table 2. Mean droplet sizes of F1–F10 were in a range of 120–200 nm except F9, which demonstrated size at 334 nm. However their PI values were not much different, which were below 0.3 except F3 and F9. At %glycerol of 0 and 50, an increase in surfactant concentration from 2.5% to 10% (w/w) resulted in a decrease in droplet size (from 164 to 139 nm and from 178 to 135 nm, respectively), which were significantly different ($p < 0.05$). However, this pronounced effect was not found with %glycerol of 75 and 100 where increasing droplet size and phase separation were found.

A possible explanation of size reduction after an increased surfactant concentration at %glycerol of 0 and 50 is that surfactants are freely moved and able to absorb around oil droplets, resulting in increasing surface to volume ratio of the particles. Moreover, surfactant that is localized to the surface of the emulsion droplet reduces interfacial free energy and provides mechanical barrier to coalescence (Reiss, 1975). At %glycerol of 75 and 100, an increase in surfactant concentration from 2.5% to 10% w/w resulted in larger droplet sizes of F9 and phase separation of F11, F12 (data not shown) were obtained. It is possible that highly viscous environment in the presence of high amount of glycerol (27.6 and 296.6 mPa s of F7 and F10, respectively), may lead to difficulty of surfactant to move and rapidly cover oil droplets during emulsification and to prevent any coalescence. Since specific surface area of the droplets is increasing dramatically during homogenization process, there is more likely that there is an insufficient surfactant to fully cover their surface (Jafari et al., 2007), resulting in coalescence of surrounding oil droplets. Chanasattru et al. (2007) has also reported an effect of co-solvent (glycerol and sorbitol concentration from 0% to 40%) on the long-term emulsion stability. A decrease in creaming index and mean particle diameter was found at low co-solvent concentration whereas glycerol appeared to give larger mean particle diameters at the higher co-solvent concentrations over 40%. Another important factor is the size and adsorption rate of the emulsifier (Schulza and Danielsb, 2000; Karbstein and Schubert, 1995). It is also possible that a surfactant used in this study, Montanov®82 (a mixture of cetearyl alcohol and cocoyl glucoside) could be slower at adsorption on oil droplets as compared with other small molecule surfactants.

3.4. Stability of nanoemulsions

Droplet size analysis of all formulations was monitored for 2 months of storage at 25 °C (Fig. 3). After 1 day of storage a phase separation was found with F11 and F12 which contain glycerol concentration of 100% (w/w) in aqueous phase (Fig. 2 Phase III). Most of the formulations revealed an increase in droplet size, which were found in a range of 120–220 nm with low PI (0.1–0.3). However, at glycerol concentration of 50% (w/w) in aqueous phase with the presence of 10% surfactant (F6), mean droplet size of nanoemulsion was significantly increased after 1 month of storage (p value <0.05). Only F10 at high glycerol concentration of 100% (w/w) in aqueous phase and 2.5% surfactant demonstrated the best stability within 2 months of storage. Similarly, Chanasattru et al. (2007) reported that an addition of increasing amounts of sorbitol and glycerol to the emulsions caused a decrease in the rate and extent of droplet flocculation as well as mean particle diameter and creaming index. In the presence of co-solvents there was still a rapid increase in mean particle diameter during the first 24 h, followed by a more gradual increase at longer times, but the extent of the increase in particle size was considerably less in the presence of high concentrations of co-solvents.

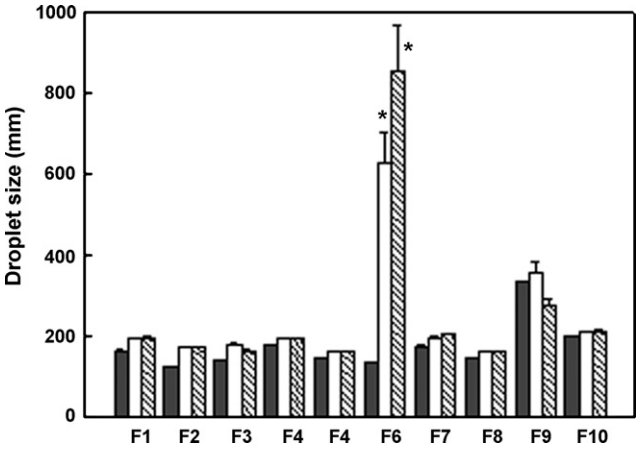

Fig. 3. Stability of citronella oil loaded-nanoemulsion on droplet size at production day (■), 1 month storage (□) and 2 months storage (▨) at 25 °C (mean ± SD, $n = 3$). The mean difference is statistically different from control (p value <0.05).

