

## FORMULATION AND CHARACTERIZATION OF [6]-GINGEROL LOADED NANOSTRUCTURED LIPID CARRIER (NLC)

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**Abstract.** Nanostructured lipid carrier (NLC) is the blend of solid lipid, liquid lipid and suitable surfactant for the purpose of encapsulated poorly water soluble drugs. [6]-gingerol is the main bioactive compound in *Zingiber officinale*, widely known as ginger in Malaysia. *Zingiber officinale* extract has been discovered to have anti-oxidant, anti-inflammatory, and anti-microbial effects to human body. The aim of this study is to develop NLC formulation for [6]-gingerol and to estimate the potential of NLC as a delivery system for these water insoluble drugs. In this work, the preparation of ginger oil loaded onto nanostructured lipid carrier (GO-NLC) was done by using ultrasonication method. The GO-NLCs were assessed by evaluating the morphology and its entrapment efficiency. The morphological study was performed by using Zetasizer Nano S and the entrapment efficiency analysis of NLC was performed using HPLC by detecting [6]-gingerol as active biomarker. The average particle size for GO-NLCs ranged from size 100 to 250 nm and the average encapsulation efficiency was  $92.7 \pm 3.03\%$ . Based on analysis, it is proved that nanostructured lipid carriers has high potential to be nanocarriers for [6]-gingerol.

**Keywords** Nanostructured lipid carrier; *Zingiber officinale*; [6]-gingerol; transdermal drug delivery; ultrasonication

## 1.0 INTRODUCTION

Ginger, the rhizome of *Zingiber officinale Roscoe*, belongs to the family *Zingiberaceae*. It is cultivated in many tropical and subtropical countries including China, India, Nigeria, Australia, Jamaica, and Haiti. Primarily, ginger has been consumed as spice throughout the globe and widely known to have medicinal values including remedy for arthritis, rheumatism, sprains, muscular aches, pains, sore throats, cramps, constipation, indigestion, vomiting, hypertension, dementia, fever, infectious disease and helminthiasis [1]. To date, more than 400 chemical constituents from ginger have been isolated or detected which include zingiberene and oleoresin [2]. The oleoresins from ginger rhizome have been found to possess many interesting pharmacological and physiological properties. One such compound, [6]-gingerol (5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-decan-3-one), is the most abundant compound found in fresh ginger. Recent studies have shown that [6]-gingerol possess anti-cancer [3], anti-inflammatory [4], anti-pyretic [5], anti-oxidant and anti-diabetic properties [6]. Ginger essential oil recently has been commercialized internationally as flavoring agent and additive for food and pharmaceutical industries. However, the lipophilic property of [6]-gingerol is unfavorable to be directly incorporated into formulation. Hence as an alternative, NLC is developed as it is composed of a solid lipid matrix with certain content of liquid lipid which useful to increase the solubility of lipophilic compound.

Nanostructured lipid carrier is a type of carrier that has been developed since early 2000 by Prof. Rainer H. Müller. NLC is composed of solid lipid core, consisting of a mixture of solid and liquid lipids, dispersed in an aqueous emulsifier solution and having nanometer range of size. NLC embraced to be successor to solid lipid nanoparticles (SLN) in term of superior drug loading capacity [7]. The molecular structure of SLN and NLC give the space to drug and the usual particle size were in the range approximately 10-1000nm. The small size of lipid nanoparticles ensures close contact to target cell and able to increase the amount of drug penetration into target cell, therefore providing greater efficacy as delivery system. NLC also has the potential to increase solubility and improve bioavailability of poor water soluble and/or lipophilic drug. Over the past several decades, much interest is focused on the design of more efficient drug delivery system to address problems such as low drug solubility. The particulate delivery systems must meet a number of characteristics including appropriate size

distribution, high drug loading, prolonged released, low cellular cytotoxicity, and cellular targeting. Therefore in this study, the preparation and characterization of NLCs encapsulates ginger oil is been highlighted. The characterization of oil-loaded nanoparticles is essential to predict their stability during storage as well as their systemic behavior. Any degradation or aggregation of nanocarriers, or the premature release of free drug before reaching the target tissues impair the purpose of nanocarriers and reduce the efficacy of drug.

Ultrasound is employed to prepare the nanostructured lipid carriers, being the hot homogenization technique the most commonly applied [8-10]. Nevertheless, NLC's preparation requires the use of appropriate devices which are not commonly available in research labs. On the other hand, ultrasonic is frequently used to disperse two immiscible phases, such as lipid and water, and can be easily applied to nanosuspensions of lipid materials. Ultrasonic is developed based on the extreme conditions generated within the collapsing cavitation bubbles of the inner phase leading to size reduction. The probes are practically self-cleaning and they account for negligible sample losses and it is can be used for high scale production. Therefore, in this study we aim to develop NLC formulation for encapsulation of ginger oil using probe ultrasonication and evaluate its morphology properties as well as encapsulation efficiency of NLC.

