EFFECT OF CONCENTRATION OF LIPID AND TEMPERATURE ON THE FORMATION OF NARINGENIN LOADED SOLID LIPID NANOPARTICLES

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Article History: **Received:** 21.04.2022 **Revised:** 16.08.2022 **Accepted:** 04.10.2022

Abstract: Naringenin is a natural flavonoid which is commonly found in large amounts of natural plants including citrus fruits, tomatoes, cherries, cocoa and grapefruit. Naringenin has a molecular weight of 272.26 and is described as poorly soluble in water. Due to its poor solubility, it will lead to low bioavailability. The aim of this study is to determine the efficiency of using solid lipid nanoparticles (SLN) method in improving solubility of Naringenin. Other than that, concentration of lipid and temperature were studied as well to determine the optimum formation of NRG-SLNs. Solubility of Naringenin is enhance by method of solid lipid nanoparticles. Materials that were used is Naringenin as active ingredient, stearic acid as lipid phase, Tween 80 as non-ionic surfactant and olive oil. As for the oil selection test, the naringenin was determined in several types of oils such as sunflower oil, eucalyptus oil, coconut oil and olive oil. The Naringenin-Solid Lipid Nanoparticles (NRG-SLNs) were prepared by separately two beakers with label of lipid phase and aqueous phase. These different beakers will be mix through some process and will be sonicate and temperature must be maintained at 85°C. On the other hand, the concentration of lipid and temperature are highlighted as the parameter in this study. Evaluation of the prepared SLNs inclusive of drug content uniformity, solubility studies, in vitro dissolution, transmission electron microscopy (TEM) and short-term stability studies. Based on the particle characterization, the particle size of NRG-SLNs ranging from 144 – 648 nm. The polydispersity index (PDI) showed values of 0.609 – 0.721. PDI is indicator if size distribution homogeneity. Zeta potential result was -0.112 mV which is nearly neutral. Other than that, the encapsulation efficiency of Naringenin encapsulated by SLNs method ranging from 77% - 82%. The solid lipid nanoparticles method was proven to significantly improve solubility and enhance stability profile of naringenin compared to the pure naringenin.

Keywords: Naringenin, solid lipid nanoparticles, concentration of lipid, temperature

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DOI: 10.31838/ecb/2022.11.09.005

INTRODUCTION

Solubility, the occurrence of dissolution of solute in solvent to form homogenous system, is one of the crucial parameters to achieve desired concentration of drugs for desired pharmacological response. Nowadays, the trends of design and structure of the drugs have led to the issue of drugs with lipophilicity, high molecular weight and poor water solubility. About 40% of new chemical entities generated in pharmaceutical sector are basically insoluble in water. Thus, solubility is a big challenge for scientist nowadays [1]. However, with fast development of insoluble drug delivery technologies, the challenge to formulate poorly water-soluble drugs can be obtained. Numerous marketed drugs were improvised to enhance efficacy, safety and patient compliance [2]

Solubility is also essential in other dosage forms, such as parenteral formulations. Poorly water-soluble drugs should require high doses to reach desired therapeutic plasma concentration. This issue can lead to waste of cost and time regards of management [1].

Thus, the low solubility of the drugs can cause unwanted effects such as poor absorption and bioavailability, insufficient solubility for IV dosing, burden shifted to patient as well since the patient have to administered with frequent high-dose and increasing consumption of cost and time.

Naringenin (figure 1) is a natural flavonoid which is predominantly found in large amounts of natural plants including citrus fruits, tomatoes, cherries, cocoa and grapefruit. Chemical named as 2,3-diyhdro-5,7-dihydroxy-2-(4-

hydroxyphenyl)-4H-1-benzopyran-4-one with molecular weight of 272.26 (C15H12O5)) [3]

Preclinical studies have shown that flavone possesses a variety of pharmacological activities such as anti-inflammatory, anticancer, antioxidant, antitumor, antiviral and antibacterial. Since it has been studied on have many benefits on healthcare, it has been approved as a therapeutic agent. Based on physicochemical analysis, naringenin has a crystallize characteristic and released slowly from oral dosage forms which lead to restrict its desired therapeutic effect. Not only that, naringenin is a hydrophobic compound with a weak oral bioavailability (-5.81%) that lead to low solubility. Therefore, solutions and managements should be provided to increase its solubility and therapeutic effect for benefit of humanity. The basic properties of naringenin molecule are insoluble in water and soluble in organic solvents such as ethanol [4]

