Effect of Concentration of Lipid with Surfactant and Ultrasonification Power on the Formation of Naringenin-Loaded Solid Lipid Nanoparticles

Nur Afiqah Mohammad Nasri[1], B.M. Helal Uddin[2], Zaidul Islam Sarker[3], Chew Yik Ling[4], Liew Kai Bin[5*]

[1]Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Cyberjaya, Cyberjaya, Selangor, Malaysia.

[2]Faculty of Pharmacy, International Islamic University Malaysia, Bandar Indera Mahkota, Kuantan, Pahang, Malaysia.

[3]Faculty of Pharmacy, International Islamic University Malaysia, Bandar Indera Mahkota, Kuantan, Pahang, Malaysia.

[4]UCSI University. No 1 Jalan Menara Gading, UCSI Heights, Cheras, Kuala Lumpur, Malaysia.

[5*]PhD, Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Cyberjaya, Cyberjaya, Selangor, Malaysia.
E-mail: liewkaia@yahoo.com

Abstract

Naringenin is a flavonoid found in abundance in natural plants, including citrus fruits, tomatoes, and grapefruit. Naringenin has a molecular weight of 272.26 and aqueous solubility of 43.83 ± 0.039 mcg/mL. It has a limited bioavailability due to its poor solubility thus limiting its application in pharmaceutical. This study was designed to determine the efficiency of Solid Lipid Nanoparticles (SLN) method in improving the solubility of Naringenin. Variables such as the concentration of lipid with surfactant and ultrasonification power were also studied to observe their effect on the formation of NRG-SLNs. This study was done using method of solid lipid nanoparticles. Initially, the Naringenin-Solid Lipid Nanoparticles (NRG-SLNs) were prepared in separate beaker for lipid phase containing stearic acid and aqueous phase containing distilled water with Tween 80. Both were brought to a temperature of 80° C. Then, aqueous phase was poured and mixed continuously at constant temperature of 80° C until a semi-transparent solution appears. The solution was then subjected to sonication for 10 minutes while maintaining the same temperature. The procedure was repeated with increasing concentration of lipid and surfactant and different sonication power. The samples were subjected to solubility study and encapsulation efficiency using Uv-Visible spectrophotometer. Zeta potential, particle size and polydispersion index were determined using Zetasizer. The sample was also studied for its short-term stability at room temperature for a period of 3 months. Based on the characterization, the maximum solubility of naringenin achieved was 0.8621 mg/ml**,** which improves from the pure naringenin. The particle size of NRG-SLNs ranged from 218 nm to 1030 nm with polydispersion index value of 0.611. The Zeta potential of the nanoparticles was -0.112 mV, which is nearly neutral. This method produces nanoparticles with encapsulation efficiency of 77.50 % to 86.21 %. The short-term stability of the NRG-SLNs was found to be good since there was only slight reduce in encapsulation efficiency with no change in colour of solution. The solid lipid nanoparticle method was successfully proven to improve the solubility and enhance the stability of naringenin, which is a lipophilic active ingredient.

Keywords: Naringenin, Flavonoid, Solid-Lipid Nanoparticles.

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INTRODUCTION

Naringenin (NRG) is a flavanone (flavonoid) that occurs naturally in citrus fruits like grapefruits, oranges and tomatoes. Its is known to have a bioactive influence on human health [1]. Naringenin has been extensively studied for its pharmacological potentials such as anti-tumor, anti-viral, anti-bacterial, anti-inflammatory and cardioprotective effect [2].

The oral bioavailability of naringenin was reported to be very low which is just approximately 8% while its half-life is estimated to be 2.6 hours. This is a consequence from the low aqueous solubility of naringenin. Aqueous solubility of naringenin is very low which is 43.83 ± 0.039 mcg/mL while it's solubility in organic solvent such as n-octanol is higher which is 440.163 ± 2.641 mcg/mL. This data shows that naringenin is almost insoluble in water [3]. Therefore, it is necessary to enhance both its preparation and delivery in order to raise its solubility, in vivo bioavailability and maintain a constant plasma concentration.

