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Research Article

ISSN: 2395 -5775

STABILITY STUDY OF GAMAVUTON (GVT-0) SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEM (SNEDDS) WITH MYRITOL AS THE OIL PHASE

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Abstract

Rheumatoid arthritis (RA) is an autoimmune disease with arthritic inflammation in adult patients. Gamavuton-0 (GVT-0) is an analog compound of curcumin that has the activity in the treatment of RA with low solubility in water, which affects the absorption into the systemic circulation.Absorption of GVT-0can be improved by makinginto the SNEDDS (Self-Nanoemulsifying Drug Delivery System) dosage form. Stability is one of the factors that affects the quality, safety, and efficacy of the SNEDDS dosage form. This study aims to determine the stability of GVT-0 SNEDDS that have been made by the previous researcher. GVT-0 SNEDDS was made using Myritol as the oil phase, Cremophor EL and Tween 20 as the surfactants, and propylene glycol as the cosurfactant. The stability study was conducted with centrifugation test, heating-cooling cycle test, freeze-thaw cycle test, endurance test, and accelerated storage test. No phase separation was showed by the result of the centrifugation test, heating-cooling cycle test, and freeze-thaw cycle test. The result of endurance test and accelerated storage test was quite well with the particle size was ≤ 200nm, the value of PDI was between 0,20-0,70, and the % transmittance was close to 100%. It can be concluded that the GVT-0 SNEDDS has good stability profile.

Key Words: Rheumatoid arthritis, GVT-0, SNEDDS, stability study

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease with an emergence of arthritic inflammation in adult patients (Singh, *et al*, 2015). This type of rheumatic is the most serious form of arthritis that causes severe joint damage (Muchid, 2006). The pain sensation that emerges on the synovial part of joints and tendon sheaths may be thickened due to an inflammation that is followed with a destruction of bones around the area of joints (Wijayakusuma, 2006).

The RA patients start their treatment with the DMARDs (Disease Modifying Anti-Rheumatoid Drugs) such as methotrexate, sulfasalazine, and leflunomide. Each DMARDs has its own toxicity so that it requires careful monitoring of therapy. The low dose of oral corticosteroids is also the part of RA treatments, with many side effects. NSAIDs are also the part of RA treatments.

However, the use NSAIDs on long term period may cause a bleeding in the GI tract. Furthermore, the effect of NSAIDs is only to relieve the symptoms of RA, not to cure the disease (Chabib, *et al*, 2016).

Gamavuton-0 (GVT-0) is an analog compound of curcumin that has the activity in the treatment of RA (Ikawati, *et al*, 2014). However, GVT-0 indicates low solubility in water, which affects the absorption into the systemic circulation thus weakening its pharmacological effects. This problem could be an obstacle to the development of GVT-0 as a drug that is used orally.

SNEDDS (Self-Nanoemulsifying Drug Delivery System) is a system of nanoparticles that could improve the solubility of a compound so that it enhances drug penetration into the site of action. The enhancement of absorption of GVT-0 using SNEDDS as the dosage form could increase the bioavailability of drug so that the drug will be more effective in the treatment of RA.

Stability of SNEDDS dosage form is influenced by the size of globules of the SNEDDS dispersed phase.

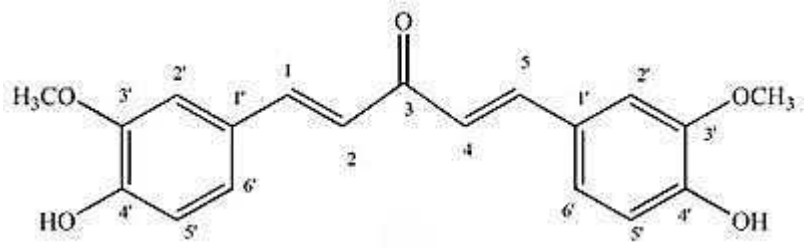

Figure 1. Structure of GVT-0 (Sardjiman, 2000).

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Received: December 27, 2016 | **Accepted:** January 18, 2017 | **Published Online:** February 28, 2017

Conflict of interest: None declared | **Source of funding:** Nil

The small size of the globules could lead to a decrease of gravitation and Brownian motion that prevents creaming or sedimentation thus increasing the physical stability of the SNEDDS. The small size of the globules could also preventflocculation. Nanoemulsion could produce very low surface tension and a big surface area between the oil and water phase (Fanun, 2014).
Based on the description above, the stability study of GVT-0 SNEDDS is necessary to be conducted. The stability study consists of centrifugation test, heating-cooling cycle test, freeze-thaw cycle test, endurance test, and accelerated storage test. This study is done to see the physical stability of the GVT-0 SNEDDS through a series of stability tests, so that provides many researchers an overview about the stable formula of SNEDDS.

MATERIALS AND METHODS

Materials

Materials were used are: GVT-0 SNEDDS (manufactured by Bagiana, 2014), aqua pro injection (General), cremophore EL (Sigma), propylene glycol (Brataco), tween 20 (Sigma), and myritol.

Methods

Formulation of the GVT-0 SNEDDS

The formula of GVT-0 SNEDDS was obtained from Bagiana, who was the previous researcher. The formula of GVT-0 SNEDDS was shown in table 1.

Table1 Formula of the GVT-0 SNEDDS (Bagiana, 2014).

Material	Function	Quantity
Gamavuton-0 (GVT-0)	Active ingredient	1 g
Myritol	Oil phase	200 mL
Propylene glycol	co-surfactant	200 mL
Cremophor EL	Surfactant	900 mL
Tween 20	Surfactant	300 mL

GVT-0 was weighed carefully, then it was dissolved into myritol until it was dissolved completely. The solution then was added with the co-surfactant using magnetic stirrer with speed of 500 rpm for 15 minutes. Then, it was added with surfactants, which were cremophor EL and tween 20.