3.5. Effect of emulsion droplet size on citronella oil release

Emulsion at glycerol concentration of 50% (w/w) in aqueous phase and 2.5% surfactant (F4) were selected as a model to investigate the effect of emulsion droplet size on citronella oil release profile (Fig. 4). An amount of released oil was determined by its marker (D-limonene) by using head space technique and GC–MS as mentioned earlier. Released amounts of D-limonene from emulsions were measured at 32 °C versus storage time. Fig. 4 demonstrated oil released versus time (h) of F4 emulsion with and without high pressure process. An increase in oil released was found over investigated time for both conditions although pronounced effect was found with the smaller size emulsion, nanoemulsion, with high pressure process. To evaluate the release rate of D-limonene in the nanoemulsion, the Higuchi's model was applied to the release time-courses of the encapsulated D-limonene as reported in the previous works of emulsion systems (Vasiljevic et al., 2006; Kapoor and Chauhan, 2008). Release kinetic parameters (k and R^2 values) were illustrated in Table 2. The k value, release rate, formulation with high pressure process ($0.1038 \pm 0.0033\% \, h^{-0.5}$) was higher than those without the high pressure process ($0.0948 \pm 0.0069\% \, h^{-0.5}$), suggesting high release

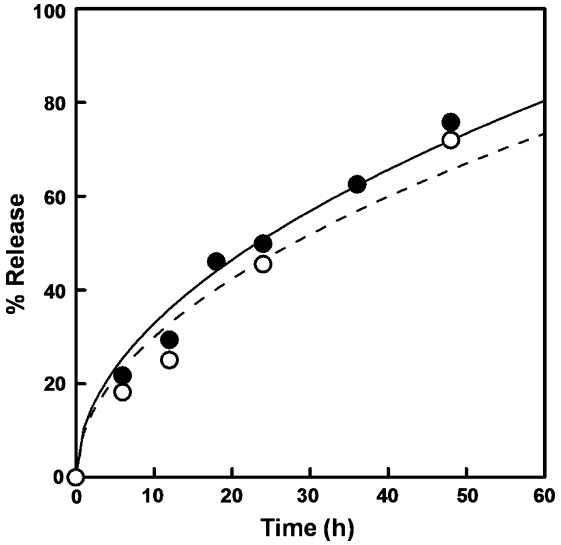

Fig. 4. Release profile of citronella oil from nanoemulsion of 50% glycerol concentration and 2.5% surfactant with (●) and without high pressure process (○).

Table 2
Droplet size, polydispersity index (PI), viscosity, release kinetic parameters (release rate (k) and R^2) and mosquito protection time of citronella oil loaded-nanoemulsion.

Formulation	Droplet size (nm) at production day	PI	Viscosity at shear rate of 120.0 s^{-1} (mPa s)	k (% $h^{-0.5}$)	R^2	Protection time (h)
F1	164 ± 2	0.13 ± 0.02	1.7 ± 0.0	0.1164 ± 0.0145	0.8896	0.8 ± 0.3
F2	125 ± 1	0.18 ± 0.01	3.4 ± 0.1	0.1226 ± 0.0096	0.8873	n/a
F3	139 ± 1	0.39 ± 0.01	236.6 ± 0.7	0.0998 ± 0.0107	0.8626	n/a
F4	178 ± 1	0.06 ± 0.00	7.6 ± 0.2	0.1038 ± 0.0033	0.9618	1.3 ± 0.3
F5	143 ± 2	0.09 ± 0.02	12.1 ± 0.1	0.0943 ± 0.0064	0.8996	2.2 ± 0.3
F6	135 ± 1	0.08 ± 0.11	131.3 ± 6.6	0.0536 ± 0.0039	0.8594	2.8 ± 0.6
F7	175 ± 6	0.08 ± 0.01	27.6 ± 0.2	0.0689 ± 0.0059	0.8543	2.3 ± 0.3
F8	147 ± 1	0.10 ± 0.02	37.8 ± 0.7	0.0706 ± 0.0047	0.8671	n/a
F9	334 ± 2	0.73 ± 0.05	n/a	0.1095 ± 0.0105	0.8097	n/a
F10	199 ± 3	0.13 ± 0.02	296.6 ± 0.8	0.0680 ± 0.0052	0.8734	2.3 ± 0.3
F4 without high pressure process	283 ± 7	0.19 ± 0.02	n/a	0.0948 ± 0.0069	0.9273	n/a