Solid lipid nanoparticle (SLN) is described as an alternative carrier system to enhance the aqueous solubility of weakly water soluble drugs. It serves as a matrix material where drug is dispersed uniformly in the particle system [4]. The proven advantages of solid lipid nanoparticles as a drug carrier are it removes the need for organic solvent in formulation, ability to incorporate both lipophilic and hydrophilic drugs, reduces chemical degradation, acts as a controlled drug release carrier system, increases physical stability, reduces cost for production, improves aqueous solubility and has potential for large scale production [5,6,7]. The objective of this study was to investigate the concentration of lipid with surfactant and

ultrasonification power on the formation of naringenin loaded solid lipid nanoparticles.

MATERIALS AND METHODS

1. Materials

Naringenin powder 98% purity was purchased from Shaanxi Yuantai Biological Co.Ltd (China), Stearic acid and Tween 80 were the gift samples from IIUM (Malaysia), olive oil and distilled water. Portable electronic balance OHAUS® Instruments (Shanghai), magnetic stirrer with heater, Laboratory Spatula, Borosilicate Glass rod, plastic dropper and Measuring cylinder, beaker, cuvette were from Thomas Scientific (USA), folded capillary zeta cell, Zetasizer from Malvern Panalytical (UK), UvLine 9400 Spectrophotometer from AHS Laboratory Supplies (Malaysia), ultrasonicator.

2. Method

Preparation of Naringenin-loaded solid lipid nanoparticles

NRG-SLNs were prepared using emulsification and solidification method with some slight modifications from the method described by Ji et al., 2016 [8]. Briefly, 0.1 g of NRG, 0.125 g Stearic acid and 0.5g olive oil are warmed together to form an organic phase at 85° C. In a separate beaker, the aqueous phase was prepared by dissolving 0.125 g Tween 80 in distilled water at 85° C. Then the aqueous phase was poured into the organic phase and subjected under mechanical stirring to become an emulsion. The suspension formed is stirred continually for 10 minutes at 85° C. The mixture was then subjected to sonification for 10 minutes. Finally, it was cooled to room temperature to obtain semitransparent NRG-SLNs. This method was repeated with increasing composition of stearic acid and Tween 80 (w/w) as seen in Table 1. The amount of NRG, olive oil and distilled water were kept constant with 0.1 g, 0.5 g and 50 mL respectively.

Table 1: Composition of NRG formulation with increasing amount of stearic acid and Tween 80

Formula code	Stearic Acid (g)	Tween 80 (g)
A1	0.125	0.125
A2	0.250	0.250
A3	0.500	0.500
A4	1.000	1.000
A5	1.500	1.500

Ultrasonification power

The optimized formulation was chosen to be subjected to various sonification power (%) as presented in Table 2 to observe its effect on formation of solid lipid nanoparticles.

Table 2: NRG-SLNs subjected to different ultrasonification power

Formula Code	Ultrasonification power (%)
C1	70
C2	60
C3	40
C4	30
C5	10

3. Characterization of NRG-SLNs

Solubility study

The solubility of the nanoparticles and the pure naringenin were determined using shake-flask method. The sample was added in excess into distilled water and it is shaken for 24 hours using the rotary shaker at a constant temperature of 37° C. After 24 hours, the sample was centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected and analyzed using Uv-Visible Spectrophotometer [9].

Particle size analysis

Photon correlation spectroscopy was used to determine the particle size of freshly prepared NRG-SLNs at 25°C using a zetasizer. The measurement was done in triplicate after the samples were diluted with double-distilled water and placed in cuvette [8].

Polydispersion index (PDI)

Polydispersity Index of various solid-lipid nanoparticle batches were analyzed using Zetasizer Nano ZS 90 (Malvern Instrument Ltd.) [10].

