Journal of Chemical and Natural Resources Engineering, Special Edition: 90-99

FORMULATION OF GERANIUM OIL LOADED SOLID LIPID NANOPARTICLES FOR MOSQUITO REPELLENT APPLICATION

S. ASNAWI[2], A. ABD. AZIZ,[2]R. ABD. AZIZ,[1] A. K. KHAMIS[1]

ABSTRACT

In recent years, essential oil has been the subject matter of many investigations due to its eco-friendly and bio-degradable nature. Geranium oil is one of the various essential oils that are used as mosquito repellent. However, stability is one of the issues related to essential oil formulations. Incorporation of essential oil in a control-release formulation could solve the problem and it also offers several advantages. In this work, a new delivery system for mosquito repellent based on the incorporation of geranium oil into solid lipid nanoparticles (SLNs) has been studied. The following has been employed for the production of geranium oil loaded solid lipid nanoparticles (GO-SLNs): 5-15% (w/w) stearic acid as the lipid, 0.5-3% (w/w) soy lecithin as the emulsifier, 0.5-3% (w/w) Tween 80 as the co-emulsifier, 5-20% (w/w) dichloromethane also as the co-emulsifier and 8% (w/w) geranium oil as the active ingredient. GO-SLNs were prepared using ultrasonic-solvent emulsification technique. GO-SLNS were characterized using transmission electron microscopy (TEM), photon cross correlation spectroscopy (PCCS), gel filtration and gas chromatography (GC). Results showed that the variation in the amount of ingredients affected geranium oil loading capacity and mean particle size. Increasing the concentrations of lipid, emulsifier and co-emulsifier resulted in the increase in the mean particle size from 46 nm to 114 nm. Geranium oil encapsulation efficiency (EE) was in the range of 92-99% (w/w). Production of high quality SLNS loaded with geranium oil has been demonstrated.

Key Words: geranium oil, solid lipid nanoparticle, particle size, encapsulation efficiency, mosquito repellent

1.0 INTRODUCTION

Solid lipid nanoparticles (SLNs) were developed at the beginning of the 1990s as alternative carriers for emulsion, liposomes and polymeric nanoparticles. SLNs were used in topical formulations, not only for pharmaceutical but also for cosmetic products [1]. The average particle size is in the range from 100 to 1000 nm, predominantly from 100 to 800 nm [2]. Many researchers reported SLNs display excellent properties such as controlled release, *in vivo* good tolerance and protection of active compound. SLNs can also favor drug penetration into the skin depending on their size. Other benefits are maintenance of sustained release to avoid systemic absorption, reduction in irritation and skin targeting potential. In addition, no biotoxicity is expected of the carrier [3].

In recent years, mosquito-borne disease has become a major international public health concern. Applying repellent to the skin is another effort in the protection against mosquito bites. In many parts of the world, N, N-diethyl-m-toluamine (DEET) is most commonly used in all insect repellent products but this active ingredient is a synthetic

[1] Chemical Engineering Pilot Plant(CEPP), Univerisiti Teknologi Malaysia, 81310 UTM Skudai, Johor Malaysia.
[2] Department of Bioprocess Engineering, Faculty of Chemical and Natural Resources Engineering Department, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia.
Tel : +607-5535525 Fax : +607-5581463
Correspondence to: Azila Abdul Aziz (azila@fkkksa.utm.my)

chemical compound that can be toxic [4]. For that reason, the introduction of plant essential oil for developing natural insect repellent formulation is rather timely. Geranium essential oil from *Pelargonium* sp. is one plant extract that can be used as an active ingredient in insect repellent formulation [5]. Its main components, which are geraniol, cintronellol, i-methnone, citronellyl formate, eugenol, and geranyl formate have potential repellency activity [6]. SLNs that are loaded with geranium oil must however be big enough so that they will not penetrate into the skin.

However, the major inconvenience of the use of oils is their chemical instability in the environment that can result in rapid evaporation and degradation of some active components. Incorporation of essential oils in controlled release formulation could solve this problem. SLNs have demonstrated the capacity to protect and prevent rapid evaporation of labile active compounds such as tocopherol acetate, retinoids and vitamin E [7].

