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Formulation and characterization of nanostructured lipid carrier encapsulated *Zingiber zerumbet* oil using ultrasonication technique

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Abstract – *This study presents the formulation of nanostructured lipid carrier encapsulated Zingiber zerumbet oil (NLC-ZZ) using ultrasonication technique. NLC is the blend of solid lipid, liquid lipid and surfactant for encapsulation of poor water soluble actives. The NLC-ZZ formulation was characterized with respect to particle size, polydispersity index (PDI), zeta-potential, encapsulation efficiency and physical morphology. The NLC-ZZ formulation had an average diameter of 96.59 nm, PDI of 0.192, zeta-potential of -39.88 mV, and encapsulation efficiency of 90%, respectively. The NLC formulation for Zingiber zerumbet oil encapsulation has been successfully developed and is suitable for transdermal delivery system due to their nano-size and stability.*

Keywords: Drug delivery, Nanostructured lipid carrier, Zingiber zerumbet, nanoparticle, ultrasonication

1.0 INTRODUCTION

Zingiber zerumbet, commonly known as pinecone or shampoo ginger is a perennial, tuberous root herb plant usually found in damp, shaded parts of lowland or hill slopes, as scattered plants. *Zingiber zerumbet* is believed to be native to India and Malaysian Peninsula. *Zingiber zerumbet* can be characterized by the presence of a pulvinus between the base of the petiole and ligule and it is also a variegated wild edible ginger with stems of approximately 1-2 m tall that erect, oblique, round, annual and are invested by the smooth sheaths of the leaves [1]. *Zingiber zerumbet* is traditionally used as spices, flavouring agent, and medicines due to their medicinal properties. The usage as widely known traditional medicine includes the treatment of inflammation, fever, toothache, indigestion, constipation, diarrhoea, sprains, to relieve pain, and as diuretic agent [2]. The volatile oil of *Zingiber zerumbet* contains zerumbone as the major component and it is a natural occurring cyclic sesquiterpene. Zerumbone component constitutes about 37% of the whole *Zingiber zerumbet* content [3]. Recent researches have revealed zerumbone potential as antioxidant, anti-pyretic, anti-cancer, antimicrobial, anti-obesity, anti-bacterial, and hepatotoxicity activity [4-6].

Nanostructured lipid carriers (NLCs) are one of the drug delivery systems which developed as active ingredient carriers in biological, pharmaceutical, medical, nutritional and cosmeceutical researches. NLC is composed of a lipid core, consisting of a mixture of solid and liquid lipids, dispersed in aqueous emulsifier solution, and has nanometer range of size [7]. The NLC

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structure protects the bioactives from enzymatic, chemical, temperature, and ionic changes in different environments, implying that the NLC composition is responsible in the fate of NLC as carrier. NLC is commonly synthesized using high pressure homogenizer, ultrasonication, emulsification, and solvent diffusion method [7-10]. This study presents the development of local plants (*Zingiber zerumbet*) oil to be encapsulated in nanostructured lipid carrier and explores the potential to be utilized in pharmaceutical and nutraceutical industry. The developed NLC carrier must establish stable physical and chemical characteristics in order to be used in different route of delivery into human body, especially transdermal and topical delivery route. In this work, we developed NLC encapsulated *Zingiber zerumbet* (NLC-ZZ) using ultrasonication technique and characterized the NLC through particle size, polydispersity index, zeta potential, and entrapment efficiency analysis as well as morphology structure of NLC-ZZ. The stability of NLC-ZZ in various storage conditions is also determined for 90 days based on particle size measurement.

2.0 METHODOLOGY

2.1 Materials

Virgin coconut oil and *Zingiber zerumbet* oil were obtained from the Institute of Bioproduct Development (Universiti Teknologi Malaysia, Malaysia). Tween 80, Sephadex G-50, and soy lecithin were obtained from Sigma-Aldrich (Selangor, Malaysia). Other chemicals and solvents used such as methanol, 2-propanol, and glyceryl monostearate were of analytical reagent grade and pharmaceutical grade. Water used in all experiments was distilled water.