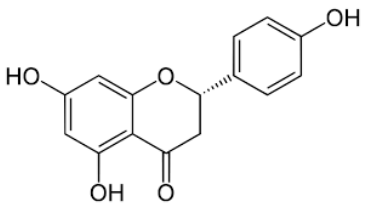

Figure 1. Structure of Naringenin

Solid Lipid Nanoparticles (SLN) are composed of solid lipids core with average photon correlation spectroscopy (PCS) diameter ranging from 50 to 1000 nm [5] . The SLN consist of purified triglycerides, complex glyceride mixtures that at solid state at room temperature and human body temperature and are stabilized by the aids of suitable surfactants such as Tween 80 [6].

SLN shown to have more advantages than previous nano-lipid carrier systems and counter on some disadvantages. As an example, SLN is same in nature with nano emulsions but present a solid lipid core as opposed to liquid lipid type. As a result, drug mobility reduces in solid lipid state compared with the oily phase, lead to enhance the controlled release of loaded drugs. Plus, SLN stability can be utilized and increase with surfactant coating [7]

On early development of SLN, it is presented as tiny and spherical particles where the outer part is covered with surfactant. It is also known of made of solid lipids at room temperature that suitable to accommodate a drug between fatty acid chains. But nowadays, it is proven that this is not happen on all events, since disc-like shape or flat ellipsoidal geometry takes place [8]

MATERIALS AND METHODS

Materials

Naringenin powder was purchased from Shaanxi Yuantai Biological Technology Co., Ltd (China), Stearic Acid, Olive Oil were provided from University of Cyberjaya, Tween 80 was purchased from Pro Prima Enterprise (Malaysia)

Method

Oil Selection Test

Determination on selection of oil that suitable with nano emulsion formulation was measured with sunflower oil, eucalyptus oil, coconut oil and olive oil. The drug should be solubilized in the best oil phase possible to provide the formulation in the proper form while avoiding precipitation and instabilities [9].

According to the findings of solubility tests, olive oil is the most suitable compared to others. The nano emulsions formulations were then tested with different compositions of oils.

The formulations were undergone to sonication using Ultrasonicator for 30 minutes at 37 °C, followed by vortexing for 15 minutes until formation of homogenous mixture occur.

Preparation of Naringenin – Solid Lipid Nanoparticles

For preparation of solid lipid nanoparticles, use two beakers separately with label of lipid phase and aqueous phase. The lipid phase consists of stearic acid, olive oil and active ingredient which is naringenin. While for aqueous phase, add distilled water with Tween 80 as surfactant. For lipid phase in the beaker, melt the stearic acid for 10 minutes at 85°C constantly using Bunsen burner. [10]

After done stirring the melted stearic acid with olive oil and naringenin, mix them by pour aqueous phase to the lipid phase. The solution must be mix well and then sonicate for 5 minutes with power less than 70% and sustain the temperature at 85°C. Once the sonicate process is done, stir the solution using magnetic stirrer and let it cool down until reach room temperature. Monitor closely and the end of the product there will be presence of turbidity due to suspensions

Concentration of Lipid to Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) are the second generation of nanoparticles that consist of solid lipids ranging from 0.1% w/w to 30% w/w and surfactant between 0.5% to 5% w/w [11]

In this parameter, the concentration of stearic acid which plays the role as solid lipid to olive oil are variables by the ratios from 1:1 to 1:5. Meanwhile the Naringenin and Tween 80 remain constant at ratio of 1. Table 1 below shows the samples and observations that can be remark from the ratio of solid lipid ranging from 1 until 5.

Table 1. Concentration of stearic acid to olive oil

Samples	Ratio	Stearic Acid	Olive Oil	Observation
B1	1: 1	0.5 g	0.5 g	- Separate layers formed - Small suspension present
B2	2: 1	1.0 g	0.5 g	- Separate layers formed - Suspension present
B3	3: 1	1.5 g	0.5 g	- Separate layers formed - Suspension present
B4	4: 1	2.0 g	0.5 g	- Separate layers formed - Suspension present
B5	5: 1	2.5 g	0.5 g	- Separate layers formed - Contain largest suspension

Based on the samples of B1 to B5, the separate layers present due to suspension forms above and the increase concentration of stearic acid led to abundant of suspensions.

Temperature to SLNs

Stearic acid was permitted to melt at 75 °C, meanwhile distilled water was boiled at 75°C in a separate beaker [12]. The magnetic stirrer was added to stir uniformly the solutions and frequently monitor the temperature at 75 °C.