## **2.0 EXPERIMENTAL**

### **2.1 Materials**

Tween 80, Sephadex G-50 and soy lecithin were obtained from Sigma-Aldrich (Selangor, Malaysia). The liquid lipid, virgin coconut oil was obtained from Institute of Bioproduct Development (UniversitiTeknologi Malaysia, Malaysia). Chinese ginger oil was purchased from Wellness Original Ingredient,(Selangor, Malaysia). Other chemical and solvents used such as methanol and glyceryl monostearate were of analytical reagent grade and pharmaceutical grade. The water used in all experiments was distilled water.

## **2.2 Methods**

### **2.2.1 Preparation of NLC**

A certain amount of solid lipid (glyceryl monostearate) and liquid lipid (virgin coconut oil), range from 20% to 30%, w/w, were blended and melted at 70°C to form a uniform and clear lipid phase. The ginger oil subsequently added to the lipid phase and ensures heating temperature always maintained at 10°C above melting temperature of solid lipid. Meanwhile, the aqueous phase was prepared by blending Tween 80 and soy lecithin according to the ratio. Immediately, the aqueous mixture was added onto lipid mixture. The pre-emulsion were homogenized using IKA Ultra Turrax® Homogenizer at 11 000 rpm for one minute. The emulsions were ultrasonicated using probe sonicator for 5 to 20 minutes durations at 40-60 amplitudes. Subsequently, the NLC dispersion was cooled in ice water bath to room temperature and stored at 4°C.

### **2.2.2 Particle size and polydispersity index analysis**

Particle size and polydispersity index analysis were performed by dynamic light scattering (DLS), also known as photon correlation spectroscopy (PCS), using a Malvern Zetasizer Nano S (Malvern instrument, UK). Prior to measurements, all samples were diluted using distilled water and vortexed for 30 seconds to generate a suitable scattering intensity. Each measurement of GO-NLCs was performed in triplicate at 25°C. Refractive indices of particles and water were 1.54 and 1.33 respectively, were used to calculate particle size distributions and polydispersity index.

### **2.2.3 Encapsulation efficiency and drug loading capacity study**

The encapsulation efficiency and drug loading capacity analyses were performed based on analysis done previously with modifications [11-12]. The GO-NLCs suspension was separated by Sephadex gel-50 column (20mm x 130mm) chromatography by washing with distilled water at flow rate of 2.0 ml/min. The GO-NLCs suspensions were diluted with distilled water at ratio of 1:5. Approximately 1 ml of the diluted solution was pipetted into the column. 15 ml

solution was collected and the first 3 ml solution was discarded to get cloudy solution (concentrated part). The collected cloudy sample and GO-NLCs suspension were each diluted with methanol with a ratio of 1:1 and sonicated in sonicator bath for 20 min to break the particle. Upon analysis by HPLC, the sonicated samples were filtered using 2µm pore size syringe filter. The particles were evaluated by determining the amount of encapsulated [6]-gingerol in NLCs using HPLC (Waters, USA). The column used was Luna 5u C18 100Å (size 250 mm x 4.6 mm). The mobile phase consisted of methanol/water (65/35, v/v) and the flow rate was adjusted to 1 ml/min. The wavelength of detection was set at 280 nm. The calibration curve ranged from 20 to 100 µg/ml.

The encapsulation efficiency (EE) was calculated using the following equations:

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

where;

$n_1$  = total concentration of [6]-gingerol in ginger oil (total amount of ginger oil in starting solution)

$n_2$  = concentration of [6]-gingerol in encapsulated ginger oil

All measurements were performed in triplicate.