Zeta potential

Zeta potential of the nanoparticles was determined using Zetasizer by employing the technique of Dynamic Light Scattering (DLS). The freshly prepared nanoparticle was diluted with distilled water and added in a folded capillary zeta cell for analysis [11].

Visual inspection

After preparation of the nanoparticle, the suspension was observed for the pattern of creaming in the cream layer and visibility of aqueous layer [12].

Encapsulation efficiency

The entrapment efficiency of the flavonoid-loaded lipid nanoparticles is determined using UviLine spectrophotometer. Firstly, the sample is prepared together with blank and centrifuged at 10,000 rpm for 10 minutes. After centrifugation, it is transferred into quartz cuvette and the absorbance is measured at wavelength of 297 nm (Naringenin). The concentration was calculated using standard curves of concentration against absorbance [12].

Short-term stability study

Naringenin-SLNs were kept at 25°C for three months at room temperature. To guarantee that the samples are free of contaminants, they must be sealed. During the first, second, and third months, the average particle size, in-vitro drug release, and physical features were regularly monitored [13].

RESULTS

1. Characterization of NRG-SLN

Solubility study

The result of the solubility profile of both pure Naringenin and its solid lipid nanoparticles formulations are as presented in Table 3. The solubility of pure Naringenin in aqueous solution was found to be slightly solubilize in water. The solubility improves with solid lipid nanoparticles formulation. Formulation A3 produced the best aqueous solubility with 0.8621 mg/ml, followed by A5, A1, A2 and A4, respectively.

Table 3: Solubility profile of pure naringenin and its solid lipid nanoparticles

Formula Code	Solubility (mg/ml)
Pure Naringenin	0.3760
A1	0.7986
A2	0.8241
A3	0.8621
A4	0.8326
A5	0.7750

Encapsulation efficiency

The results of encapsulation efficiency were tabulated in Table 4. The maximum encapsulation efficiency was 86.21% which was from sample A3. While the minimum reading was 77.50% from sample A5.

Table 4: Result of encapsulation efficiency of each sample formulation

Sample	Absorbance	Concentration of unencapsulated Naringenin	Total concentration of naringenin (mg/ml)	Encapsulation Efficiency (%)
A1	0.576	0.2014	1	79.86
A2	0.480	0.1759	1	82.41
A3	0.337	0.1379	1	86.21
A4	0.448	0.1674	1	83.26
A5	0.665	0.2250	1	77.50

Particle size analysis and Polydispersiom index

The results of D_{50} and PDI are presented in table 5. The particle size ranges from 218 nm to 1030 nm, which indicates the particles produced are in nano-size range. Polydispersion index obtained were from 0.611 to 1.000 because of the wide size distribution among particles.

Table 5: Tabulated results of particle size analysis and Polydispersion index

FORMULA CODE	D50 (nm)	POLYDISPERSION INDEX,PDI
A1	1030	0.974
A2	218	1.000
A3	398	0.611
A4	430	1.000
A5	562	0.648

Zeta potential

The charges on the surface of particle is expressed as zeta potential. Zeta potential is influenced by chemical structure of all molecules involved in the formulation. The result of Zeta Potential was -0.112 mV, which indicates that the surface charge of the particles is nearly neutral.

Visual inspection of nanoparticles

Formulation A3 has the best appearance with no visibly large particles presence and has only slight creaming of oil phase at the top. As the concentration of stearic acid increases, more creaming is presence as seen in formulation A5. The results were tabulated in Table 6 below.

Table 6: Visual inspection of NRG-SLNs with increasing concentration of stearic acid and Tween 80

FORMULA CODE	STEARIC ACID (g)	TWEEN 80 (g)	OBSERVATION
A1	0.125	0.125	- White suspension formed but with yellow clumps (olive oil) on top which indicates that solution is not emulsified due to small amount of emulsifier.
A2	0.25	0.25	- Light creamy colour with oily phase on top. -Nano particle is formed.
A3	0.5	0.5	-No visible particles which indicates nanoparticle has formed. -Slight turbid suspension with less creaming (oil phase) on top.
A4	1.0	1.0	-White colour suspension that is more viscous. More creaming appears due to high ratio of oily phase.
A5	1.5	1.5	-Suspension is dominant with creaming of oily phase.