On the trials, the sonicate power is maintained at 50%. The samples on different temperatures with the observation is provided in Table 2 below.

Table 2. Variables in Temperature

Product	Stearic Acid	Tween 80	Temperature	Observation
D1	0.5 g	0.5 g	60°C	- Big clumps present - Creamy color - No separate layer
D2	0.5 g	0.5 g	70°C	- At this state, the SLN formation has formed - Presence of small white clumps
D3	0.5 g	0.5 g	80°C	- Formation of SLN - Less suspension
D4	0.5 g	0.5 g	90°C	- Formation of SLN - Lesser suspension

Drug content uniformity

Method for drug content uniformity measurements is reliable with the Ultraviolet/visible (UV/Vis) spectrophotometry technique. The absorption has been determined along with the given wavelength for Naringenin – SLNs related with its concentration. Using this technique required the material being analyzed to have a suitable absorption within this spectral range and for the other materials present to not obstructing the absorption. However, spectral overlap can generally be managed by spectral deconvolution or appropriate curve-fitting quantitative methods.

Solubility Studies

For solubility method of the Naringenin-SLNs, shake flask method was used. To achieve equilibrium solubility, the sample added in excess to 2 ml of distilled water and the suspension present above the solution was shaken for 24 hours on a rotary shaker at consistent temperature which is 37°C. The saturated solutions were measured using AHS Laboratory Supplies UviLine 0400 Spectrophotometer

Stability Studies

Naringenin-SLNs was stored at room temperature 25°C for 3 months. The samples must be sealed to ensure no contaminants. Meanwhile the average particle size, in-vitro drug release and physical characteristics were observed continuously during 1, 2 and 3 months [13].

Visual Inspection

Visual inspection or light transmittance technique to investigate the purity of samples. Plus, visual inspection is necessary to assess potential physical instability issues during processing and storage [14]. Moreover, the prepared solid lipid nanoparticles were also tested for its purity by phase separation through visual inspection.

Potential crystallization of stearic acid, precipitation and mold formation were also analyzed by visual inspection.

Zeta Potential

Zeta potential is measured by add up the solution to a cell that have two gold electrodes. The particles will move toward the electrode with the opposite charge once the voltage has been applied.

A Doppler technique is used to measure the particle velocity as a function of voltage. A laser beam travels through the cell, and the intensity of scattered light modulates at a frequency equivalent to the particle speed as particles move through the laser beam.

According to a previous report, particles can be dispersed stably when the absolute value of the zeta potential is <30 Mv [4]

Particle Size Analyzer

Based on studies, the particle size distribution is a crucial property used to conclude the stability of colloidal systems

For preparation of particle size analyzer, add 10 drops of sample Naringenin-SLNs in the cleaned cuvette. After that, put the cuvette that contained the sample carefully in the particle size analyzer to be evaluated. The sizing method can be categorized into two parts which is stream-scanning and field-scanning techniques. The stream-scanning provides extra experimental parameters. Instead of measured the number of particles in the samples, it will as well provide a better resolution, lower quantification limits and better interpretation of data. Otherwise, the field-scanning technique is time saving, sturdier and more corresponded for online/in-line applications (Etzler et al., 2004). The interpretation of the data will give the Polydispersity Index (PDI) as an indicator of the homogeneity of the particle size distribution [15].

RESULTS

Characterization of NRG-SLNs

Particle Characterization

For characterization, particle size distribution is one of the major properties that has to be highlighted to evaluate the stability of the colloid system [16]. The globule size and distribution, as well as zeta potential values, are essential parameters that can affect a nano emulsion's in vivo stability when formulating and optimizing it. Nanoparticle sizes are crucial as they ensure faster and better permeation of drug across absorption barriers due to their ability to provide a high surface area and free energy. The particle size prepared for Naringenin – Solid Lipid Nanoparticles was found ranging from 144 nm to 1420 nm with PDI of 0.609 ± 0.359. PDI is the indicator of size distribution homogeneity, and the homogenous nature of the formulation comprises of narrow size distribution and uniform size (Table 3).

As for the zeta potential, based on previous studies has proved that particles can be dispersed stable when the absolute value of the zeta potential is > 30 mV. However, the value of zeta potential for optimized Naringenin – SLN in this study was -0.112 mV which is nearly neutral as can be seen from Figure 2 below.