of essential oil from the nanoemulsion. By looking into droplet size of both conditions, the result from Table 2 demonstrated that a significant size reduction was found from F4 with high pressure homogenization (178 ± 1 nm) as compared to F4 without the higher process (283 ± 7 nm). Additionally, high pressure homogenization process obviously raised homogeneity comparing between the formulations with and without high pressure process as shown by PI value of 0.06 ± 0.00 and 0.19 ± 0.02, respectively. These results suggested that the faster release of D-limonene from formulation with high pressure process is most likely due to smaller droplet size and an increase in the surface area of the oil droplets in the emulsion (Floury et al., 2000; Yuan et al., 2008; Tan and Nakajima, 2005). In addition to the higher surface area of D-limonene to diffuse, an accelerated dissolution of the D-limonene into the carrier solution and diffusion through the liquid to evaporate could be expected. Similar observation was reported on all-trans-retinol acetate nanoemulsion where the amount of retinol dissolved in 30 min increases when the particle size decreases (Taha et al., 2004). According to the Higuchi's model, the n value, which represented release mechanism, was assumed to be 0.5, indicating that the mechanism of the oil release was diffusion controlled.

3.6. Effect of surfactant on citronella oil release

An effect of surfactant concentration on release profile was observed on nanoemulsion at glycerol concentration of 50% (w/w) in aqueous phase (F4, F5 and F6). The release profiles of citronella oil from nanoemulsion were observed over storage time as shown in Fig. 5. By fitting with Higuchi's equation, kinetic parameters are shown in Table 2. At 10% (w/w) surfactant the lowest diffusion rate (k value of 0.0536% $h^{-0.5}$) can be obtained comparing to 2.5% and 5% (w/w) surfactant formulation, which were 0.1038% and 0.0943% $h^{-0.5}$, respectively. The results indicated that at high surfactant concentration, a slow release of the citronella oil from the emulsion can be obtained. However, for the emulsion droplet size, increasing surfactant concentration from 2.5% to 5% and 10% resulted in reduced surface tension of the droplets, leading to small size (178 ± 1 to 143 ± 2 and 135 ± 1 nm, respectively). The results of release rate were opposed with the results of emulsion size effect as mentioned before. The smaller size of emulsion from the higher surfactant decreased the release rate. These results indicated that the release behavior could be attributed not only to the effect of droplet size on the % release but also to the concentration of surfactant. The oil droplets emulsion could be abundantly covered with the surfactant molecules at the high surfactant concentration. Moreover, it is possible that in a presence of high surfactant concentrations there are also excess surfactants that may inhibit diffusion of citronella oil to the carrier solution.

3.7. Effect of glycerol concentration on citronella oil release

Release study of glycerol concentration was performed by using 2.5% (w/w) surfactant based formulations (F1, F4, F7 and F10) as a

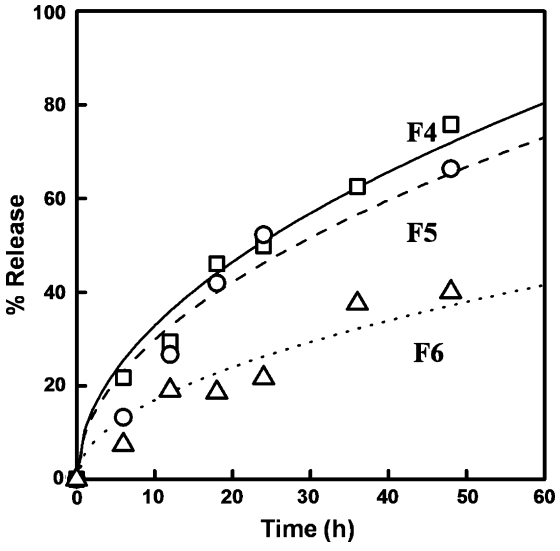

Fig. 5. Release profile of citronella oil from nanoemulsion of 50% glycerol concentration with high pressure process at varying surfactant concentration of 2.5% (F4), 5% (F5) and 10% (F6).