Formulation C1 (Sonification power 70%) produced the smallest particles (204 nm) and the solution appeared semi-transparent compared to Formulation C5 (Sonification power 10%) that had the largest particle size (1032 nm) with whitish solution and visibly large particles suspended in solution. The higher the sonification power, the smaller the particle size of SLNs produced. This is proven in Table 7 below.

Table 7: The appearance and particle size of NRG-SLN with different sonification power

FORMULA CODE	SONIFICATION POWER (%)	PARTICLE SIZE (nm)	OBSERVATION
C1	70	204	Solution is semi-transparent
C2	60	386	Slightly cloudy solution formed
C3	40	662	Cloudier solution with some larger particles suspended
C4	30	694	Solution is whitish with visibly large particles
C5	10	1032	Solution is whitish with visibly large particles

Short-term stability study

The stability study of NRG-SLNs was tabulated in Table 8. The visible appearance and encapsulation efficiency of the sample was observed for a period of 3 months. After 1 month, the solution appeared to have little to no sediment of particles at the bottom. The encapsulation efficiency dropped slightly from 86.21% to 84.90 %. At the period of 3 months, colour of solution remained and there were aggregates that can be easily redispersed with shaking. The encapsulation efficiency was 80.98%.

Table 8: The tabulated result of stability study of NRG-SLNs

Parameters	1 month	2 months	3 months
Visible Appearance	Milky solution with very little sediment at the bottom. No change in colour	Milky solution with Presence of aggregates that can be redisperse by shaking. No change in colour	Milky solution with Presence of aggregates that can be redisperse by shaking. No change in colour
Encapsulation efficiency (%)	84.90	81.65	80.98

DISCUSSION

1. Solubility of NRG-SLNs

With the solid lipid nanoparticle formulation, the solubility of naringenin was improved significantly compared to free naringenin. The improvement can be contributed by a few factors such as reduction in particle size and presence of surfactant.

Effect of Particle Size on Solubility

The saturation solubility of tiny particles, particularly those in the nanometer range, might be greatly boosted. The Noyes-Whitney equation contributed to the enhancement of dissolving velocity by increasing saturated solubility and increasing surface area. This was due to decreasing particle size, as defined by the Noyes–Whitney equation, which stated that as particle size decreased, the surface area to volume ratio increased. Because of the larger surface area, there was more contact with the solvent molecules, resulting in higher solubility. Because of the enormous surface area, reducing the particle size enhances the rate of solution [14].

Effect of Presence of Surfactant on Solubility

In general, the solubility of a poorly water soluble molecules can be increased in the presence of surfactant. This may be due to reduction in surface tension by the surfactant. Molecular structure of naringenin has a bend that tend to make the molecule to become slightly amphiphilic thus allowing it to become solubilized in surfactant [15].

2. Encapsulation Efficiency

The low entrapment efficiency from formulation A1 might be due to insufficient amount of stearic acid and Tween 80 to contain the naringenin particles. However, excess amount of stearic acid causes naringenin to accumulate in lipid layer

formed on top, instead of incorporating into nanoparticles.

With increasing lipid concentration, encapsulation efficiency rose considerably. This is because the amount of lipophilic active encapsulation into lipid particles is determined by the amount of lipid available. At high lipid concentration, more lipid was available for drug encapsulation, resulting in increased encapsulation efficiency [16].

Tween 80 was chosen as the surfactant over Brij 78, Tween 80, Tween 40, and Lutrol F-68 because of its hydrophilic character, which allowed for improved drug entrapment efficiency. (Dhawan et al., 2011). The presence of sufficient surfactant ensures that the active ingredient remains in the lipid particles [16].