Table 3. Shows the samples with particle size and PDI

Formula code	Particle size	Polydispersity Index (PDI)
B1	1420	1.000
B2	648	0.721
B3	1500	1.000
B4	144	0.609
B5	159	0.782

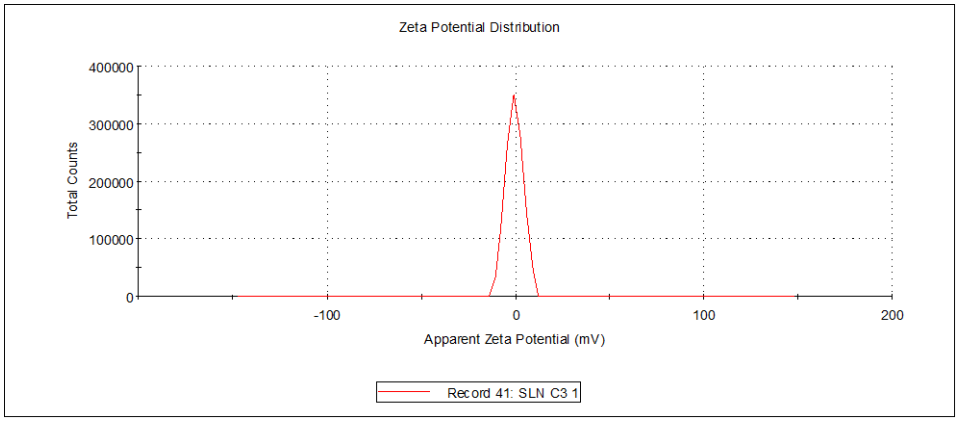

Figure 2. Shows the zeta potential value for NRG-SLNs

Optimization of Naringenin - Solid Lipid Nanoparticles

The Naringenin – Solid Lipid Nanoparticles formulation was optimized through procedure and formulation optimization. After that, the optimized formulation of naringenin loaded with solid lipid nanoparticles (table 4) were identified for characterization, solubility, stability, dissolution studies and Transmission Electron Microscope (TEM).

Table 4. Optimized formulation of the NRG-SLN

Formula Code	Naringenin (g)	Oil (g)	Water (mL)	Surfactant (g)	Lipid (g)
NRG-SLN	0.1 g	0.5 g	100 mL	1.0 g	2.0 g

Concentration of Lipid and Temperature to Naringenin-Solid Lipid Nanoparticles

Considering lipids are proven as oral drug absorption enhancers, lipid-based drug delivery methods appear promising [17]. From trials and investigations of increasing lipid content over 5-10% (w/w) resulted in larger mean particle sizes and broader size distributions. An increment in the concentration of lipid gradually has greatly minimizes the emulsification capacity of surfactants, which results in the aggregation of particles. Noted as well, during this trials, other materials concentrations were remained constant.

Considering on other parameters, the increase on concentration of lipid has led PDI to gradually increased as well. An increase in PDI was noticed due to an increment in the heterogeneity of developed nanoparticles

Table 5. Samples with different lipid concentrations

Samples	Lipid Concentration (g)	Particle Size (nm)	PDI
B4	2.0 g	144	0.609
B5	2.5 g	159	0.782

Temperature affects the formation of Naringenin-Solid Lipid Nanoparticles

Generally, higher temperatures cause smaller particle sizes due to accelerate the degradation rate of the drug and the nanoparticles. Not only that, rises in temperatures will as well influence the mobility and the hydrophilicity of all emulsifiers to a different extent. As in these studies, the emulsifier that was used is Tween 80.

The solubility improves when increased temperature and increased surfactant concentrations are used. Therefore, when low production temperatures and low surfactant concentrations are used little or no burst effect occurs [7]

The mean particle sizes from 80 nm up to 1000 nm can be produced when the temperature that applied is at optimum state (table 6). Higher temperatures would increase the kinetic energy of a system, in combination with a reduced zeta potential what will leads to SLN aggregation.

In addition, increase in temperature will likely lead to changes in crystalline structure of the lipid that will potentially reduce the zeta potential

On the other hand, the formation of SLN with low temperature can lead to negative result. Apparently, the components in lipid nanoparticles did not solubilize completely and visually many presences of big clumps.

