CHEMICAL ENGINEERING TRANSACTIONS
VOL. 83, 2021
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Jeng Shiun Lim, Nor Alafiza Yunus, Jiří Jaromír Klemeš

ISBN 978-88-95608-81-5; **ISSN** 2283-9216
DOI: 10.3303/CET2183080

Formulation and Characterization of Nanostructured Lipid Carrier Encapsulate Lemongrass Oil Using Ultrasonication Technique

Jia Yuan Ma[a], Rosnani Hasham[a,*], Zaitul Iffa Abd Rasid[b], Norhayati Mohamed Noor[b]

[a]School of Chemical and Energy Engineering, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia
[b]Institute of Bioproduct Development, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia
r-rosnani@utm.my

Lemongrass with scientific name *Cymbopogon citratrus* was widely found in tropical country. The aroma scent released by the lemongrass able to relieve anxiety and rejuvenate us. Lemongrass oil is suitable to use as one of the car freshener ingredient. However, the oil is easily evaporating when expose to high temperature environment. In order to preserve the fragrance release, the objective of this study is to formulate the nanostructured lipid carrier (NLC) encapsulated lemongrass oil using the ultrasonication technique. The characterization of the NLC-Lemongrass were particle size, polydispersity index (PDI), zeta potential, efficiency of the loading and the release rate. The NLC-Lemongrass oil formulation had an average mean particle size of 43.86 nm, PDI of 0.28, zeta potential -42.9 mV and efficiency of the loading 82.13 %, respectively. In addition, after 25 d, the component of the lemongrass oil inside the NLC able to remain at 53.24 %. Therefore, the NLC was successfully encapsulate the lemongrass oil and prolong the aroma release at high temperature environment.

1. Introduction

Cymbopogun citratus or known as lemongrass is commonly used in food preparation in Southeast Asia. The lemongrass is aromatic plant and able to grow up to 1.5 m (Charles, 2012). The fresh lower bulb of the lemongrass stalk is usually used in Malay or Thai food cooking, while the other part of this plant can be extracted into essential oil. The aroma scent of the lemongrass essential oil is fresh and lemony. The scent is suitable to be used as a car freshener to uplifting, refresh and scented the air. The oil of the lemongrass is yellowish and contain more than 75 % of biological active agent which is citral (Ha et al., 2008). Citral is one of the chemical marker compound in lemongrass oil, and contains broad biological properties such as antimicrobial, antifungal, anti-inflammatory and mosquito-repelling properties (Balti et al., 2017). However, citral is a fragile compound and easily lose its stability when exposed to high temperature environment. Encapsulation of citral is vital in order to protect the therapeutic properties and retain the properties over time.
The nanostructured lipid carrier (NLC) is the mixture of the liquid lipid, solid lipid and surfactant to encapsulate the lemongrass oil. NLC able to ensure the high loading capacity and maintain the sustainable release of the oil (Tamjidi et al., 2013). NLC also able to maintain the physical stability of the particle. This is due to partially crystallized and less ordered crystalline structure of the NLC (Tamjidi et al., 2013). By the structure of the NLC, high loading capacity of oil is able to be loaded inside the core of the solid lipid (Tamjidi et al., 2013). The ultrasonication technique is the technique that generate ultrasonic waves inside the liquid suspension (Taurozzi et al., 2012). It is referred as direct sonication since the ultrasound probe is directly immerse into the liquid suspension. Higher effective energy output is generated into the liquid suspension and effectively break the cluster into nano particle. Broad range of the condition of the formulation needed to be consider to determine the optimal sonication. This is because the effect of the variety of parameters of the sonication will affect the dispersion state of the liquid suspension (Lorimer et al., 2006). In this study, lemongrass oil was loaded into NLC system using ultrasonication technique.