2.2 NLC-ZZ preparation

The composition of ingredients in NLC was prepared following the formulation in Table 1, in which lipid percentage is varied, while *Zingiber zerumbet* and surfactant composition are kept constant. First, certain amount of solid lipid (glyceryl monostearate) and liquid lipid (virgin coconut oil) were blended and melted at 50°C to form a uniform and clear lipid phase. The *Zingiber zerumbet* oil was subsequently added to the lipid phase and the heating temperature was ensured to maintain at 10°C above the melting temperature of glyceryl monostearate. Meanwhile, the aqueous phase was prepared by blending distilled water, Tween 80 and soy lecithin according to the correct ratio. Immediately, the aqueous mixture was added into the lipid mixture to form a pre-emulsion mixture. The pre-emulsion was then homogenized using IKA Ultra Turrax® homogenizer at 11 000 rpm for one minute. Then, the pre-emulsion mixture was ultrasonicated using probe sonicator for 20 minutes at 50 amplitudes. Subsequently, the NLC dispersion was cooled in ice water bath to room temperature (25±1°C) and stored at 4°C.

2.3 Particle size, polydispersity index analysis, and zeta potential analysis

Particle size, polydispersity index (PDI) and zeta potential analysis were performed using dynamic light scattering (DLS) method, also known as photon correlation spectroscopy (PCS) using a Malvern Zetasizer nano ZSP (Malvern instrument, UK). The NLC-ZZ sample was put in a standard capillary electrophoresis cell equipped with gold electrodes. The NLC-ZZ suspension was diluted (1:10) and vortexed to avoid multiple scattering effects and directly placed in the module. Each measurement was performed in triplicate at 25°C. Refractive indices of nanoparticles and water were set at 1.54 and 1.33, respectively.

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2.4 Encapsulation efficiency analysis

The encapsulation efficiency (EE) was calculated using Folin-Ciocalteu colorimetric method [11]. The NLC-ZZ suspension was separated by Sephadex gel-50 using mini spin column. Mini centrifuge was employed to separate encapsulated *Zingiber zerumbet* oil to unencapsulated *Zingiber zerumbet* based on size. The collected encapsulated ZZ sample and NLCs suspension were each diluted with a solvent (ethanol and 2-propanol) with a ratio of 1:3 and sonicated in a sonicator bath for 20 min to break the NLC wall. 500µL distilled water was added to 125 µL sample (separated encapsulated ZZ and NLC-ZZ suspension) and 125 µL Folin-Ciocalteu reagent. The mixture was left to stand for six minutes. Afterwards, 1.25 mL of 7% Na_2CO_3 was added to the mixture. About 1 mL distilled water was added to make a 3 mL solution. The solution was incubated for 90 minutes in the dark before the measurement was taken. The measurement was done at 760 nm wavelength (using gallic acid as reference) using a UV-vis spectrophotometer. A standard curve for gallic acid was prepared by dissolving 25 mg gallic acid in 25 mL distilled water. Concentrations of 0 to 450 µg/mL gallic acid were used to construct a calibration curve. Therefore, the percentage of encapsulation efficiency was calculated using the following equation (1):

$$\text{EE}\ (\%) = \frac{n_1}{n_2} \times 100 \quad (1)$$

where:

n_1 = total concentration of phenolic content in encapsulated *Zingiber zerumbet* oil

n_2 = concentration of phenolic content in nanostructured lipid carrier encapsulated *Zingiber zerumbet* suspension

2.5 Transmission electron microscopy

Transmission electron microscopy (TEM) was used to observe NLC-ZZ dispersion by using negative staining method. NLC-ZZ dispersion was spread on a 200-mesh copper grid coated with carbon membranes for about 3 min. Subsequently, any excess droplets were removed using filter paper. The grid was then placed above a drop of phototungstic acid solution (2%, w/w) for about 2 min and excess droplets were removed using filter paper. The grid was then observed using Hitachi H-7110 electron microscope (Japan).