However, the Naringenin-Solid lipid nanoparticles formation were successfully formed at its temperature of 80°C. The sample D3 and D4 was let to be cool down for 24 hours before

undergoing particle size test. The value particle size of 250 nm and 97 nm were obtained which shows good particle size

Table 6. Differences in temperature from 4 samples

Sample	Temperature	Particle size
D1	60°C	1600 nm
D2	70°C	438 nm
D3	80°C	250 nm
D4	90°C	97 nm

Encapsulation Efficiency

The standard curve is presented in figure 3 and the encapsulation efficiency is presented in table 7. For spectrophotometers, the useful absorbance range is from 0.1 to 1.0. However, if the absorbance value greater than or equal to 1.0 means the solution is too concentrated. Therefore, the samples must be diluted. Based on the EE tests conducted, the EE of optimized NRG-SLN formulation was 81.36 %. This showed that little significant loss of drug during formulation process. Thus, this result has proved that the method of preparation of NRG-SLN is efficient and beneficial.

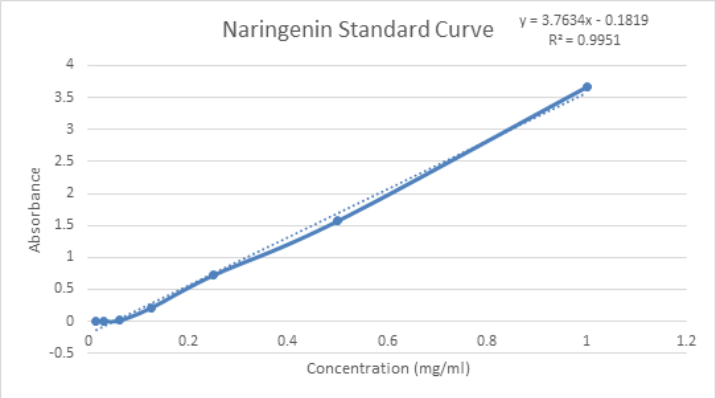

Figure 3. Absorbance against Concentration

Table 7. Encapsulation efficiency with constant total concentration of NRG

Sample	Concentration of free naringenin (mg/mL)	Total concentration of naringenin (mg/mL)	Encapsulation Efficiency (%)
B1	0.2284	1	77.16 %
B2	0.2059	1	79.41 %
B3	0.2157	1	78.43 %
B4	0.1864	1	81.36 %
B5	0.1747	1	82.53 %

Solubility Studies

For solubility test, the study was tested between pure naringenin, and solid lipid nanoparticles samples loaded with naringenin. Based on the results that can be observe from Table 8 below, it shows that the Naringenin-Solid lipid nanoparticles have increased in solubility than pure naringenin itself**.**

Table 8. Solubility profile of pure Naringenin with NRG-SLN

Sample	Solubility (mg/ml)
Pure Naringenin	0.3760
B1	0.7534
B2	0.7620
B3	0.7137
B4	0.7582
B5	0.7460

Short Term Stability Studies

These studies were planned to examine the stability of Naringenin – Solid Lipid Nanoparticles over a certain period. So as to observe the effect of the storage temperature on the stability, NRG – SLN were monitored on two aspects, which is the physical and chemical stability in period of 3 months of storage without presence of sunlight at room temperature (25°C). The sample is observed at interval of 1 month, 2 months and 3 months. The results of the stability studies of NRG-SLN for 3 months are shown in Table 9. In these studies, we found that the color, particle size and zeta potential had no significant effect on storage up to 3 months. This means that the method of production of NRG-SLN is stable and appropriate. The good stability may be due to the slow transition of lipid in SLNs, the low particle size, and steric effect of Tween 80.

Table 9. Short Term stability studies for 3 months

Parameters	1 month	2 months	3 months
Visual Appearance	Milky solution with very little suspension at the bottom. No change in color	Milky solution with presence of aggregates that can be redisperse by shaking. No change in color	Milky solution with presence of aggregates that can be redisperse by shaking. No change in color

Particle Size (nm)	144	156	162
Drug Content (%)	81	79	79

DISCUSSION

Effect of Stirring Speed to Particle Size

For formation of NRG – SLN, certainly there are factors that plays important role in its solubility enhancement. One of the factors is stirring speed. If the speed level of stir the nanoparticle solution is low, the particle size will increase, and its stability will be affected as well. However, if the stirring speed is too high, they will be foam presents above the solutions that will affect the surfactant's emulsifying effect. Therefore, the stirring to the NRG-SLN solution should be at moderate state. It was found that the stirring speed for NRG-SLN was confirmed to be 1,500 rpm [4]

Characterization of NRG-SLN formulation

Particle Size Reduction

The value of particle sizes from the recorded five samples ranges from 144 nm to 648 nm, where the samples of the NRG-SLN formulation trend from low to high lipid level, with the constant concentration of Tween 80 as the surfactant. However, the sample of B1 and B3 have particle size more than 1000 nm which can be assume as large particle sizes. This could be due to both samples did not filter properly before undergo particle size test. Another possible factor is the stearic acid did not fully soluble during formulation due to inconsistent temperature.