Paper Received: 15/08/2020; Revised: 13/11/2020; Accepted: 20/11/2020
Please cite this article as: Ma J.Y., Hasham R., Abd Rasid Z.I., Mohamed Noor N., 2021, Formulation and Characterization of Nanostructured Lipid Carrier Encapsulate Lemongrass Oil Using Ultrasonication Technique, Chemical Engineering Transactions, 83, 475-480 DOI:10.3303/CET2183080

2. Methodology

2.1 Materials

Lemongrass oil (LO) and virgin coconut oil were obtained from the Institute of Bioproduct Development (Universiti Teknologi Malaysia, Malaysia). Tween 80, SephadexG-50, soy lecithin, Ethanol and Glyceryl Monostearate were obtained from Sigma-Aldrich (Selangor, Malaysia). Water used throughout the experiments was distilled water.

2.2 NLC-lemongrass oil (NLC-LO) preparation

Table 1 shows the composition ingredients used to prepare the NLC-LO. The lipid percentage of the formulation was chosen as a manipulated variable. Meanwhile, the percentage of the lemongrass oil and the composition of the surfactant were used as fixed variables. The solid lipid (Glyceryl monostearate) and liquid lipid (virgin coconut oil) were mixed in the beaker. The mixture of the solid lipid and liquid lipid were heated at 70 °C to form a clear lipid mixture. The lemongrass oil was subsequently added into the lipid mixture. The heating temperature of the lipid mixture with lemongrass oil was maintained at 70 °C. The distilled water, tween 80 and soy lecithin were added in another beaker as aqueous phase and heated at 70 °C. The pre-emulsion mixture was formed by mixing the aqueous and the lipid mixture. Homogenizer (IKA ULTRA Turrax T25, Germany) was used to homogenize the pre-emulsion mixture at 12,000 rpm for 1 min. The pre-emulsion mixture was ultra-sonicated for 20 min at 50 amplitudes using the probe sonicator (Fisherbrand™ Model 705 Sonic Dismembrator, USA). To prevent overheating of the NLC-dispersion, ice water bath was used immediately to cool down the NLC-LO dispersion to room temperature (25±1 °C) and stored in the refrigerator (4-8 °C).

2.3 Particle size, polydispersity index analysis and zeta potential analysis

Photon correlation spectroscopy (PCS) or dynamic light scattering (DLS) method using a Malvern Zetasizer Nano ZSP (Malvern instrument, UK) were used for the analysis of particle size, polydispersity index (PDI) and zeta potential. Standard capillary electrophoresis cell was used for the determination. The diluted NLC-LO suspension in distilled water (1:10) was vortexed to avoid multiple scattering effects and directly placed in the cell. All the measurement was performed in triplicate at room temperature.

Table 1: Composition (%) for NLC-LO preparation

Formulation NLC-LO	Composition (%)					
	Lemongrass Oil	Solid Lipid	Liquid Lipid	Soy lecithin	Tween 80	Distilled water
A1	1.00	1.00	4.00	7.00	7.00	80.00
A2	1.00	2.00	3.00	7.00	7.00	80.00
A3	1.00	2.50	2.50	7.00	7.00	80.00
A4	1.00	3.00	2.00	7.00	7.00	80.00
A5	1.00	4.00	1.00	7.00	7.00	80.00

2.4 Encapsulation efficiency analysis

Sephadex gel-G50 was used to separate the entrapped and unentrapped lemongrass oil from NLC-LO suspension. 0.5 mL of the NLC-LO was added into the mini spin column packed with Sephadex gel-G50. The mini size centrifuge was run for 12 min at 3500 rpm. 0.5 mL of distilled water was added and continued spin. The collected encapsulated lemongrass oil were diluted with solvent (Ethanol) (n1). The absorbance measurement was measured at 393 nm wavelength (using lemongrass oil in ethanol as reference) using a UV-Vis spectrophotometer. A standard curve for the lemongrass oil was prepared by dissolving 10 mg lemongrass oil in 10 mL ethanol. A calibration curve was constructed at concentration of 0 to 1,000 µg/mL lemongrass oil. The percentage of the encapsulation efficiency was calculated using the following Eq(1):

$$EE(\%) = \frac{n1}{n2} \times 100 \quad (1)$$

Where n1 is the concentration of lemongrass oil entrapped in NLC-LO and n2 is the total concentration entrapped and unentrapped of lemongrass oil in NLC-LO suspension.