2.6 NLC-ZZ stability study

5.0 mL volume of NLC-ZZ samples is placed in amber-coloured glass vials after preparation. The vials were stored at temperature 25°C, 10°C, and -2°C. The average particle size was taken on the 1st, 30th, 60th and 90th day from preparation day. The analysis was done in triplicate.

3.0 RESULTS AND DISCUSSION

Zingiber zerumbet oil loaded onto nanostructured lipid carriers was successfully prepared by an ultrasonication technique at temperature 70°C. An oil-in-water nanoemulsion was subsequently obtained after adding heated aqueous phase into the oil phase at similar temperature. NLC-ZZ was obtained immediately by dispersing the *Zingiber zerumbet* oil and ultrasonicated for 20 min. NLC-ZZ appeared as milky white solution after the sonication process and immediately quenched in cold bath to prevent further aggregation of nanoparticles. The prepared NLC-ZZ was stored at 4°C and utilized for further characterization. The

composition of lipid and *Zingiber zerumbet* oil tabulated in Table 1 was based on preliminary study previously done. The smallest NLC-ZZ size achieved was obtained using 5% lipid in the formulation. Therefore in this study, the lipid composition was maintained at 5% lipid, while the ratio between solid lipid and liquid lipid was varied. Five formulations were prepared and named as formulation A1, A2, A3, A4 and A5. The physical characterization was done based on the measurement of nanoparticle size, polydispersity index, zeta potential and encapsulation as recorded in Table 1. Usually, the stability can be observed through the characterization of nanoparticles in which no aggregation occurred for long time storage.

Table 1: NLC-ZZ composition (%) and the responded characterization. Data presented in mean ± standard deviation (SD) (n=3).

Formulation NLC-ZZ	Composition (%)			Physical characterization			
	Zingiber zerumbet Oil	Solid lipid	Liquid lipid	Particle size [nm] ± SD	Polydisperisity index ± SD	Encapsulation efficiency [%]± SD	Zeta Potential [mV] ± SD
A1	1.00	1.00	4.00	80.47±1.33	0.188±2.72	88.03±5.85	-38.9±2.11
A2	1.00	2.00	3.00	82.02±5.76	0.187±0.14	85.94±10.29	-42.3±0.35
A3	1.00	2.50	2.50	85.79±2.35	0.210±2.20	92.11±4.35	-37.8±2.49
A4	1.00	3.00	2.00	105.57±10.94	0.194±0.76	89.42±8.99	-39.8±5.76
A5	1.00	4.00	1.00	129.12±0.52	0.182±0.55	94.51±5.57	-40.6±2.58

NLC core has stacks of solid lipid and liquid lipid in its particle matrix, therefore the size may depend significantly on the lipid percentage in formulation. The lipid composition in this NLC-ZZ preparation was fixed at 5% composition but at various percentages of solid and liquid lipids, while the surfactant (Tween 80 and Soy Lecithin) composition was fixed throughout the experiment. In Table 1, the particle size achieved for the formulations was in nano-sized range, measuring from 80.47 nm to 129.12 nm, respectively. The particle size measured by Malvern Zetasizer Nano ZSP was basically presented as *z*-average diameter which denotes as hydrodynamic diameter. Referring to Table 1, the particle size was found to increase with the increment of solid lipid (glyceryl monostearate) in composition NLC-ZZ. The increase in size trend was observed from formulation A1 to A5. Similar observation was recorded in few researches in which the percentage of solid lipid was controlled to certain requirement to obtain the targeted size [7-9]. On the other hand, high solid lipid percentage, for example in formulation A4 and A5, may acquire extra sonication power during the preparation of NLC-ZZ as compared to formulation A1. Since the ultrasonication power and time was fixed throughout the experiment, NLC-ZZ with high solid lipid percentage has significantly increased in particle size as more sonication power and time is needed to further reduce the size [12]. This is due to the less dispersion energy offered per unit lipid which leads to bigger particle size.