Besides, the polydispersity index (PDI) for most of the samples ranging from 0.609 to 0.721 while the sample B1 and B3 PDI values are 1.000. The PDI indicates the homogeneity of the sample. The lower the PDI means the better the homogeneity. Thus, the factor that may affect the PDI is the instrumental condition. Example of it is the sonication. It could be due to imprecise duration of sonication, sonication pulses and amplitude [18].

Zeta Potential

Nanoparticles with a zeta potential value ranging from −10 to +10 mV are measured as nearly neutral. Otherwise for nanoparticles with value of zeta potentials higher than +30 mV are known as strongly cationic while value of lesser than −30 mV is considered as strongly anionic [19]. Based on these studies, the value of zeta potential for the NRG-SLN formulation is nearly neutral where the value is -0.112 mV. Followed with the zeta potential value, certain parameters that may affect the physical stability of nanoparticles are material properties and type of surfactants been utilized. In this experiment, the surfactant that has been used is the Tween 80.

Presence of Surfactant

For the formulation of Naringenin-Solid Lipid Nanoparticles, the Tween 80 were added as surfactant. Surfactant concentration are one of the compositions that may affect the NRG-SLN entrapment efficiency. The effect of the concentration of Naringenin on entrapment efficiency was studied by maintaining the amount of Tween 80 while the concentrations of lipid are varying. Besides, the surfactant was administered in production of solid lipid nanoparticles play a crucial action on physical stability of nanoparticles and extent of the drug dissolution [20]. The Tween 80 stabilize the SLN structure by minimize the interfacial tension between two phases which is the hydrophobic surface of lipid and the aqueous phase [21]

In this study, the samples of formulation NRG-SLN has zeta potential of -0.112 mV which is nearly neutral. This is because the surfactant that was utilized is Tween 80 which is non-ionic surfactant. This zeta potential value is similar to previous report from Schubert et al., 2015 that stated the value of zeta potential for non-ionic surfactant ranges between -23.4 mV to -0.9 mV. However, the non-ionic surfactant may form weak repulsive forces between particles due to nearly neutral charge and probable to easier aggregates in the long time although it is at good stability. Therefore, the usage of two surfactant system which contain non-ionic surfactant and ionic surfactant can be recommended as they have relatively more stable than by only one surfactant due to synergic effects [20]

Physical Characteristics of Lipid Phase

In this experiment, the formulations of NRG-SLN were tested with ranges of temperatures from 60°C to 90°C to determine the suitable temperature that is appropriate for the formulations. Apparently from the particle size and visual inspection that can be notice, the sample D1 has 1600 nm of particle size and sample D2 with 438 nm have presence of white clumps remain in the sample that can be seen. One of possible reason is, the lipid phase which consist of stearic acid permissible to melt at 75°C [12]. Since D1 and D2 sample was tested with temperature of 60°C and 70°C, the stearic acid did not fully melt which led to increase in particle size and present of clumps. On the other hand, the other samples showed result of forming nanoparticles formulation with particle size ranging from 97 nm to 250 nm depends on its temperature. In order to maintain its molten state during preparation process, the temperature should be > 75°C and, finally the optimized emulsifying temperature was selected to be 90°C.

Encapsulation Efficiency

The encapsulation efficiency of NRG-SLN samples that were recorded are ranging from 77.16% to 82.53%. Based from previous study by [10], the EE of SLN were found ranging from 71% to 85%. The formulation of B1 resulted with low entrapment efficiency compared to the other samples can possibly be due to inadequate concentration of stearic acid. As been showed at Table 3.4b, the encapsulation efficiency improves as lipid concentration increased. This is because of increment solubilizing agents for highly lipophilic drugs. Thus, it offered more spaces for Naringenin to be accommodate [22]

Stability Test

The utilization of the surfactant which is Tween 80 promotes stabilization to the NRG-SLN formulation by slow transition of lipid and steric effect. The steric effect of Tween 80 was adequate to cover exterior of nanoparticles efficiently and prevent from aggregation during homogenization method [23]

In this study, the storage for the samples were executed at room temperature (25°C). According to stability tests from previous study, the stability test on storing the formulations at 4°C have better stability compared at room temperature 25°C [24]. This is because at room temperature storing, can lead to higher decreased entrapment and increased particle size due to the coalescence of the nanoparticle into a microparticle.