General ingredients to formulate stable SLNs include solid lipids, emulsifiers and water. The lipids that are normally used are tristearin, imwitor, stearic acid and cetyl palmitate. All classes of emulsifiers have been used to stabilize the lipid dispersion. A combination of emulsifiers might prevent particle agglomeration more efficiently compared to the use of a single emulsifier[2]. Different ingredients and compositions can affect SLNs particle size. Tiyaboonchai *et al.* [8] showed that the particle size of SLNs increased when each composition of the ingredients was increased. This information is useful in cases where it is important to produce large SLNs to ensure that they stay on skin surface. SLNs which are bigger than 800 nm has been shown to exhibit significantly less penetration into skin compared to SLNs with the particle size of around 100 nm [9].

In this work, geranium oil-SLNs were produced using ultrasonic-solvent emulsification technique. The primary goal here was to produce large stable particles for mosquito repellent product applications. Different compositions of the components were expected to affect the mean diameter of the SLNs particle sizes. Additionally, the size, morphology and the encapsulation efficiency of the SLNs were studied.

2.0 MATERIALS AND METHODS

2.1 Chemicals

Pure geranium oil was purchased from Peacock, USA. Citronellol, geraniol, eugenol, geranyl formate, citronellyl formate and i-menthone were purchased from MERCK. Stearic acid, extracted pure as a lipid, was purchased from Scharlau (Spain), the surfactant soybean lecithin was obtained from Across Organics (USA), and co-surfactant Tween 80 was obtained from Sigma (Spain). Dichloromethane and ethanol were obtained from MERCK. All chemicals were used as received.

2.2 Methods

2.2.1 Geranium oil Characterization

The geranium oil was stored at 4°C until use. The quality-quantitative analysis of the geranium oil was performed using gas chromatography (GC) and gas chromatography/mass spectrometer (GC/MS). GC analyses were performed by employing a Perkin-Elmer gas chromatography (Model 8500) fitted with flame ionization detector (FID), and using a bonded phase fused silica capillary column BP-1 (25 m length

x 0.5 mm i.d.; film thickness 0.25 μm) coated with polydimethylsilixone. Nitrogen at a flow rate of 40 ml/min (linear velocity 34 cm/s) and 10 psi inlet pressure was the carrier gas employed. Experiments were conducted from 60 to 220°C at 5°C/min ramp rate with a final hold time of 10 min. Injector and detector were maintained at 250°C and 300°C, respectively. The samples (0.1-0.2 μl) were injected neat with 1:80 split ratios. The oil components were identified by comparing their relative retention time (RT) with those of authentic samples or by comparing their retention index (RI) relative to the series of n-hydrocarbons, and computer matching against commercial library and homemade library mass spectra made up of pure substance and components of known oils and MS literature data.

2.2.2 Preparation of Solid Lipid Nanoparticle

SLNs were prepared using ultrasonic-solvent emulsification technique at 45°C-50°C. 5-15 % (w/w) stearic acid and 8 % (w/w) geranium oil were mixed with 5-15 % (w/w) dichloromethane and heated to 50°C. 0.5-3 % (w/w) emulsifiers (soy lecithin/Tween 80) were dispersed in 10mL distilled water with magnetic stirring at the same temperature. After evaporating most of the solvents, the water phase was added to the oil phase drop-by-drop at 50°C followed by magnetic stirring for 10 min. Then the coarse emulsion was subjected to 55 W of ultrasonic treatment for 5 min using a high-intensity probe ultra sonicator with water bath (0°C). The dispersions were immediately dispersed in bulks of distilled water (0°C) followed by magnetic stirring to remove traces of organic solvents, if any. After the solvents had completely evaporated, the geranium oil-SLNs suspensions were filtered through a 0.45μm membrane in order to remove the impurity materials (e.g. metal) carried during ultrasonication and then stored at 4°C.