Table 1 shows the formulation NLC-ZZ A1 to A5 that has the polydispersity index of less than 0.20, which denotes as uniformity between the particle sizes. It also plays an important role in determining the stability of nanoparticle besides nanoparticle size characteristics. Basically polydispersity index presents the width of the particle size distribution which ranges from 0 to 1 and gives us the insight of homogeneity of NLC-ZZ dispersion. The zeta potential also measured by similar Zetasizer Nano ZSP, shows negative charges for all NLC-ZZ formulation A1 to A5 which are favorable since it indicates long term physical stability and particle adhesive properties. Additionally, a system having ±30 mV zeta potential is considered as a stable formulation if dispersed in a liquid as colloidal dispersion [13]. High negative or positive

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value of zeta potential will cause nanoparticles to repel each other and prevent the tendency of aggregation.

The encapsulation efficiency is one of the important aspects in developing drug delivery carrier. It is presented as the percentage of encapsulation of actives inside the carrier. As referred in Table 1, encapsulation efficiency for all NLC-ZZ formulation shows promising encapsulation efficiency in which they had more than 80% encapsulation of *Zingiber zerumbet* oil. Although the encapsulation efficiency has no significant difference for various lipid concentration ratios, the efficiency was expected to increase with the increase of lipid composition. Formulation A5 shows the highest efficiency of 94.51% in which provides more space for *Zingiber zerumbet* encapsulation, however the size was the biggest compared to other formulation. The encapsulation process was due to the formation of lipid matrix by solid lipid and liquid lipid which contain imperfect crystal lattice, therefore provide space for *Zingiber zerumbet* oil loading [14].

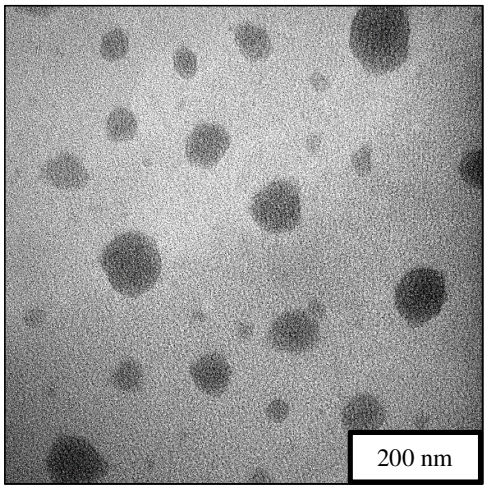

Figure 1: Morphology of NLC-ZZ formulation A1 observed under transmission electron (magnification of 150 000).

The TEM micrograph in Fig. 1 portrays that *Zingiber zerumbet* oil loaded NLCs are spherical in shape with smooth morphology and also show no aggregation of particle. The spherical NLC-ZZ is in nanometer-sized range and has good size distribution. The mean diameters observed were between 50 nm to 90 nm. The observation was similarly recorded by Gomes et al. [15] and Manea et al. [16], in which they found the lipid nanoparticles exhibited spherical shape and smooth surface, regardless the type of lipid used in the experiment. Different measurement principle and different sample preparation has caused the difference in size obtained by the TEM and particle sizer [17].