2.5 Morphology study using transmission electron microscopy (TEM)

A negative staining method was used to observe the morphology of the NLC-LO. 200 mesh copper grid coated with the carbon membrane was used for the spreading of the NLC-LO dispersion for about 3 min. Filter paper was used to remove any excess droplets. A drop of phototungstic acid solution (2 wt%) was placed on the grid for 2 min. The grid was then observed by using the transmission electron microscope (Hitachi H-7110, Japan).

2.6 NLC-LO physical stability test

After preparation, 10 ml volume of the NLC-LO samples were placed in the glass vials. The vials were stored at room temperature 25 °C. The particle size was taken at day 1, day 17 and day 14.

2.7 Investigation the release rate of NLC-LO and blank lemongrass oil

10 ml of the prepared NLC-LO and 10 ml of blank lemongrass oil were used for the release study. The release rate of the lemongrass oil from NLC-LO and blank lemongrass oil were investigated for 3 weeks at 45 °C. Certain time intervals for the sampling steps were carried out. Beakers that contained sampling were removing out from the oven after certain time interval and dissolving the content with ethanol. UV-VIS Spectrophotometer was used to determine the content of the lemongrass oil. The NLC- LO and blank lemongrass oil both were replicated for three parallel measurements. The unreleased fraction of the lemongrass oil in the NLC-LO or lemongrass oil (pure) were measured using this method.

3. Results and discussion

3.1 Effect of lipid concentration on particle size

From the Figure 1, it was observed that the size of particle NLC-LO was decreased from 1 wt% solid lipid to 3 wt% of liquid lipid. This result can be supported by Sun et al. (2009) and Swidan et al. (2018) who mentioned that the increase of solid lipid and liquid lipid will subsequently decrease the mean particle size and increase the mean particle size, respectively. However, the particle size for NLC-LO increase at 4 wt% solid lipid and 1% liquid lipid. This proved that the particle size would continue increase its size after 4 wt% solid lipid and 1 wt% liquid lipid. This was due to the 4 wt% solid lipid and 1 wt% liquid lipid might not the right proportion for reducing the size of the particle.

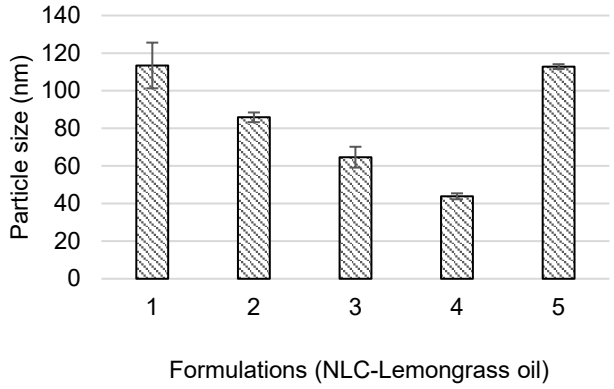

Figure 1: Size of the NLC-lemongrass oil

Based on the study done by Rosli et al. (2015) and Suhaimi et al. (2017), the size of the particle was depend on the different lipid percentage inside the formulation. The power and time for ultrasonication was fixed throughout the experiment and formulation with 3 % solid lipid and 2 % liquid lipid had effectively reduced the size of the particle. Based on the study, more sonication time and power was needed for other formulation with different lipid percentage in order to further reduce the size of the particle (Loo et al., 2013).

3.2 The size distribution of the NLC-LO affects the uniformity of the particle size

The polydispersity index was used to investigate the uniformity and stability of the particles (Table 2). Formulation A1 to A5 showed the polydispersity index were less than 0.3 which indicated that the particles were uniform and stable. Based on the research done by Adedokun et al. (2014), polydispersity index (PDI) at more than 50 % would not be accurate and stable for the width of the size distribution. Low PDI would be

better uniformity and stability for the particle size (Adedokun et al., 2014). Aggregation and agglomeration of particle would have happened if the PDI is more than 50 % (Singh et al., 2016) and narrow distribution of particle size would obtain if the PDI value was less than 50 % (Baumann, 2009).