2.2.3 Characterization of Solid Lipid Nanoparticles

Encapsulation Efficiency

Encapsulation efficiency (EE %) is expressed as a percentage of the total amount of *geranium* oil found in the formulation at the end of the preparation procedure. One milliliter geranium oil SLNs was separated using Sephadex-G50 column (13.5 cm x 2.0 cm) and washed with distilled water at a flow rate of 2.0ml/min. The entrapped and free geranium oil was respectively collected at continuous volume intervals of 2.0ml. The collected samples were extracted and diluted with 3ml methanol for 1 hour in an ultrasonic bath. After extraction, the water was hydrated using sodium sulfate and then the geranium oil content in methanol was filtered through 0.2μm filter before being measured using GC (as described in Geranium oil Characterization), where the most important component of the geranium oil, geraniol, was used as a "lead".

The percentage of geranium oil incorporation was then calculated using a calibration curve from the range of 0.0003-0.0024 g/ml of standard geraniol which was dissolved in methanol. EE was calculated according to the following equation:

$$EE = \left[\frac{T - S}{T} \right] \times 100\% \quad (1)$$

*** T = Total quantity of incorporated and non-incorporated oil in the SLNs
*** S = the non-incorporated oil quantity separated with gel chromatography

Particle Size Analysis

The mean particles size and particle size were measured using photon cross correlation spectroscopy (PCCS) employing a NANOPHOX (Sympatec GmbH) using a 3D cross correlation technique controlled by WINDOX 5 software. The aqueous SLNs were diluted with distilled water before starting the measurement. Percent of dilution of samples was in the range of 400-500. The particle size was the average of 3 measurements.

Transmission Electron Microscopy (TEM)

TEM was used to characterize the structure of geranium oil-SLNs. SLNs were placed on a carbon-coated copper grid (slide) and then a drop of 1% phosphotungstic acid was dropped on the SLNs. The superfluous phosphotungstic acid on SLNs was wiped off by filter paper. The TEM images were obtained using a Tecnai G2 20 TEM (FEI Corp. German). The image was obtained when a projector shined a beam of light through the slide and as the light passed through it was subjected to changes by the structure and object on the slide. These effects resulted in only certain parts of the light beam transmitted through certain parts of the slide. This transmitted beam was then projected onto the viewing screen, forming an enlarged image of the slide. Image obtained from a TEM are two dimensional sections of the material.

3.0 RESULTS AND DISCUSSION

3.1 Geranium oil Characteristics

The essential oil of geranium oil has a pale yellow color and a rose mint odor. Table 1 shows the composition of the most abundant molecules of the essential oil. Monoterpene geraniol, citronellol, eugenol, isomethone and linalool represented more than 25% of the essential oil. Geraniol (7.89%) and citronellol (7.73%) were the main components for the geranium oil. These components are believed to exhibit potential as mosquito repellents [10].

Table 1 Main Components of Geranium oil as Determined by GC*

Components	Retention time R_f	Area %
Linalool	15.304	4.53
Isomenthonel	16.521	2.82
B-Citronellol	20.780	7.73
Geraniol	22.184	7.89
Eugenol	27.529	5.42

*GC indicates gas chromatography

3.2 Morphology

Geranium oil loaded solid lipid nanoparticle were successfully prepared using ultrasonic-solvent emulsification technique at a temperature range of 45°C – 50°C. A water-in-oil microemulsion was spontaneously obtained as recognized by the formation of a cloudy solution after the addition of the heated water phase into the oil phase of the same

temperature. The SLNs were obtained after sonicating the warm microemulsion in a water bath at 0°C and then dispersing the cold nanoemulsion into cold water with the aid of a homogenizer. The cold water facilitated rapid lipid crystallization and prevented lipid aggregation. Transmission Electron Microscopy (TEM) revealed that geranium oil loaded SLNS were spherical in shape (Figure 1).

Figure 1 TEM micgrographs of geranium oil loaded solid lipid nanoparticles consisting of stearic acid 5% (w/w), Tween 80 and soy lecithin 0.5% (w/w) and dichloromethane 15% (w/w)

3.3 Particle size

The mean particle size of the geranium oil loaded SLNs were determined by PCCS. The results showed that the amount of lipid, emulsifier and co-emulsifier were critical parameters for the size of the nanoparticles.