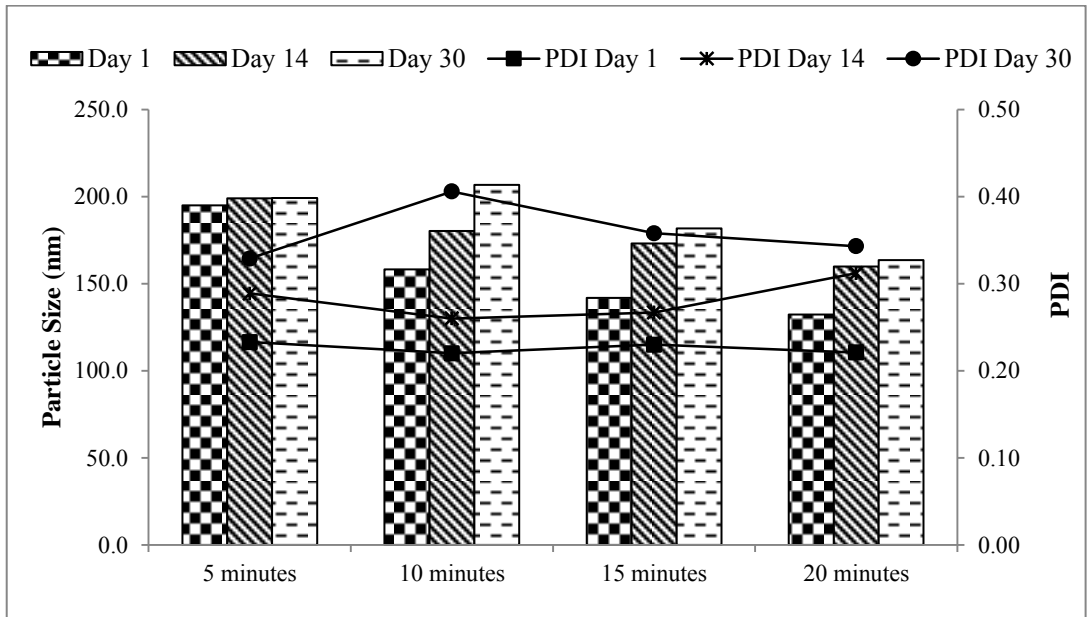
### **3.0 RESULTS AND DISCUSSION**

Ginger oil loaded nanostructured lipid carriers were successfully prepared by an ultrasonication of nanoemulsion at 70°C. An oil-in-water nanoemulsion was spontaneously obtained after adding heated aqueous phase into the oil phase at similar temperature. GO-NLCs were obtained immediately by dispersing the ginger oil and ultrasonicated for certain period of time. Milky white solution, GO-NLCs (Figure 3.1) were produced after the sonication and fast-quenched in cold bath to prevent further aggregation of nanoparticles. The prepared GO-NLCs were stored at 4°C and utilized for further characterization. For characterization analysis, particle size and polydispersity index were taken for different sonication time and power as well as for different percentage of lipid used in the formulation. The results were expressed in Figure 3.2 to 3.4 and Table 3.1, respectively.



**Figure 3.1:** Nanostructured lipid carrier loaded with ginger oil

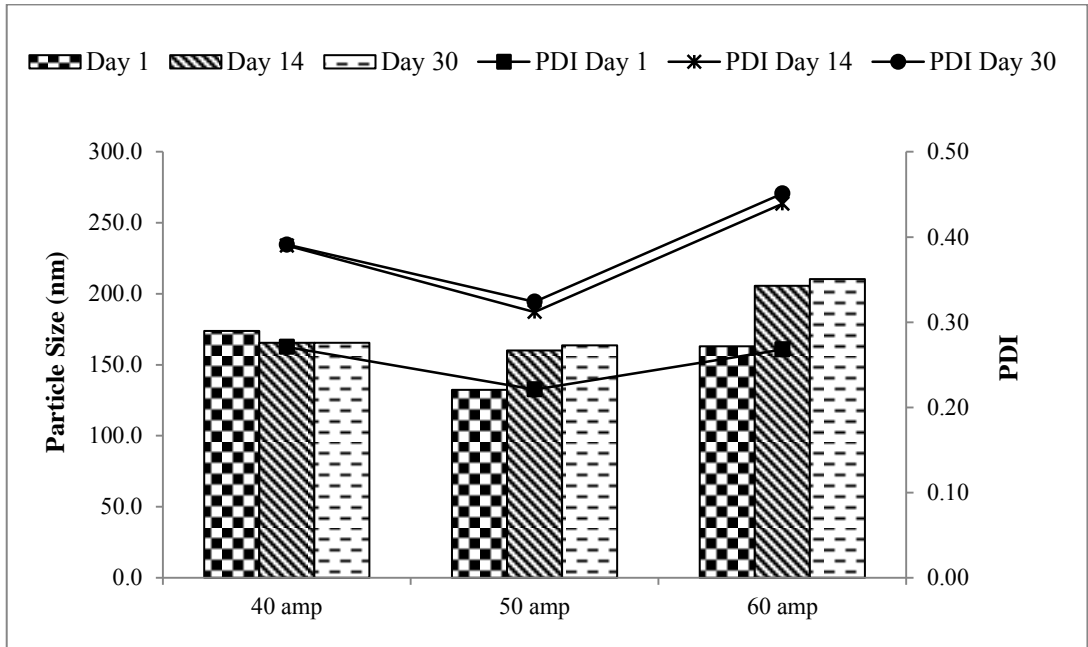
Particle size of the NLC is presented by z-average diameter, which is basically mean hydrodynamic diameter of the particles. In this formulation technique, sonication was used to breakdown the coarse emulsion drops to nano-scale size (nanoemulsion). Duration of sonication step in the process is considered to be a critical processing parameter since it involves the input of energy into the system. In this study, the GO-NLCs prepared using four different sonication times (5, 10, 15, and 20 min) and the power setting of 50 amplitude were evaluated. The formulation ratio for this experiment was kept constant for all four samples and maintained at 20% lipid percentage. The particle size and polydispersity index data for the four batches at initial time point, 14<sup>th</sup> days and 30<sup>th</sup> days were shown at Figure 3.2. The short term stability studies up to 30 days were demonstrated to know the stability of nanoparticles at room temperature.



