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Submission date: 08-Feb-2023 11:21AM (UTC+0700)
Submission ID: 2009071170
File name: Artikel_2023_gendis.pdf (291.55K)
Word count: 3476
Character count: 17618

Natural Sciences Engineering & Technology Journal Vol 3 Issue 2 2023

Natural Sciences Engineering & Technology Journal (NASET Journal)

Journal Homepage: https://nasetjournal.com/index.php/nasetjournal

In vitro Naringenin SNEDDS Release Test by Dissolution

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ARTICLE INFO

Keywords:
SNEDDS
Naringenin
Triacetin
Tween 80
Transcutol p

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/nasetjournal.v3i2.29

ABSTRACT

Naringenin is the main flavanone in grapefruit which has anti-inflammatory, anti-cancer, hepatoprotective and antilipid peroxidation effects. Its low solubility in water causes dissolution and low bioavailability when taken orally. This study aims to increase the solubility and bioavailability of naringenin by using the SNEDDS technique. Initial characterization to determine the optimum formula was carried out using the D-optimal mixture design method, namely by optimizing the composition of SNEDDS which consisted of triacetin as the oil phase, tween 80 as surfactant and transcutol P as cosurfactant as an independent factor and SNEDDS characterization included emulsification time, drug loading, size globules and percent transmittance in response. The optimization results showed that the optimum formula was obtained at the composition of 10% triacetin, 70% tween 80 and 20% transcutol P. The dissolution test showed that the SNEDDS of naringenin was capable of dissolution (Q_{30}) of 87,50% ±1,73 at the 30th minute and the f_2 value of 28,93 so it can be concluded that the dissolution profile between the SNEDDS of naringenin and the naringenin capsules is not identical.

1. Introduction

Naringenin is a flavonoid Citrus is abundant in fruits such as grapes, grapefruit, blood orange, lemon, pamelo, and tamarind. NRG has been reported to have several effects on biological systems, such as antioxidant, anti-inflammatory, anticancer, anti-fibrogenic, and antiatherogenic. However, other studies have found the administration of this drug to be hampered by its. Extreme water insolubility and low bioavailability Single doses of naringenin 150, 300, 600, and 900 mg are safe and well-tolerated in humans. At these doses, the metabolite naringenin appears in the circulation and disappears after 24 hours. Naringenin at a concentration of 8 µM is effective on human major adipose and major adipose tissue. Consuming 300 mg twice a day will be sufficient to elicit a physiological response. So one way to overcome the problem of bioavailability of naringenin is to formulate it into SNEDDS with the composition of triacetin oil, surfactant tween 80, and cosurfactant transcutol P. Recently, lipid-based formulations have emerged as the best and most effective solution for delivering drugs with low solubility, among others, SNEDDS has proved to be a promising technology to increase the systemic bioavailability of low water solubility drugs or

phytoconstituents. SNEDDS is an isotropic mixture of the active ingredient, oil, surfactant, and usually one or more hydrophilic cosolvents or emulsifiers that come into contact with an aqueous medium by light shaking, forming a fine oil-in-water nanoemulsion with droplet sizes ranging from 20 to 200 nm. The maximum solubility of the drug in the oil phase is very important to keep the drug in the dissolved form and prevent the deposition of the drug during dissolution in the intestinal lumen. The higher the solubility of the drug in oil, it is certain that the smaller the amount of oil used in the formulation and consequently, the smaller the amount of surfactant and cosurfactant needed to emulsify the drug in oil droplets. It has been observed that the solubility of naringenin is higher in semi-synthetic carrier oils than in natural oils. Among several oils, triacetin was found to dissolve a maximum amount of naringenin of 145 ± 0.51 mg/ml. The solubility in triacetin is significantly higher compared to other oils.[1-5]

Nonionic surfactants are generally considered safer than ionic surfactants and are generally acceptable for oral administration. Surfactant is the main component that can determine the self-emulsification mechanism and droplet size. Co-surfactant works as a solubilizing agent for drugs in oil and is able to help surfactants to stabilize dispersion oil. Failure to select the appropriate oil, surfactant, and cosurfactant can lead to errors in the SNEDDS formulation.

The use of Tween 80 is widely used in research on o/w type SNEDDS formulations to increase the solubility of drugs that have poor water solubilities, such as mefenamic acid, atorvastatin, insulin, pitavastatin, and glimepiride drugs. The use of surfactant tween 80 and Transcutol as co-surfactants showed that the SNEDDS preparation was proven to increase the dissolution of the active substance. The formula is determined using Design-Expert software by selecting the D-optimal mixture design. The independent variables were included along with the upper and lower limits of each variable, namely oil (10 – 30%), surfactant (50 – 80%), and co-surfactant (10 – 30 %). The critical parameters that were observed to see the success of the SNEDDS formula were the parameters of emulsification time, drug loading, percent transmittance, and particle size. Dissolution testing is emerging as a very important tool in the pharmaceutical industry. The dissolution test is used extensively in formulation development, monitoring of manufacturing processes, and as a control. The value of Q is the amount of active substance dissolved as listed in each monograph, expressed in percentage levels on the label. Another parameter used for dissolution evaluation is dissolution efficiency (ED). The dissolution efficiency value is the AUC (area under the curve) value of the amount of drug dissociated per unit of time.[6-9] This study aims to increase the solubility and bioavailability of naringenin by using the SNEDDS technique.