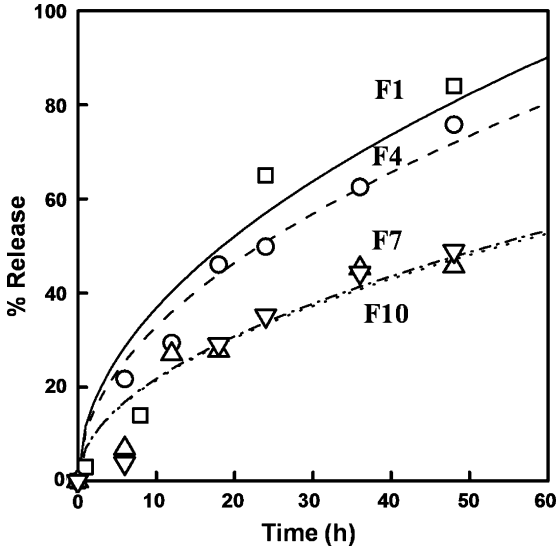

Fig. 6. Release profile of citronella oil from nanoemulsion of 2.5% surfactant with high pressure process at varying glycerol concentration of 0% (w/w) (F1), 50% (w/w) (F4), 75% (w/w) (F7) and 100% (w/w) (F10) in aqueous phase.

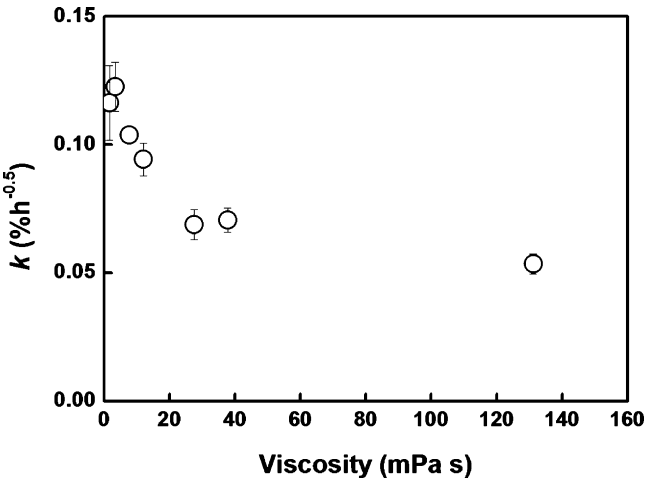

Fig. 7. Relation between viscosity of citronella oil loaded-nanoemulsion and release rate.

model to determine the effect of glycerol concentration. It was seen from Fig. 6 that glycerol amount greatly influenced the retention of citronella oil. The formulations could be divided into two groups according to their *k* value. The first group composed of F1 and F4 with low amount of glycerol (0% and 50% (w/w) in aqueous phase) and high *k* value (0.1164% and 0.1038% $h^{-0.5}$, respectively). The second group composed of F7 and F10 with high amount of glycerol (75% and 100% (w/w) in aqueous phase) and low *k* value (0.0689% and 0.0680% $h^{-0.5}$, respectively). Regarding similarity in obtained droplet sizes, it is possible that at high glycerol concentration the vicinity is more viscous, which may then be less diffusion of citronella oil into carrier solution, resulting in slow release of the oil. A relationship of viscosity of nanoemulsion and *k* value was plotted (Fig. 7). The result confirms a good relationship between viscosity and release rate in which an increase in viscosity resulted in a decrease in release rate of citronella oil. Apart from an effect of viscosity, a partition coefficient of citronella oil should be taken in to account. Its affinity toward aqueous phase would be much lower as compared to oil phase, especially in the presence of high glycerol concentration.

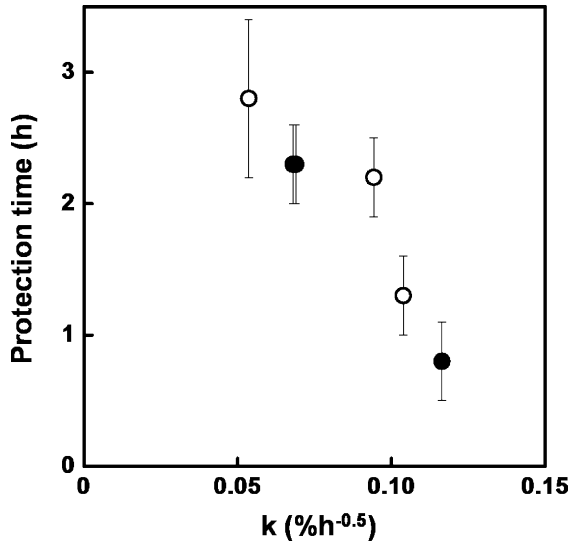

Fig. 8. Relation between release rate and mosquito repellent time of citronella oil loaded-nanoemulsion at varying concentration of glycerol (●) and surfactant (○).