Table 2: Percentages of the solid lipid and liquid lipid (%) and the polydispersity index of the NLC-lemongrass oil (%)

Formulation	Lemongrass Oil	Solid lipid (GMS)	Liquid lipid (VCO)	Polydispersity index (%)
A1	1.00	1.00	4.00	27.17 ± 2.47
A2	1.00	2.00	3.00	29.00 ± 0.92
A3	1.00	2.50	2.50	24.67 ± 2.96
A4	1.00	3.00	2.00	27.53 ± 3.01
A5	1.00	4.00	1.00	28.93 ± 1.38

3.3 The zeta potential for NLC-LO

A4 formulation of NLC-lemongrass oil was furthered analysed for the zeta potential. The zeta potential for the A4 formulation NLC-LO showed charge value of -42.9 ± mV. Long term stability of the particle had significantly relationship with the zeta potential. Better stability of the nanoparticles, if the nanoparticle emulsion had higher or bigger value of zeta potential. This could explain by the electrostatic repulsion between the particles. Bio colloids could be prevented from the agglomeration of the nanoparticles emulsion. Nanoparticle with more than -30 mV showed good stability and less than or equal to -60 mV showed a very good physical stability (Uprit et al., 2013).

3.4 The encapsulation efficiency of the NLC-LO

The lemongrass oil was used as the active material inside the core of the particle and it was presented as the oil encapsulated inside the core of the particles. The encapsulation efficiency for A4 NLC-LO formulation was 82.13 ± 2.83 % . The efficiency was expected to be increased if the concentration of the solid lipid increased. With more than 80 % encapsulation efficiency, it proved that NLC successfully entrapped the oil and able to preserve the therapeutic properties of the lemongrass oil. Based on the study conducted by Souaza et al. (2014), the imperfect crystal lattice provide space for the loading of the lemongrass oil. The encapsulation process successfully carried out due to the blending of the solid lipid and liquid lipid to form the lipid mixture.

3.5 The morphology of NLC-lemongrass oil formulation

Figure 2 shows that the TEM morphology of the NLC-LO for A4 NLC-LO preparation. All particles were in spherical shape and smooth morphology. From the figure, no aggregation and agglomeration were observed. The spherical NLC-Lemongrass was in nanometer sized range. However, the mean diameters observed were in the range of 200 nm. Due to unclear solution of the prepared lemongrass oil sample, some of the nano size particle which less than 100 nm cannot zoom in to more than 5,000 magnifications to capture the image. When more than 5,000 magnifications were zoom in to these particles, unclear and blur images were obtained. These result can be proven by the study of Zheng et al. (2013), he mentioned that different size would obtained by the TEM and particle sizer by the different sample preparation and measurement principle.

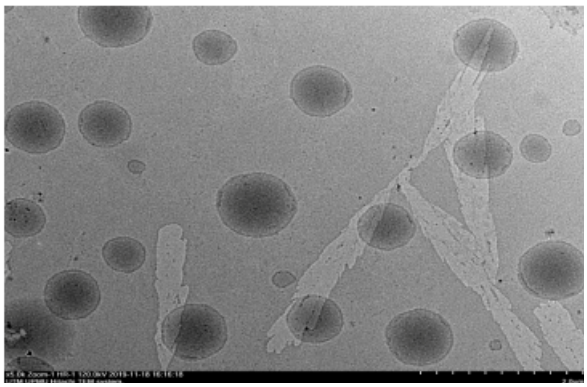

Figure 2: Morphology of NLC-lemongrass oil formulation A4 observed under the transmission electron microscopy (Magnification of 5,000).

3.6 NLC-LO physical stability test

Table 3 shows the physical stability of NLC-LO formulation for 14 d at 25 °C based on particle size measurement. It was observed that particle size for NLC-LO stored in 25 °C was considered stable. After 14 d, the particle size still maintain less than 100 nm. Therefore, encapsulation of the particle by NLC had successfully developed a stable and uniform particle.

Table 3: NLC-lemongrass oil formulation A4 stability based on the particle size observed for 14 d in the storage at room temperature.