The effect of stearic acid concentration on the particle size was evaluated by varying the concentration from 5%, 10% to 15% (w.w) while maintaining the amount of soy lecithin and Tween 80 at 0.5 % (w/w) and dichloromethane at 15% (w/w). The result showed that when increasing lipid concentration from 5-15% (w/w), size distribution with the mean particle size increased from 46 to 86 nm (Table 2). This finding was in agreement with Tiyaboonchai *et al.* [8] who reported that increasing the lipid content over 5-12.5% (w/w) in most cases will result in large particle sizes. Therefore, 5% (w/w) stearic acid was found to be an optimum concentration for the formulation of the SLNs.

Table 2 Effect of stearic acid concentration on the particle size of geranium oil loaded solid lipid nanoparticle

Stearic acid (%, w/w)	Mean Particle Size (nm ± S.D.)
5	46.03±1.33
10	56.35±0.51
15	86.46±0.44

Composition of the SLNSs: Tween 80 and soy lecithin, 0.5% (w/w); dichloromethane, 15% (w/w)

The effect of the amount of emulsifier and co-emulsifier, soy lecithin and Tween 80, on the particle size was studied by varying the amount of each emulsifier from 0.5, 1.5 to 3 % (w/w) while maintaining the amount of stearic acid at 5% (w/w) and dichloromethane at 15% (w/w), respectively. An optimum particle size with narrow size distribution was obtained with 0.5% (w/w) of each emulsifier. When the amount of the soy lecithin and Tween 80 were increased, the particle sizes were also increased (Table 3 and 4).

Table 3 Effect of soy lecithin concentration on the particle size of geranium oil loaded solid lipid nanoparticle

Soy Lecithin (%, w/w)	Mean Particle Size (nm ± S.D.)
0.5	46.03±1.33
1.5	77.63±0.14
3.0	114.22±9.28

Composition of the SLNs: stearic acid, 5% (w/w); Tween 80, 0.5% (w/w); dichloromethane, 15% (w/w)

Table 4 Effect of Tween 80 concentration on the particle size of geranium oil loaded solid lipid nanoparticle

Tween 80 (%, w/w)	Mean Particle Size (nm ± S.D.)
0.5	46.03±1.33
1.5	86.27±1.72
3.0	60.17±0.71

Composition of the SLNs: stearic acid, 5% (w/w); soy lecithin, 0.5% (w/w); dichloromethane, 15% (w/w)

The effect of dichloromethane on particle size was studied by varying the amount of dichloromethane from 5%, 10%, 15% and 20% (w/w) while maintaining the amount of stearic acid at 5% (w/w), soy lecithin at 0.5% (w/w) and Tween 80 at 0.5% (w/w). Dichloromethane act as a co-emulsifier and geranium oil co-solvent. As expected, the results revealed that when increasing the concentration of dichloromethane from 5% to 15%, the particle size decreased from 73 to 46 nm, respectively (Table 5). However, the

increase of the dichloromethane concentration to more than 15% (w/w) resulted in a larger mean particle size, 65 nm, and a broad size distribution.

Table 5 Effect of dichloromethane concentration on the particle size of geranium oil loaded solid lipid nanoparticle

Dichloromethane (%, w/w)	Mean Particle Size (nm ± S.D.)
5	73.43±3.39
10	57.82±0.59
15	46.03±1.33
20	65.57±1.99

Composition of the SLNs: stearic acid, 5% (w/w); Tween 80 and soy lecithin, 0.5% (w/w)

The variation in the particle size of SLNS may be due aggregation as a result of intrinsic thermodynamic instability of the nanoparticle system with dispersed molecules of the surfactant and co-surfactant in the lipid matrix that finally resulted in the absorption of the emulsifier to the particle surface [11]. At very low concentrations, the emulsifier is absorbed directly onto the surface of the particles. However, at high concentrations of the emulsifier, compression of the emulsifier molecules at the particle surface with formation of loops and tails become prominent and finally lead to the bridging between the primary nanoparticles.

3.4 Determination of geranium oil encapsulation efficiency

High geranium oil incorporation efficiency in the range of 93-99 % (w/w) was demonstrated. The experiment results indicate that the concentration of lipid, emulsifier and co-emulsifier have critical effects on the geranium oil incorporation efficacy (Figure 2). Geranium oil is poorly soluble in water. They are soluble in alkaline medium and dichloromethane. The effect of the amount of lipid on the entrapment efficacy was studied by maintaining the amount of surfactant while varying the amount of lipid. The result showed that the entrapment efficiency increased as the amount of lipid, surfactant and cosurfactant increased (Figure 2).