CONCLUSION

This study focuses on improving the solubility of poorly aqueous-soluble drug Naringenin by incorporated with solid lipid nanoparticles method. Based on the results and findings of the study, the physicochemical characterization and short-term stability were investigated. The in-vitro dissolution studies showed that the drug release of the SLN has better efficacy than Naringenin alone. On the other hand, studies have showed that the optimum temperature is 90°C and suitable concentration of lipid to be administered is 2.0 g to form most appropriate SLN formulation. Thoroughly, it shows that solid lipid nanoparticle method provides a promising delivery system for enhancement of the solubility and bioavailability of poorly soluble drugs such as Naringenin.

REFERENCES

i. K. T. Savjani, A. K. Gajjar, and J. K. Savjani, "Drug Solubility: Importance and Enhancement Techniques," ISRN Pharm., vol. 2012, no. 100 mL, pp. 1–10, 2012, doi: 10.5402/2012/195727.

ii. S. Kalepu and V. Nekkanti, "Insoluble drug delivery strategies: Review of recent advances and business prospects," Acta Pharm. Sin. B, vol. 5, no. 5, pp. 442–453, 2015, doi: 10.1016/j.apsb.2015.07.003.

iii. L. L. Gadaga and D. Tagwireyi, Critical Review of the Guidelines and Methods in Toxicological Research in Africa. 2014. doi: 10.1016/B978-0-12-800018-2.00003-0.

iv. P. Ji et al., "Naringenin-loaded solid lipid nanoparticles: Preparation, controlled delivery, cellular uptake, and pulmonary pharmacokinetics," Drug Des. Devel. Ther., vol. 10, pp. 911–925, 2016, doi: 10.2147/DDDT.S97738.

v. L. Battaglia, E. Peira, M. Trotta, and M. Gallarate, "Solid lipid nanoparticles prepared by coacervation method as vehicles for ocular cyclosporine," Artic. J. Drug Deliv. Sci. Technol., 2011, doi: 10.1016/S1773-2247(12)50016-X.

vi. A. C. Silva, A. Kumar, W. Wild, D. Ferreira, D. Santos, and B. Forbes, "Long-term stability, biocompatibility and oral delivery potential of risperidone-loaded solid lipid nanoparticles," Int. J. Pharm., vol. 436, no. 1–2, pp. 798–805, Oct. 2012, doi: 10.1016/J.IJPHARM.2012.07.058.

vii. N. Schöler, H. Hahn, R. H. Müller, and O. Liesenfeld, "Effect of lipid matrix and size of solid lipid nanoparticles (SLN) on the viability and cytokine production of macrophages," Int. J. Pharm., vol. 231, no. 2, pp. 167–176, Jan. 2002, doi: 10.1016/S0378-5173(01)00882-1.

viii. J. Mazuryk et al., "Rapamycin-loaded solid lipid nanoparticles: Morphology and impact of the drug loading on the phase transition between lipid polymorphs," Colloids Surfaces A Physicochem. Eng. Asp., vol. 502, pp. 54–65, Aug. 2016, doi: 10.1016/J.COLSURFA.2016.05.017.

ix. B. Gaba et al., "Vitamin E Loaded Naringenin Nanoemulsion via Intranasal Delivery for the Management of Oxidative Stress in a 6-OHDA Parkinson's Disease Model," Biomed Res. Int., vol. 2019, 2019, doi: 10.1155/2019/2382563.

x. L. Wang et al., "Paclitaxel and naringenin-loaded solid lipid nanoparticles surface modified with cyclic peptides with improved tumor targeting ability in glioblastoma multiforme," Biomed. Pharmacother., vol. 138, no. February, p. 111461, 2021, doi: 10.1016/j.biopha.2021.111461.

xi. S. Nanda, A. Nanda, S. Lohan, R. Kaur, and B. Singh, "Nanocosmetics: performance enhancement and safety assurance," Nanobiomaterials Galen. Formul. Cosmet. Appl. Nanobiomaterials, pp. 47–67, Jan. 2016, doi: 10.1016/B978-0-323-42868-2.00003-6.