3. Particle Size Analysis and Polydispersion Index

The results from particle size analysis shows that the solid-lipid nanoparticles produced were in nanometer range (218 nm to1030 nm). The contributing factors to produce nanoparticles are high energy input such as high production temperature, high stirring rate, long emulsification time and strong ultrasonification power. Aside from the production parameters, the lipid matrix, surfactant mix, and lipid and aqueous phase viscosity all impacted the procedure's outcome

The limited size distribution and consistent size verified the homogenous nature of the formulation, as shown by the PDI. According to a prior publication, when the absolute value of the zeta potential is >30, particles can be disseminated stably [17].

Influence of Stirring Speed on Particle Size

Another aspect that influences nanoparticle size is the stirring speed. If the speed is too slow, the particle size will rise and the surfactant's stability will suffer; if the speed is too fast, a considerable amount of foam will be formed, which will reduce the surfactant's emulsifying action. Finally, the ideal stirring speed was determined to be 1,500 rpm [18].

Influence of Homogenization Time on Particle Size

Increased homogenization time resulted in a significant increase in particle size. The particle size was lowered to an optimum at 10 minutes, but starts to adversely increased by time. Although extending the period of homogenization breaks up the emulsion droplets into smaller droplets, there is a significant potential for droplets to coalesce when they collide, which is exacerbated by the increased surface area of the predominantly generated tiny droplets. An experiment by clearly shows that increased homogenization time resulted in nanoparticles with various sizes and distribution due to agglomeration [19].

According to kinetic characteristics, tiny particles are forced together at relatively fast speeds and more efficiently interact with one another, resulting in a simple agglomeration process.

Influence of Lipid and Emulsifier Concentration on Particle Size

The effect of lipid phase, which is stearic acid on the particle size was evaluated with varying the concentration from 5% up to 12.5% (w/w). The results of this study proved that upon increasing lipid concentration, the particle size together with polydispersion index increases. (Tiyaboonchai et al., 2007) An intrinsic thermodynamic instability of the nanoparticle system with scattered molecules of the surfactant and co-surfactant in the lipid matrix might explain the observed aggregation, leading in emulsifier adsorption to the particle surface. The emulsifier is adsorbed directly onto the surface of the particles at very low concentrations. However, at high emulsifier concentrations, compression of the emulsifier molecules at the particle surface, leading to the production of loops and tails and eventually bridging between the main nanoparticles [20].

Furthermore, a high surfactant concentration lowers surface tension and improves particle partitioning during sonication. Due to the reduction in particle size, the increase in surface area during sonication happens relatively quickly. As a result, kinetic considerations must be taken into account. There are more surfactant molecules that can stabilise the new surface that is formed by the reduction in particle size as the lipid to surfactant ratio is raised [21].

Influence of Temperature on Particle Size

Higher temperatures cause the inner phase's viscosity to drop, resulting in smaller particle sizes. High temperatures, on the other hand, hasten the drug's and carrier's deterioration [22].

In previous study, the temperature was gradually raised from 30 to 90 °C with increments of 20 °C to evaluate the influence of water temperature on particle size. With an increase in water temperature, a decrease in particle size was observed. When the temperature of the water was elevated from 30 to 90 °C, the particle size fell from 9.71.2 m to 16705 nm. This appears self-evident, as adding colder water causes the melt to quench, resulting in quicker aggregation and bigger particles, as well as an increase in viscosity. Lipid crystallisation is an unavoidable part of the process. When water at the same temperature as the molten lipid mixture is utilised for pre-emulsion formation, quenching and crystallisation can be avoided [23].