2. Methods

Materials and instruments

The tools used are analytical balance, measuring cup, volumetric flask, beaker glass, micropipette, cuvette, magnetic stirrer, UV Vis spectrophotometer, Particle Size Analyzer (PSA), ultrasonicator, 10 ml glass vial, type 2 USP dissolution device (basket type) 1 chamber, centrifuge. The materials used were naringenin, 96% ethanol, triacetin, tween 80, transcutol P, distilled water, metanol p.a, HCl 0,1 N (merck).

Optimization formula

The SNEDDS formula was made according to the proportions obtained from the software design expert and then adjusted for the manufacture of 10 ml SNEDDS. Comparison of the composition of triacetin = 10-30%, tween 80 = 50-70% and transcutol P = 20-40%.

Making SNEDDS Naringenin

Pipette each component of SNEDDS, namely triacetin, tween 80, and transcutol according to the calculations in the formula table in D–optimal mixture design for 10 ml of preparation, then mixed using an ultrasonicator for 10 minutes and then stirred with a

magnetic stirrer with a rotation of 500 rpm until a homogeneous (single-phase) isotropic mixture is formed. The SNEDDS formed was added with naringenin little by little until the saturated condition was indicated by the presence of turbidity in the SNEDDS. SNEDDS naringenin was saturated and then centrifuged at 5000 rpm for 45 minutes. The results of the SNEDDS naringenin supernatant were stored in vials and protected from sun exposure, and stored at room temperature. The results of the supernatant were tested for characteristics including emulsification time, drug loading, percent transmittance, and measurement of globule size.

Determination of the optimum formula

The optimum formula was selected based on the test results of each characteristic. The conditions that must be met from each characteristic are emulsification time or nanoemulsion formation time < 1 minute; The higher the drug loading value or the drug content in SNEDDS, the better. The concentration of naringenin as a free radical scavenger in the FRAP (ferric reducing antioxidant power) test was 60 µg/mL, so the Naringenin level was expected to be close to this figure.[6] The percent transmittance value is close to 100%, which means that the SNEDDS clarity level is close to that of distilled water and has a nanoemulsion droplet size of < 100 ηm.

Dissolution test

The dissolution profile was tested using a basket-type instrument (dissolution tester 1 USP basket type) with 900 mL of 0.1N Ph 1.2 HCl solution as a medium. The basket rotation speed is 100 rpm, and the media temperature is 37 ± 0.5℃. Sampling using a syringe was carried out at 5, 10, 15, 30, 45, and 60 minutes for as much as 5 mL. Each sampling solution is replaced with the same medium so that the volume in the chamber remains. The absorbance of the sample solution was measured using a 0.1N HCl solution blank with a UV spectrophotometer at a wavelength of 299 ηm. The absorption measurement was repeated three times for each test time, and then the parameter values of Q_{30} and f_2.

3. Results and Discussion

Preparation of SNEDDS Naringenin

The SNEDDS formula consists of three components, namely triacetin, tween 80, and transcutol P, then determines the SNEDDS formula using D-optimal mixture design software on Design Expert 12. The three constituent components are included in the mixture component with a quadratic model, and Four responses were included, namely emulsification time, drug loading, globule size, and percent transmittance, so that sixteen runs were obtained from formulas. Based on the design of the formula, 10 mL of SNEDDS preparation was made by mixing the three constituent materials into a vial and then putting them in an ultrasonicator for 10 minutes which functions to mix and reduce particle size. The sonication method using ultrasonic waves can speed up the contact time between the components that make up SNEDDS, even at room temperature. These waves surround the sample, so that cavitation bubbles are generated, which cause nano-diameter particles. The homogeneous SNEDDS mixture was then mixed using a magnetic stirrer at room temperature, and the speed was kept constant, then, naringenin was added until a slightly cloudy SNEDDS was obtained, and there was undissolved naringenin. This indicates that the addition of naringenin is saturated. The mixing process with a magnetic stirrer aims to increase homogeneity, reduce globule size and assist the process of dissolving naringenin in SNEDDS. The saturated SNEDDS preparation was then centrifuged for 45 minutes at a speed of 5000 rpm to obtain a clear supernatant free of impurities. The clear supernatant was then tested for SNEDDS characteristics, including emulsification time, drug loading, percent transmittance, and globule size. In this study, the results of the SNEDDS supernatant were thick, yellow, and clear. The results of observations and characterization of critical parameters of SNEDDS are presented in Table 1.