xii. Surekha R and Sumathi T, "An Efficient Encapsulation of Thymoquinone Using Solid Lipid Nanoparticle for Brain Targeted Drug Delivery: Physicochemical Characterization, Pharmacokinetics and Bio-Distribution Studies," Int. J. Pharm. Clin. Res., vol. 8, no. 12, pp. 1616–1624, 2016, Accessed: Jun. 18, 2022. [Online]. Available: www.ijpcr.com

xiii. V. K. Venishetty, R. Samala, R. Komuravelli, M. Kuncha, R. Sistla, and P. V. Diwan, "β-Hydroxybutyric acid grafted solid lipid nanoparticles: A novel strategy to improve drug delivery to brain," Nanomedicine Nanotechnology, Biol. Med., vol. 9, no. 3, pp. 388–397, Apr. 2013, doi: 10.1016/J.NANO.2012.08.004.

xiv. S. Jacob et al., "Lipid Nanoparticles as a Promising Drug Delivery Carrier for Topical Ocular Therapy—An Overview on Recent Advances," Pharm. 2022, Vol. 14, Page 533, vol. 14, no. 3, p. 533, Feb. 2022, doi: 10.3390/PHARMACEUTICS14030533.

xv. M. Danaei et al., "Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems," Pharm. 2018, Vol. 10, Page 57, vol. 10, no. 2, p. 57, May 2018, doi: 10.3390/PHARMACEUTICS10020057.

xvi. W. Zhang et al., "Dynamic variation in physicochemical properties of activated sludge floc from different WWTPs and its influence on sludge dewaterability and settleability," Colloids Surfaces A Physicochem. Eng. Asp., vol. 467, pp. 124–134, Feb. 2015, doi: 10.1016/J.COLSURFA.2014.11.027.

xvii. S. Chakraborty, D. Shukla, B. Mishra, and S. Singh, "Lipid – An emerging platform for oral delivery of drugs with poor bioavailability," Eur. J. Pharm. Biopharm., vol. 73, no. 1, pp. 1–15, Sep. 2009, doi: 10.1016/J.EJPB.2009.06.001.

xviii. R. Kumar, A. K. Thakur, P. Chaudhari, and N. Banerjee, "Particle Size Reduction Techniques of Pharmaceutical Compounds for the Enhancement of Their Dissolution Rate and Bioavailability," J. Pharm. Innov., Jun. 2021, doi: 10.1007/S12247-020-09530-5.

xix. U. Hasanah, M. S. Miran, M. M. Rahman, and M. M. Islam, "Simultaneous reductions of production loss and environmental burden through the treatment of loose leather with non-toxic manganese dioxide nanoparticles," J. Clean. Prod., vol. 318, p. 128541, Oct. 2021, doi: 10.1016/J.JCLEPRO.2021.128541.

xx. K. Karn-orachai et al., "Surfactant effect on the physicochemical characteristics of γ-oryanol-

containing solid lipid nanoparticles," Colloids Surfaces A Physicochem. Eng. Asp., vol. 488, pp. 118–128, Jan. 2016, doi: 10.1016/J.COLSURFA.2015.10.011.

xxi. H. A. Ebrahimi, Y. Javadzadeh, M. Hamidi, and M. B. Jalali, "Repaglinide-loaded solid lipid nanoparticles: effect of using different surfactants/stabilizers on physicochemical properties of nanoparticles," DARU J. Pharm. Sci., vol. 23, no. 1, Sep. 2015, doi: 10.1186/S40199-015-0128-3.

xxii. J. Emami, H. Mohiti, H. Hamishehkar, and J. Varshosaz, "Formulation and optimization of solid lipid nanoparticle formulation for pulmonary delivery of budesonide using Taguchi and Box-Behnken design," 2015.

xxiii. L. Hu, Q. Xing, J. Meng, and C. Shang, "Preparation and Enhanced Oral Bioavailability of Cryptotanshinone-Loaded Solid Lipid Nanoparticles", doi: 10.1208/s12249-010-9410-3.

xxiv. S. Shidhaye, R. Vaidya, S. Sutar, A. Patwardhan, and V. Kadam, "Solid Lipid Nanoparticles and Nanostructured Lipid Carriers – Innovative Generations of Solid Lipid Carriers," Curr. Drug Deliv., vol. 5, no. 4, pp. 324–331, 2008, doi: 10.2174/156720108785915087.