Influence of Sonication Time on Particle Size

The sonication time (ST) had a significant impact on particle size and Polydispersion index (PI). With increasing ST, size and PI both reduced dramatically. These findings are plausible since SLNs' ultimate particle size was determined by sonication, which converted coarse emulsion drops to nanoparticles droplets. More sonication energy was applied to the SLN dispersions with a longer sonication period, resulting in smaller nanoparticle size and a narrower size distribution [16].

4. Zeta Potential

Zeta potential is referred to the electric potential in the interfacial double layer at the site of the sliding plane against a point in the bulk fluid distant from the interface in a colloidal system. The potential difference between the dispersion medium and the stationary layer of fluid linked to the dispersed particle is expressed by this term. Zeta potential is often used as a way to characterize double-layer properties. The higher zeta potential value is associated with greater stability of a colloidal formulation [24].

The zeta potential of solid lipid nanoparticles formed read at -0.112 mV, which can be considered as nearly neutral. Zeta potential can be influenced by the chemical molecules present in the formulation. The ionic property of surfactant used which was Tween 80 can affect the surface charge of particles formed because they are coated with the surfactant. Tween 80 is a non-ionic surfactant with Hydrophile-Lipophile Balance (HLB) value of 16.7. A nano-emulsifying formulations requires surfactant with HLB value of more than 12 [25].

As a result, the obtained zeta potential range was insufficient for electrostatic stabilisation. However, the combination of electrostatic and steric stabilisation of the SLN formulations can still be expected since Tween 80 can give further steric stabilisation of particles [26].

5. Visual Appearance of Naringenin Solid Lipid Nanoparticles

The emulsifier used has a significant influence on the SLN quality. Increased emulsifier concentration aids in surface tension reduction and particle partitioning during homogenization. Increased surface area is achieved by reducing particle size. The initial dispersion must have an excessive amount of emulsifier during SLN preparation in order to quickly cover the new surfaces created during High Pressure Homogenization; otherwise, agglomeration of uncovered new lipid surfaces would occur. For different kinds of surfactants, the time required to redistribute emulsifier between new particle surfaces and micelles varies. It has been discovered that low molecular weight surfactants require less time to redistribute, while high molecular weight surfactants take longer [21].

The Effect of Ultrasonification Power

The ultrasonication techniques affect the surface and structure of nanoparticles (act as repulsive forces) and prevent the agglomeration of particles to achieve stable nanofluids. At higher ultrsonification, nanoparticles overcome the adhesion force and resulted in higher stability from particle distribution [27].

Nanoparticles prefer to agglomerate rather than diffuse in fluids because of their surface energy. The ultrasonication method can disperse nanoparticles in suspensions and break up agglomerations. However, knowing the appropriate amount of sonication time to overcome the surface energy of nanoparticles is crucial for effective dispersion. The common notion that longer ultrasonication of nanofluids is required for greater nanoparticle dispersion may be incorrect, as several recent studies have found that there may be an optimal period for improved dispersion stability, which may not be the longest ultrasonication time investigated [27].

6. Stability Study

The presence of surfactant-stabilizer such as Tween 80 gives further stabilization by steric repulsion. The steric effect reduces particles aggregation, thus producing a stable formulation [28].

According to stability tests from previous study, storing at room temperature (i.e. 25°C) results in decreased entrapment and increased particle size due to the coalescence of the previously nanoparticulate system into a microparticulate system. As a conclusion, the preferred storage condition for SLNs was discovered to be refrigeration (i.e. 2–8°C). Earlier research has shown that the ideal storage temperature for SLNs is 4°C [29].

As a result, it was discovered that SLNs made using this improved production approach may yield increased drug incorporation for lipophilic active such as naringenin.

CONCLUSION

Naringenin alone has a low effectiveness but the incorporation into solid lipid nanoparticles has proven to improve its solubility profile and stability. The optimal sonification power was observed above 50% while the best concentration of stearic acid and Tween 80 were at 0.5g each. It clearly demonstrates that solid lipid nanoparticle can be utilized as a delivery technique to improves the solubility of a poorly soluble ingredient like naringenin

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