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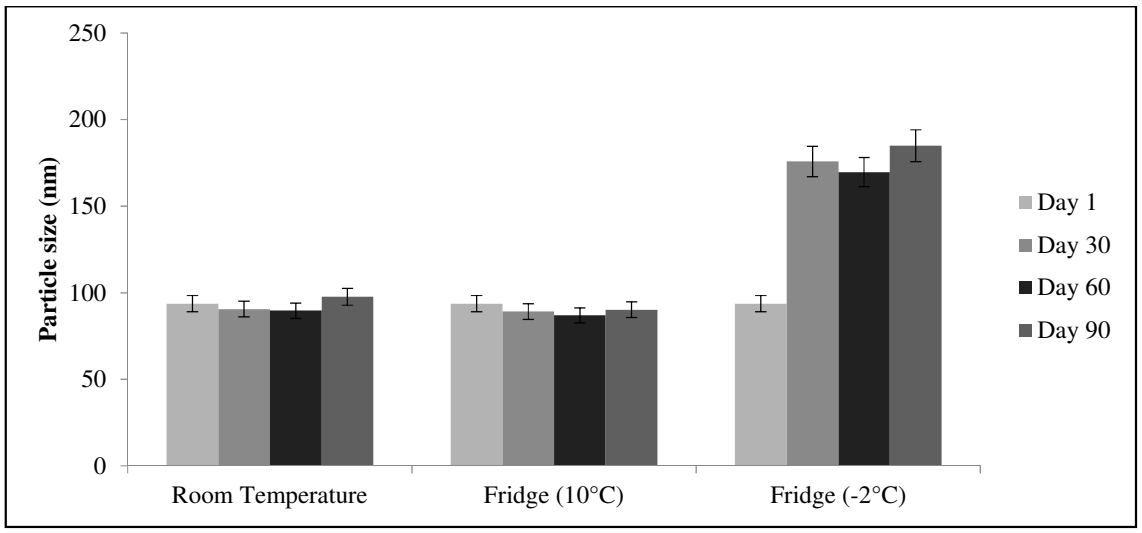

Figure 2: NLC-ZZ formulation A1 stability based on particle size observed for 90 days in storage at room temperature, 10°C, and -2°C. Data presented in mean ± standard deviation (SD) (n=3).

Figure 2 shows the stability of NLC-ZZ formulation A1 for 90 days in three different storage temperatures based on particle size measurement. It is observed that particle size for NLC-ZZ stored in 10°C was the most stable as the size difference from day 1 to day 90 is almost insignificant. The trend is followed by NLC-ZZ stored in room temperature however, slight increase was observed on day 90. The NLC-ZZ stored in -2°C freezer experienced remarkable size increment and might be due to the aggregation of lipid particle. The stability in room temperature and 10°C has given insight on promising stability for the incorporation of NLC-ZZ in cosmeceutical, nutraceutical and pharmaceutical product.

4.0 CONCLUSSION

In this work, nanostructured lipid carrier encapsulated *Zingiber zerumbet* oil has been successfully developed using ultrasonication technique with various lipid composition. The NLC-ZZ exhibited nanometer size, stable polydispersity index and also zeta potential charge. The encapsulation efficiency shows the ability of lipid carrier to encapsulate *Zingiber zerumbet* oil more than 80% efficiency. Due to the size and stability of NLC-ZZ, it is suitable for topical and transdermal delivery since it can enhance the penetration to the deeper layer of skin. The utilization of local plant herbs in this experiment also revealed the potential of *Zingiber zerumbet* oil to be used in pharmaceutical and nutraceutical application. Optimization of these NLC-ZZ mixture formulations can be done to assist in feasible large scale production.

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REFERENCES

[1] M. Sabu, Revision of the genus Zingiber in South India, Folia Malesiana, 4 (2003) 25-52.

[2] N.J. Yob, S.M. Jofrry, M.M. Affandi, L.K. Teh, M.Z. Salleh, Z.A. Zakaria, Zingiber zerumbet (L.) Smith: A Review of Its Ethnomedicinal, Chemical, and Pharmacological Uses, Evidence-based Complementary and Alternative Medicine 2011 (2011) 1-12.

[3] N.P. Damodaran, S. Dev, Studies in sesquiterpenes—XXXVII: Sesquiterpenoids from the essential oil of zingiber zerumbet smith, Tetrahedron, 24 (1968) 4113-4122.