**Figure 3.2:** Effect of sonication time on particle size and polydispersity index of GO-NLCs

As shown in Figure 3.2, preparation of GO-NLCs with different sonication times yielded particles with range of sizes from 132 to 195 nm which were measured right after the preparation. In the result, the particle size was slightly decreased with the increases of sonication time. Longer sonication time provides more energy for breakdown of large particles. However, longer sonication duration caused temperature of nanoemulsion to increase and this may elevate the evaporation rate of ginger oil. Longer sonication times also lead to aggregation of particles, thus producing larger size of nanoparticles. Therefore, the 20 min sonication was enough to control the temperature rise. Short term stability study also revealed that the size of GO-NLCs prepared with different sonication time was slightly increased over the studied time, 30 days. The GO-NLCs were left in room temperature and particle size measurement was taken on 14<sup>th</sup> and 30<sup>th</sup> day. The increased size was attributed to particle aggregation. Polydispersity index (PDI) indicates the width of the particle size distribution, which ranges from 0 to 1. Theoretically, monodisperse populations indicates PDI = 0. It should be noted that the PDI values for the studied formulations were lower than 0.3, which is considered an optimal value for the dispersion and homogeneity of these nanoparticles [13]. In general, at initial time point of preparation, all GO-NLCs

produced have PDI value less than 0.3. However, the PDI values increased as it left in room temperature for 30 days.