Day	Particle size (nm)
1	39.1
7	42.3
14	53.64

3.6 Release rate of NLC-lemongrass oil and lemongrass oil.

Figure 3 shows the lemongrass oil release profile for 25 d at 45 °C. The release rate of lemongrass oil for the NLC-lemongrass oil was 35.18 % however the release rate for the blank lemongrass oil was 59.91 %. From the above result, it proved that slow release rate of lemongrass oil with small release rate of lemongrass oil (Feczkó et al., 2010). Based on the result from the Figure 3, there was small release rate for the lemongrass oil for about 3 % to 6 % from day 1 until day 25. This result is in agreement with Phunpee et al. (2017), the lemongrass oil was very volatile and unstable compound and when exposed to high temperature it also can cause the loses of some of their therapeutic properties. Therefore, based on the result above, NLC is able to encapsulate the lemongrass oil and prolong the release rate of the oil. This is also supported by previous study, where the encapsulation of lemongrass oil was able to prolong the evaporation rate of lemongrass oil and retained over time (Baker et al., 2018). Besides, based on Guimarães and Ré (2011), NLC could avoid the expulsion of active substance and able to increase loading capacity of actives.

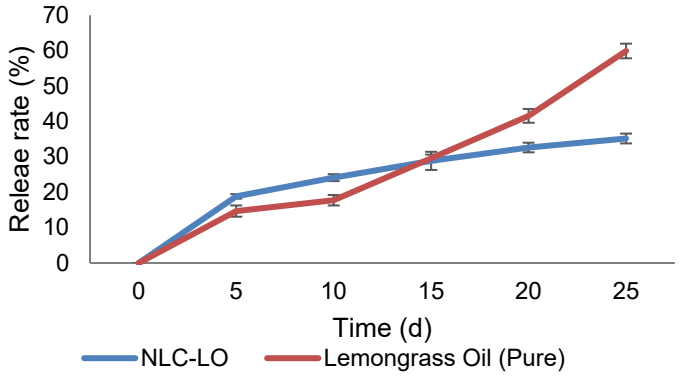

Figure 3: Release rate of NLC-lemongrass oil and blank.

4. Conclusions

From the findings, this project was successfully developed the nano-encapsulated system of lemongrass oil by nanostructured lipid carrier and incorporated with ultra-sonication technique. The particle size of all developed NLC-Lemongrass oil were found less than 100 nm. Formulation A4 was selected for further experiment due to its smallest particle size (43.855 ±1.58 nm) compare to other formulations. Moreover, this formulation also shows low polydispersity index (27.53 ±3.01 %) and good zeta potential value (-42.9 mV). Besides, it also has high encapsulation efficiency (82.13 ± 2.83 %) that indicate the strong ability of the nanostructured lipid carrier to encapsulate the lemongrass oil. Due to the smaller size and stable formula, the slow release rate of lemongrass oil was also obtained. These results indicated that even at high temperature, the nanostructured lipid carrier is able to prolong the release rate of the lemongrass oil. The result also reveals that after 25 d, the lemongrass oil was remain more than 50 % in the NLC system. Taken together, the use of essential oil in car-freshener products could reduce the exposure of volatile organic compounds from synthetic fragrances, as well as promote a healthy environment to the consumer.

Acknowledgement

The authors gratefully appreciate Higher Institution Centres of Excellence (HICOE) grant (R.J130000.7846.4J266) and Research Universty grant (RUG) (Q.J130000.2509.10H97) for financial support of this study.

References

Adedokun, M.O., Lawal, S.B., Ubochi, G.O., 2014, Formulation and in vitro evaluation of ibuprofen-loaded poly (D , L-lactide-co-glycolide) Microparticles, Tropical Journal of Pharmaceutical Research, 13(10), 1571–1576.

Baker B.P., Grant J.A., Malakar-Kuenen R., 2018, Lemongrass Oil Profile: Active Ingredient Eligible for Minimum Risk Pesticide Use <from http://hdl.handle.net/1813/56119> accessed 23.11.2020.