3.8. The relation between the release characteristics and mosquito repellent efficiency

The relation between the mosquito protection time and release characteristics was shown in Fig. 8 at varying surfactant and glycerol concentrations. For both variables (surfactant and glycerol concentrations), release rate related well to the protection time where a decrease in *k* value tended to prolong mosquito protection time. According to the diffusion controlled release mechanism of nanoemulsion in this study, the prolonged protection time could be obtained at high glycerol and surfactant concentration.

4. Conclusions

The high pressure homogenization process, surfactant and glycerol concentration demonstrated their influence on release kinetics and mosquito protection time in addition to the nanoemulsion droplet size and the stability. High pressure homogenizer process reduces droplet size and polydispersity of oil droplet. The emulsion composition also affected to the stability of encapsulated oil over storage time. The release of essential oil could be effectively controlled by changing the amount of surfactant and glycerol. An increase in surfactant concentration led to decreasing droplet size, increasing of homogeneity and extension of release and protection time. Moreover, the release of citronella oil from high amount of glycerol was much slower than that from the low glycerol amount, resulting in sustained mosquito protection time. Therefore, the optimal size of oil droplets and viscosity of medium in nanoemulsion should be recommended for stability during storage and ability to control the release of essential oil as well as the *in vivo* activity.

Acknowledgements

This research was financially supported by National Nanotechnology Center (NANOTEC), Thailand (Research grant number B21 CR0167 10RDCR01). The authors are grateful for GC–MS support by Traditional Thai Medicine Development Center, The Institute of Traditional Thai Medicine Department for Development of Traditional and Alternative Medicine, Thailand; and for mosquito repellent test by Department of Medical Sciences, Ministry of Public Health, Thailand.

References

Boonyabancha, S., Suphapathom, K., Srisurapat, A., 1997. Repellent effect of essential oils on *Aedes aegypti*. Bull. Dept. Med. Sci. 39, 61–66.
Bouchemal, K., Briançon, S., Perrier, E., Fessi, H., 2004. Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimisation. Int. J. Pharm. 280, 241–251.
Chanasattru, W., Decker, E.A., McClements, D.J., 2007. Inhibition of droplet flocculation in globular-protein stabilized oil-in-water emulsions by polyols. Food Res. Int. 40, 1161–1169.
Chanasattru, W., Decker, E.A., McClements, D.J., 2009. Influence of glycerol and sorbitol on thermally induced droplet aggregation in oil-in-water emulsions stabilized by β-lactoglobulin. Food Hydrocolloid 23, 253–261.
Chokechaijaroenporn, O., Bunyapraphatsara, N., Kongchuensin, S., 1994. Mosquito repellent activities of ocimum volatile oils. Phytomedicine 1, 135–139.
Coleman, R.E., Robert, L.L., Roberts, L.W., Glass, J.A., Seeley, D.C., Laughinghouse, A., Perkins, P.V., Wirtz, R.A., 1993. Laboratory evaluation of repellents against four anopheline mosquitoes (Diptera: Culicidae) and two phlebotomine sand flies (Diptera: Psychodidae). J. Med. Entomol. 30, 499–502.
Curtis, C.F., 1992. Personal protection methods against vectors of disease. Rev. Med. Vet. Entomol. 80, 543–553.
Fermamdez, P., André, V., Rieger, J., Kuhnle, A., 2004. Nano-emulsion formation by emulsion phase inversion Colloids and Surfaces A: Physicochem. Eng. Aspects. 251, 53–58.
Floury, J., Desrumaux, A., Lardières, J., 2000. Effect of high-pressure homogenization on droplet size distributions and rheological properties of model oil-in-water emulsions. Inn. Food Sci. Emerg. Technol. 1, 127–134.
Fradin, M.S., 1998. Mosquitoes and mosquito repellents: a clinician's guide. Ann. Int. Med. 128, 931–940.