Table 1. Results of observations and measurements of the SNEDDS formula

Formula	Composition			WE (seconds)	DL (µm/mL)	Transmittance (%)	Globule Size (nm)
	A	B	C				
1	10	60,07	29,92	30,39 ± 0,095	23,84 ± 0,052	30,22±0,025	25,70±0,100
2	10	50	40	58,15 ± 0,254	25,08 ± 0,010	25,61±0,028	32,23± 0,152
3	10	55,21	34,78	32,26 ± 0,902	29,46 ± 0,173	39,33±0,020	27,23± 0,251
4	30	50	20	32,30 ± 0,041	34,46 ± 0,015	25,90±0,173	332,63± 0,251
5	10	70	20	15,17 ± 0,047	67,73 ± 0,073	90,66±0,292	23,33± 0,416
6	14,88	50	35,11	40,66 ± 0,336	61,93 ± 0,041	44,18±0,160	19,70± 0,200
7	16,68	56,71	26,60	50,35 ± 0,186	64,65 ± 0,015	46,78±0,080	20,13± 0,351
8	19,98	60,01	20	38,31 ± 0,216	26,93 ± 0,058	93,69±0,020	11,40± 0,400
9	20,00	50,13	29,85	80,07 ± 0,457	20,15 ± 0,050	89,39±0,083	44,70± 0,264
10	10	60,07	29,92	55,87 ± 0,272	19,32 ± 0,020	76,21±0,104	12,93± 0,416
11	23,68	53,78	22,52	40,40 ± 0,060	8,40 ± 0,0404	81,34±0,392	282,43± 0,416
12	19,98	60,01	20	46,29 ± 0,095	31,95 ± 0,015	79,74±0,072	11,53± 0,351
13	10	70	20	20,52 ± 0,424	42,90 ± 0,020	84,83±0,040	11,46± 0,550
14	16,68	56,71	26,60	49,90 ± 0,403	56,36 ± 0,025	90,27±0,068	11,86± 0,802
15	20,00	50,13	29,85	78,12 ± 0,112	17,55 ± 0,050	92,12±0,062	42,53± 0,550
16	13,51	63,23	23,24	48,20 ± 0,229	50,58 ± 0,070	89,84±0,040	11,30± 0,700

Determination of the optimum formula

Optimization of formula in pharmaceutical preparations, including SNEDDS preparations, is to determine the level of variable stable and strong products with high-quality characteristics that can be produced. Optimization was carried out by entering the upper and lower limit values for the concentration of triacetin oil, surfactant tween 80, and cosurfactant transcutol P using expert design software and inputting the parameters and criteria, which can be seen in the Table 2.

Table 2. Optimum formula criteria

Parameter	Criteria	Lower limit	Upper limit
Triacetin	In range	10	30
Tween 80	In range	50	70
Transcutol P	In range	20	40
Emulsification time	In target	15.17	80.07
Drug loading	Maximize	8.4	67.73
Globule size	Minimize	11.3	332.6
Percent transmittance	Maximize	25.61	93,69

Optimization with a D-optimal mixture design is then determined by the optimal formula with predetermined criteria. The optimal formula obtained was then remade with three replications and characterized, including emulsification time, drug loading, globule size, percent transmittance, zeta potential, and in vitro release test using the dissolution method. The composition of the optimum formula and the results of the characterization of the optimum formula SNEDDS naringenin can be seen in Table 3:

Table 3. The results of the measurement of the optimal formula response

No	Triacetin	Tween 80	Transcutol P	WE (seconds)	DL (mg/L)	PSA (nm)	% Transmittance	Desirability
1	10.16	69.842	20.00	18.58 ± 0.62	51.13 ± 4.53	14.8	88.74 ± 2.27	0.942

Observations and measurements of the emulsification time in the optimum formula obtained the emulsification time of 18.58 ± 0.62 seconds with a clear and homogeneous visual appearance. From the results of measurements and visual observations of nanoemulsions, it can be concluded that the optimum formula nanoemulsion is in grade A (the system forms nanoemulsions quickly < 1 minute with a clear appearance).

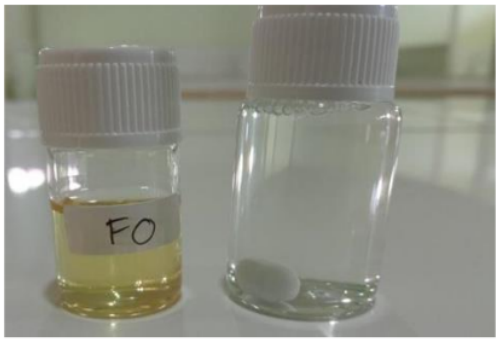

Figure 1. Nanoemulsion appearance of the optimum formula of SNEDDS naringenin

After visual observation, the percent transmittance was measured to determine the clarity level of the nanoemulsion. In measurements using UV Vis spectrophotometry, the percentage of transmittance of the optimum formula was 88.74% ± 2,275, which can be concluded that the nanoemulsion has a clarity level that is close to good clarity because it is above 80%.