Balti M., Hadrich B., Kriaa K., 2017, Lab-scale extraction of essential oils from Tunisian lemongrass (Cymbopogon flexuosus), Chemical Engineering and Processing: Process Intensification, 124.

Baumann L., Saghari S, Weisberg E., 2009, Cosmetic dermatology principles and practice. 2nd Ed. The McGraw-Hill Companies. Inc., New York, United States.

Charles J.D., 2012, Antioxidant Properties of Spices, Herbs and Other Sources, Springer, New York, United States.

Feczkó, T., Kokol, V., Voncina, B., 2010, Preparation and characterization of ethylcellulose-based microcapsules for sustaining release of a model fragrance, Macromolecular Research, 18(7), 636–640.

Guimarães K.L., Ré M.I., 2011, Lipid Nanoparticles as Carriers for Cosmetic Ingredients: The First (SLN) and the Second Generation (NLC). In: Beck R., Guterres S., Pohlmann A. (eds) Nanocosmetics and Nanomedicines, Springer, Berlin, Heidelberg.

Ha H.K.P., Maridable J., Gaspillo P., Hasika M., Malaluan R., Kawasaki J., 2008, Essential oil from lemongrass extracted by Supercritical Carbon Dioxide and steam distillation, Philippine Agricultural Scientist, 91, 36–41.

Lorimer J.P., Mason T.J., Cuthbert T.C., Brookfield E.A., 2006, Effect of ultrasound on the degradation of aqueous native dextran, Ultrasonic Sonochem, 2(1), 55-57.

Phunpee S., Ruktanonchai U., Yoshii H., Assabumrungrat S., Soottitantawat A., 2017, Encapsulation of lemongrass oil with cyclodextrins by spray drying and its controlled release characteristics, Bioscience, Biotechnology, and Biochemistry, 81, 1–6.

Rosli N.A, Hasham, R., Abdul Aziz, A., Aziz, R., 2015, Formulation and characterization of nanostructured lipid carrier encapsulated Zingiber zerumbet oil using ultrasonication technique, Akademia Baru, 11(1),16–23.

Singh T.G., Dhiman S., Jindal M., Sandhu I., Chitkara M., 2016, Nanobiomaterials: Applications in biomedicine and biotechnology, Applications of Nanobiomaterials, 1, 401-429.

Souza J.M., Caldas A.L., Tohidi S.D., Molina J., Souto A.P., Fangueiro R., Zille A., 2014, Properties and controlled release of chitosan microencapsulated limonene oil, Revista Brasileira de Farmacognosia, 24(6), 691–698.

Suhaimi S.H., Hasham R., Rosli N.A., 2017, Optimization of Orthosiphon stamineus loaded onto nanostructured lipid carrier using d optimal mixture design, Chemical Engineering Transactions, 56, 1141-1146.

Sun R., Shi J., Guo Y., Chen L., 2009. Studies on the particle size control of gelatin microspheres, Frontiers of Chemistry in China, 4(2), 222–28.

Swidan S.A., Mansour Z.N., Mourad Z.A., Elhesaisy N.A., Mohamed N.A., Bekheet M.S., Badawy M.A., Elsemeiri M.M., Elrefaey A.E., Hassaneen A.M., 2018, DOE , Formulation , and Optimization of Repaglinide Nanostructured Lipid Carriers, Journal of Applied Pharmaceutical Science, 8(10), 8–16.

Tamjidi F., Shahedi M., Varshosaz, J., Nasirpour A., 2013, Nanostructured lipid carriers (NLC): A potential delivery system for bioactive food molecules, Innovative Food Science & Emerging Technologies,19, 29-43.

Taurozzi J.S., Hackley V.A., Wiesner, M., 2012, Preparation of nanoparticle dispersions from powdered material using ultrasonic disruption <https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.1200-2.pdf> accessed 23.11.2020.

Uprit S., Sahu R.K., Roy A., Pare A., 2013, preparation and characterization of minoxidil loaded nanostructured lipid carrier gel for effective treatment of alopecia, Saudi Pharmaceutical Journal, 21(4), 379-385.

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