Fradin, M.S., Day, J.F., 2002. Comparative efficacy of insect repellents against mosquito bites. N. Engl. J. Med. 357, 13–18.
Gutieĭrrez, J.M., Gonzaĭlez, M., Sole, I., Pey, C.M., Nolla, J., 2008. Nano-emulsions: new applications and optimization of their preparation. Curr. Opin. Colloid Interface Sci. 13, 245–251.
Jafari, S.M., He, Y., Bhandari, B., 2007. Optimization of nano-emulsions production by microfluidization. Eur. Food Res. Technol. 225, 733–741.
Kapoor, Y., Chauhan, A., 2008. Ophthalmic delivery of Cyclosporine A from Brij-97 microemulsion and surfactant-laden p-HEMA hydrogels. Int. J. Pharm. 361, 222–229.
Karbstein, H., Schubert, H., 1995. Developments in the continuous mechanical production of oil-in-water macro-emulsions. Chem. Eng. Proc. 34, 205–211.
Kim, J.K., Kang, C.S., Lee, J.K., Kim, Y.R., Han, H.Y., Yun, H.K., 2005. Evaluation of repellency effect of two aatural aroma mosquito repellent compounds, citronella and citronellal. Entomol. Res. 35, 117–120.
Müller, G.C., Junnila, A., Kravchenko, V.D., Revay, E.E., Butlers, J., Schlein, Y., 2008. Indoor protection against mosquito and sand fly bites: a comparison between citronella, linalool and geraniol candles. J. Am Mosq. Control Assoc. 24, 150–153.
Qiu, H., McCall, J.W., Wonjun, H., 1998a. Formulation of topical insect repellent *N* N-diethyl-m-toluamide (DEET): vehicle effects on DEET in vitro skin permeation. Int. J. Pharm. 163, 167–176.
Qiu, H., Jun, W., McCall, J.W., 1998b. Pharmacokinetics, formulation, and safety of insect repellent N N-diethyl-3-methylbenzamide (DEET): a review. J. Am. Mosq. Control Assoc. 14, 12–27.
Reiss, H., 1975. Entropy-induced dispersion of bulk liquids. J. Colloid Interface Sci. 53, 61–70.
Schulza, M.B., Danielsb, R., 2000. Hydroxypropylmethylcellulose (HPMC) as emulsifier for submicron emulsions: influence of molecular weight and substitution type on the droplet size after high-pressure homogenization. Eur. J. Pharm. Biopharm. 49, 231–236.
Solans, C., Esquena, J., Forgiarini, A., Uson, N., Morales, D., Izquierdo, P., Azemar, N., GarcÍa- Celma, M.J., 2003. Absorption and Aggregation of Surfactants in Solution. In: Mittal, K.L., Dinesh, O.S. (Eds.), Nano-emulsions: Formation, Properties and applications. Marcel Dekker, New York, pp. 525–554.
Solans, C., Izquierdo, P., Nolla, J., Azemar, N., Garcia-Celma, M.J., 2005. Nano-emulsions. Curr. Opin. Colloid Interface Sci. 10, 102–110.
Taha, E.I., Al-Saidan, S., Samy, A.M., Khan, M.A., 2004. Preparation and in vitro characterization of self-nanoemulsified drug delivery system (SNEDDS) of all-trans-retinol acetate. Int. J. Pharm. 285, 109–119.
Tan, C.P., Nakajima, M., 2005. β-Carotene nanodispersions: preparation, characterization and stability evaluation. Food Chem. 92, 661–671.
Tawatsin, A., Wratten, S.D., Scott, R.R., Thavara, U., Techadamrongsin, Y., 2001. Repellency of essential oils from plants against three mosquito vectors. J. Vector Ecol. 26, 76–82.
Trongtokit, Y., Rongsriyam, Y., Komalamisra, N., Apiwathnasorn, C., 2005. Comparative repellency of 38 essential oils against mosquito bites. Phytother. Res. 19, 303–309.
Vasiljevic, D., Parojcic, J., Primorac, M., Vuleta, G., 2006. An investigation into the characteristics and drug release properties of multiple W/O/W emulsion systems containing low concentration of lipophilic polymeric emulsifier. Int. J. Pharm. 309, 171–177.
WHO. 1996. Report of the WHO informal consultation on the evaluation and testing of insecticides. CTD/WHOPES/IC/96.1, Control of Tropical Diseases Division. World Health Organization, Geneva, 69.
Yang, P., Ma, Y., 2005. Repellent effect of plant essential oils against Aedes albopictus. J. Vector Ecol. 30, 231–234.
Yuan, Y., Gao, Y., Zhao, J., Mao, L., 2008. Characterization and stability evaluation of β-carotene nanoemulsions prepared by high pressure homogenization under various emulsifying conditions. Food Res. Int. 41, 61–68.