In the measurement of drug loading, the optimum formula with three replications obtained absorption of 0.753, 0.778, and 0.712 after being calculated using a linear regression equation obtained naringenin levels of 52.13 mg/L; 55.53 mg/L, and 46.55 mg/L and the average value of the optimum drug loading formula was 51.41 ± 4.53 mg/L.

Measurement of the globule size of the optimum formula in this study obtained a globule size of 14.8 nm with a polydispersity index of 0.366. This shows that the globule size in the optimum formula has met the criteria for nanoparticle characterization, namely 10-200 nm, and the polydispersity index value describes the uniformity of the size of the nanoemulsion, which is polydisperse in which the

particle size produced is uniform but has various shapes.[10]

Dissolution test

Determination of the percent dissolution content of SNEDDS naringenin by means of a UV spectrophotometer at a wavelength of 309 nm. The parameters observed are the value of Q_{30} and the similarity factor (f_2). The results of the release test with the dissolution of the SNEDDS preparation of naringenin and the active substance naringenin can be seen in Table 4:

Table 4. Percentage of drug dissolution (Q_{30}) SNEDDS of naringenin and active substance naringenin

Time (minutes)	% Dissolution	
	SNEDDS Naringenin	Naringenin capsules
5	64.40 ± 8,40	27.39 ± 2.47
10	72.88 ± 4.10	45.31 ± 5.53
15	84.26 ± 3.30	53.32 ± 3.55
30	87.50 ± 1.73	69.88 ± 4.73
45	93.90 ± 4.40	71.90 ± 7.03
60	95.18 ± 3.29	78.22 ± 2.13
Q_{30} value (%)	87.50 ± 1.73	69.88 ± 4.73

The results of the SNEDDS naringenin dissolution test are described by a straight line equation between time versus % drug dissolution dissolved in 0.1 N HCl media. This depiction of the drug release profile at 30 minutes can also be referred to as the Q_{30}. The dissolution graph of SNEDDS naringenin with the active substance naringenin has almost the same pattern, namely an increase in dissolution from 5 to 60 minutes.

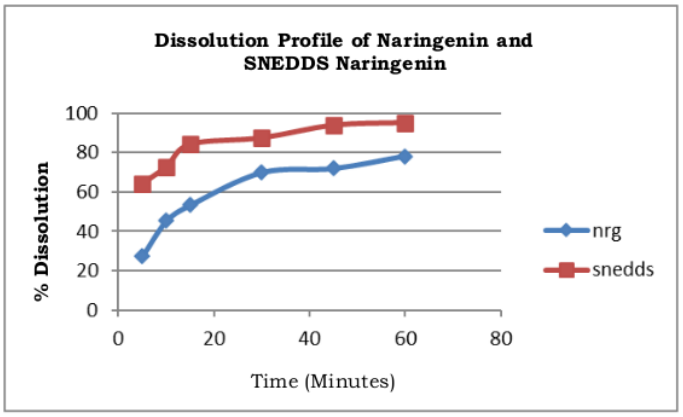

Figure 2. Graph of the dissolution profile of naringenin and SNEDDS naringenin

At the beginning of the minute, the experiment showed that the SNEDDS formula of naringenin at 5 minutes had been dissolved at a high concentration, namely at 64.40% ± 8.40. Then at 15 minutes, the SNEDDS of naringenin was more than 80% dissociated. The high percentage of dissolution at the beginning of the test time illustrates that SNEDDS can quickly form spontaneous nanoemulsions when in contact with liquid. Based on the Q_{30} obtained in the SNEDDS naringenin formula of 87.50% ± 1.73, it can be concluded that the formula meets the requirements for the Q_{30} that within 30 minutes, it must dissolve at least 80%. The dissolution profiles of the naringenin SNEDDS preparations and naringenin capsules were compared using the f_2 value, where the f_2 value = 50 or greater indicated the similarity of the 2 dissolution

profiles. The dissolution test showed a value of 28.93 or less than 50, so it can be concluded that the dissolution profiles of the two preparations are not identical.[11]

4. Conclusion

The optimal proportion of triacetin, tween 80, and transcutol P in the manufacture of SNEDDS naringenin using the D-optimal mixture design method is in the ratio 10.16 : 69.84 : 20. The results of the in vitro release test of SNEDDS preparation with dissolution test on the formula optimum have a Q_{30} of 87.50% ± 1.73 and f_2 of 28.93.

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