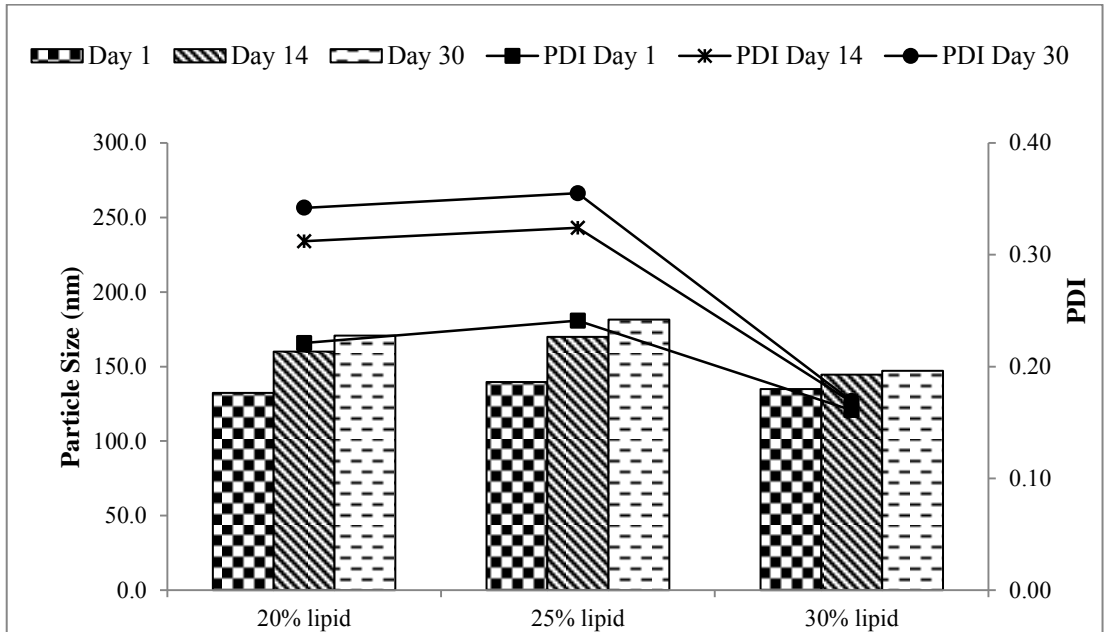


**Figure 3.3:** Effect of sonication power on particle size and polydispersity index of GO-NLCs

As in Figure 3.3, the sonication power between 40-60 amplitude has produced GO-NLCs with particle size that ranges between 132nm to 173nm which subsequently measured after preparation. For these particular experiments, the GO-NLCs were prepared by the same formulation and the sonication time were kept constant for 20 min. Short stability test for 30 days has revealed that the production of GO-NLCs by using 40 amplitude sonication exhibits reduction in particle size from 173.8nm to 165.4nm in 14 days. However, the GO-NLCs didn't experienced further reduction in term of particle size in day 30, which proved the short term stability of nanoparticles over time in room temperature. For GO-NLCs which were prepared using 50 and 60 amplitude of sonication power, the particle size exhibits significant increment, which ranged between 132.3nm to 163.6nm and 163.0nm to 210.3nm, respectively. For PDI value, all GO-NLCs which were produced with different sonication power have increased dramatically over the storage time. At initial time, the three samples shown PDI value lower



than 0.3, but after 30 days, it has increased above 0.3 which indicates the NLC's homogeneity were decreased compared to the fresh prepared NLCs.



**Figure 3.4:** Effect of lipid concentration in formulation on particle size and polydispersity index of GO-NLCs

In Figure 3.4, at initial time of preparation the particle size achieved for all formulation from 20% lipid to 30% lipid were 132.3nm, 139.7nm and 134.8nm, respectively. The preparations of GO-NLCs by using 30% of lipid in formulation has shown the lowest particle size increment which found to be 134.8 nm, 144.6 nm, and 147.1 nm respectively. At initial time of preparation, there is no significant difference for particle size with the change of lipid percentage. The GO-NLCs prepared from 30% lipid also exhibits no significant changes in particle size and PDI over 30 days as compared to GO-NLCs prepared from 20% and 25% lipid. The sonication time (20 min) and power (50 amp) were kept constant for all formulation of 20%-30% lipid percentage. Hence, the stability of GO-NLCs with high lipid content is better compared to one with slightly low lipid content. However, high lipid content may cause difficulty in handling due to semi-solid properties of GO-NLCs prepared. Thus, to overcome this problem, different types of surfactant or co-surfactant can be chosen to improve the properties of NLCs.

As depicted in Figure 3.2 to 3.4, the short stability study for 30 days has shown that GO-NLCs exhibits negligible particle size increases since it remain in nanosize range (<250 nm). [6]-gingerol is heat and light sensitive substance. Thus, it is important to maintain their stability during storage especially NLC as carrier for [6]-gingerol.

**Table 3.1:** Encapsulation efficiency of GO-NLCs at different lipid concentration

Sample	Encapsulation efficiency (%)
GO-NLC, 20% lipid	89.28
GO-NLC, 25% lipid	92.16
GO-NLC, 30% lipid	96.64

The encapsulation efficiency was expressed as percentage of the starting active ingredients and it was determined after separation of the free active ingredients by mini column Sephadex G-50. Entrapment efficiency obtained was in range of 89.28 to 96.64% reflected that almost all the ginger oil entrapped inside the lipid carrier. The satisfying values of encapsulation efficiency obtained in all sample can be attributed to the particular inner structure of NLC, where the combined use of a solid lipid matrix with a liquid lipid gives rise to a less compact structure, therefore able to accommodate loading of ginger oil. Although the encapsulation efficiency has no significant difference for various concentration of lipid in NLC formulation, the efficiency was expected to be high as lipid concentration increase. This is due to more lipids which can accommodate oil molecules at high concentration of lipid.

#### 4.0 CONCLUSIONS

In the present study, [6]-gingerol loaded nanostructured lipid carrier with particle size of 100-250 nm could be successfully obtained. After 30 days storage at room temperature, the mean particle size remained in the nanosize range and less than 250nm in all GO-NLCs formulation. High encapsulation efficiency has proved that NLC able to encapsulate ginger oil and possible to cope with various administration route of drug delivery. Advantages of using this ultrasonication approach include easy manufacturing process with mild preparation conditions,

use of biocompatible lipids, production in aqueous media avoiding organic solvents, and high encapsulation parameters. Extension of storage life and stability of GO-NLCs formulation may be achieved by manipulating the type of surfactant and ratio of lipid formulation. In future experiments, we hope that GO-NLCs can be further improvised and characterized to make it viable to be exploited in cosmeceutical and nutraceutical applications.

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