The geranium oil incorporation efficacy increased with the increasing amount of stearic acid, soy lecithin and dichloromethane (Figure 2). The increase was not so significant for Tween 80. Most probably, when the amount of emulsifier and co-emulsifier increased at a constant amount of lipid, the particles have a large surface to absorb all surfactant and co-surfactant molecules, which will result in the formation of a micellar solution of the active ingredients. Hence the solubility of the active ingredients in the water phase will be decreased [12].

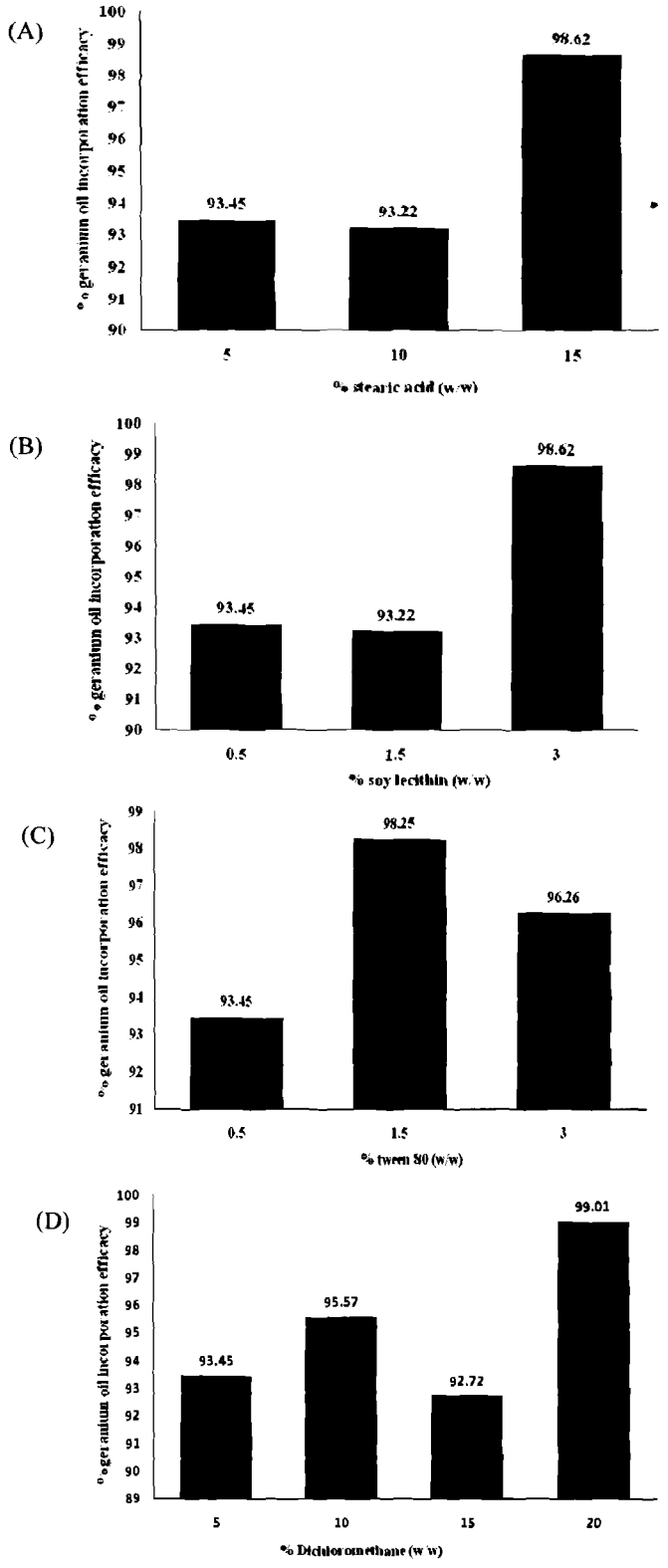

Figure 2 Incorporation efficiency of geranium oil on the surface of SLNs formulation with different amounts of (A) stearic acid; (B) soy lecithin; (C) Tween 80; (D) dichloromethane