[4] M.N. Somchit, A.A. Bustamam, A. Zuraini, Anti-pyretic and Analgesic Activity of Zingiber zerumbet, International Journal of Pharmacology 1 (2005) 277-280.

[5] R. Prasannan, K.A. Kalesh, M. K. Shanmugam, A. Nachiyappan, L. Ramachandran, A.H. Nguyen, A.P. Kumar, M. Lakshmanan K.S. Ahn, G. Sethi, Key cell signaling pathways modulated by zerumbone: Role in the prevention and treatment of cancer, Biochemical Pharmacology 84 (2012) 1268-1276.

[6] Y.I. Mohamed, I.A.W. Siddig, M.E. Manal, S.A. Adel, M.M. Syam, Attenuation of cisplatin-induced hepatotoxicity in rats using zerumbone, Research Journal of Biological Sciences 4 (2009) 777-784.

[7] R.H. Müller, R.D. Petersen, A. Hommoss, J. Pardeike, Nanostructured lipid carriers (NLC) in cosmetic dermal products, Advance Drug Delivery Review 59 (2007) 522-530.

[8] J. Pardeike, S. Weber, T. Haber, J. Wagner, H.P. Zarfl, H. Plank, A. Zimmer, Development of an itraconazole-loaded nanostructured lipid carrier (NLC) formulation for pulmonary application, International Journal of Pharmaceutical 419 (2011) 329-338.

[9] A.P. Nayak, W. Tiyaboonchai, S. Patankar, B. Madhusudhan, E.B. Suoto, Curcuminoids-loaded lipid nanoparticles: novel approach towards malaria treatment, Colloids and Surfaces B: Biointerfaces 81 (2010) 263-273.

[10] H. Shete, V. Patravale, Long chain lipid based tamoxifen NLC. Part I: Preformulation studies, formulation development and physicochemical characterization, International Journal of Pharmaceutics 454 (2013) 573-583.

[11] S. Kaur, P. Mondal, Study of total phenolic and flavonoid content, antioxidant activity and antimicrobial properties of medicinal plants, Journal of Microbiology and Experimentation 1 (2014) 1-6.

[12] C.H. Loo, M. Basri, R. Ismail, H.L.N. Lau, B.A. Tejo, M.S. Kanthimathi, H.A. Hassan, Y.M.Choo, Effect of compositions in nanostructured lipid carriers (NLC) on skin hydration and occlusion, International Journal of Nanomedicine 8 (2013) 13-22.

[13] F. Han, S. Li, R. Yin, H. Liu, L. Xu, Effect of surfactant on the formation and characterization of a new type of colloidal drug delivery system: nanostructured lipid carriers, Colloids and Surfaces A: Physiochemical and Engineering Aspects 315 (1-3) (2008) 210-216.

Journal of Advanced Research in Applied Mechanics
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[14] E.B. Suoto, S.A. Wissing, C.M. Barbossa, R.H. Müller, Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery, International Journal of Pharmaceutics 278 (2004) 71-77.

[15] M.J. Gomes, S. Martins, D. Ferreira, M.A. Segundo, S. Reis, Lipid nanoparticles for topical and transdermal application for alopecia treatment: development, physicochemical characterization, and in vitro release and penetration studies, International Journal of Nanomedicine 9 (2014) 1231-1242.

[16] A.M. Manea, B.S. Vasile, A. Meghea, Antioxidant and antimicrobial activities of green tea extract loaded into nanostructured lipid carriers, Comptes Rendus Chimie 17 (2014) 331-341.

[17] M. Zheng, M. Falkeborg, Y. Zheng, T. Yang, X. Xu, Formulation and characterization of nanostructured lipid carriers containing a mixed lipids core, Colloids and Surfaces A: Physiochemical and Engineering Aspects 430 (2013) 76-84.