4.0 CONCLUSIONS

Geranium oil loaded SLNs were successfully prepared using ultrasonic-solvent emulsification technique. The amount of lipid and emulsifier were found to be crucial factors in obtaining particles with acceptable size and encapsulation efficiency. The formulation that consisted of stearic acid 5% (w/w), Tween 80 0.5% (w/w), dichloromethane 15% (w/w) and soy lecithin 3% (w/w) was chosen for further investigation as the size of the SLNs obtained were over 100 nm and the EE was at a respectable 98%. The size of the SLNs must not be too small as this might aid penetration into skin.

ACKNOWLEDGEMENTS

We would like to thank Mr. Nordin Ismail and Mr. Johadi (Department of Chemical, Faculty of Science, UPM), Mr. Mohamed Mohd Salleh (Institute of Ibnu Sina, UTM) and Miss Nur Fashya Musa (CEPP, UTM). This project was funded under eScienceFund (project no: 02-01-06-SF0075), Ministry of Science, Technology and Innovation, Malaysia.

REFERENCES

[1] Wissing, S. A. and R. H. Muller. 2003. Cosmetic Application for Solid Lipid Nanoparticle (SLN). *International Journal of Pharmaceutics.* 254: 65-68.

[2] Mehnert, W. and K. Mader. 2001. Solid Lipid Nanoparticles Production, Characterization and Applications. *Advanced Drug Delivery Reviews.* 47: 165-196.

[3] Muller, R. H., M. Radtke, and S. A. Wissing. 2002. Solid Lipia Nanoparticle (SLN) and Nanostructured Lipid Carriers (NLC) in Cosmetic and Dermatological Preperations. *Advanced Drug Delivery Reviews.* 1: 131-155.

[4] Qui, H., J. W. McCall and H. W. Jun. 1998. Formulation of Topical Insect Repellent N, N-Diethyl-m-toluamide (DEET): Vehicle Effect on DEET in Vitro Skin Permeation. *International Journal of Pharmaceutics.* 163: 167-179.

[5] Balchin, M. L. 2002. History of Nomenclature, Usage and Cultivation of Geranium and *Pelargonium* species. In: Balchin, M. L. *Geranium and Pelargonium: The genera Geranium and Pelargonium.* Londan & New York: Taylor & Francis. 5-10

[6] Omolo, M. O., D. Okinyo, I. O. Ndiege, W. Lwande and A. Hassanali. 2004. Repellency of Essential Oils of Some Kenyan Plants against *Anopheles gambiae. Photochemistry.* 65: 2797-2802.

[7] Lai, F., S. A. Wissing, R. H. Muller and A. M. Fadda. 2006. Artemisia arborescens L Essential oil-Loaded Solid Lipid Nanoparticle for Potential Agriculture Application: Preperation and Characterization. *AAPS PharmSciTech.* 7: 1-8

[8] Tiyaboonchai, W., W. Tungpradit and P. Plianbangchang. 2007. Formulation and Characterization of Curcuminoids Loaded Solid Lipid Nanoparticle. *International Journal of Pharmaceutics.* 337: 299-30

[9] Verma, D.*D., S. Verma, G. Blume and A. Fahr. 2003. Particle Size of Liposomes Influnces Dermal Delivery of Substances Into Skin. *International Journal of Pharmaceutics.* 258: 141-151.

[10] Zaki, Z.M., N. M. A. Azah, A. A. Said and M. Z. Faridz. 2005. Four Monoterpene Compounds: Repellency Evaluation Againts Aedes aegypti. *Malaysian Journal of Science*. 24: 225-228.

[11] Goppert, T. and R. H. Muller. 2005. Protein adsorption patterns on Poloxamer and Poloxamine-stabilized solid lipid nanoparticle (SLNS). *Eur. J. Pharm. Biopharm.* 60: 361-372

[12] Gohel, M., M. Panchal and V. Jogani. 2000. Novel mathematical method for quantitative expression of deviation from the Higuchi model. *AAPS Pharm. Sci. technol.* 1: 1-6