Centrifugation test

GVT-0 SNEDDS formula was diluted 100 times with aqua pro injection. Then, it was centrifugated using the centrifugator with speed of 3500 rpm for 30 minutes and then it was observed visually to check the phase separation (Sawant, *et al*, 2011).

Heating-cooling cycle test

The stable formula resulted from the centrifugation test was used to conduct the heating-cooling cycle test that was conducted by six cycles at the temperature of 4°C and 40°C with storage of the formula was not less than 48 hours. The formula must be stable at this temperature. Then, the formula was centrifugated with speed of 3500 rpm for 15 minutes and then it was observed visually to check the phase separation (Sawant, *et al*, 2011).

Freeze-thaw cycle test

The stable formula resulted from the heating-cooling cycle test was used to conduct the freeze-thaw cycle test that was conducted with six cycles at the temperature of -20°C and 25°C with storage of the formula was not less than 48 hours. The formula must be stable at this temperature. Then, the formula was centrifugated with speed of 3500 rpm for 15 minutes and then it was observed visually to check the phase separation (Sawant, *et al*, 2011).

Endurance test

The stable formula resulted from the freeze-thaw cycle test was used to conduct the endurance test. The formula was diluted with the dilutions of 25, 50, 100, and 250 times with aqua pro injection. Then, the change of% transmittance, polydispersity index (PDI), and particle size of the formula was evaluated (Sawant, *et al*, 2011).

Accelerated storage stability test

The stable formula resulted from the endurance test was used to conduct the accelerated storage test, which was conducted for 1 month with the storage condition of 40°C ± 2°C/75% RH ± 5% RH. Then, the change of % transmittance, polydispersity index (PDI), and particle size of the formula that was diluted 250 times was evaluated at weeks 0, 1, 2, 3, and 4 (Anonymous, 2013).

RESULTS AND DISCUSSION

Centrifugation test

The test is conducted to determine the stability of SNEDDS after it is changed to an emulsion form. Table 2 indicates that the three replications of the formula were able to maintain their stability after they were centrifugated with no phase separations occurred.

Table 2 Result ofthe centrifugation test

Replication	Phase behavior
1	No phase separation
2	No phase separation
3	No phase separation

Based on the Stokes Law, some factors such as particle size of the dispersed phase, the density difference between the phases, and the viscosity of outer phase are related to the speed of dispersed phase separation of an emulsion. The density difference between the dispersed phase and outer phase should be as low as possible and the outer phase should have quite high viscosity. A decrease of surface tension and increase of

viscosity could be achieved with the addition of surfactant to an emulsion (Anton & Vandamma, 2011).

Heating-cooling cycle test

The test as one of the thermodynamic studies in this study is conducted to observe the effects that are caused by heating, cooling, and centrifugation onthe thermodynamic stability of SNEDDS (Patel, *et al*, 2008). Table 3 indicates that there was no phase separation in all replications of the formula that were used to conduct the heating-cooling cycle test.

Table3 Result of the heating-cooling cycle test

Replication	Phase behavior
1	No phase separation
2	No phase separation
3	No phase separation

An emulsion tends to be stable at the temperature of 40°C - 45°C. However, it will be unstable at the temperature of 50°C -65°C despite few hours of storage. An emulsion would be more aqueous at the high temperature, whereas at the room temperature, an emulsion would be more viscous. The solubility of surfactant is more sensitive in either the oil phase or the water phase thus freezing is more potential to break the emulsion (Anton & Vandamma, 2011).

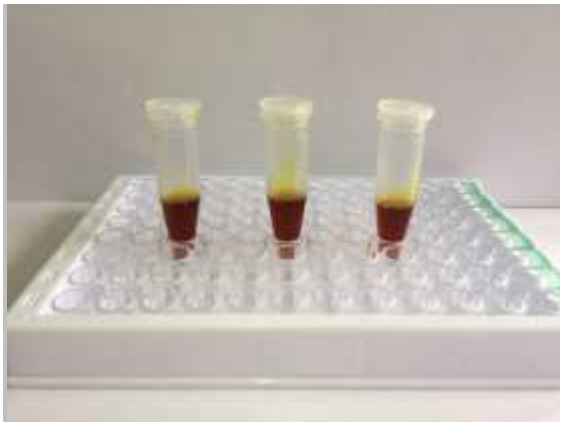

Figure 2 Result of the heating-cooling cycle test.

The test is also conducted to observe the thermodynamic stability of the SNEDDS. The difference between the freeze-thaw cycle test and the heating-cooling cycle test is the temperatures that are used.

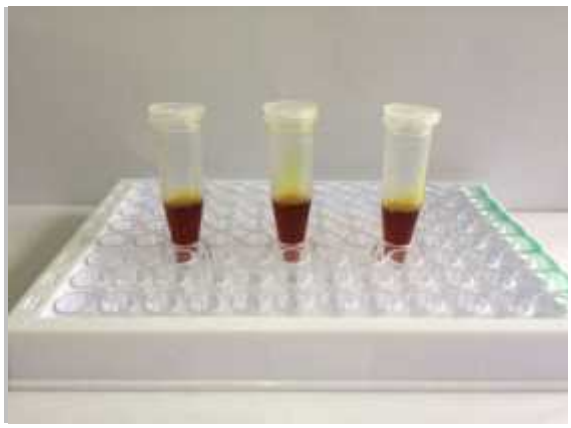

Figure 3 Result of the freeze-thaw cycle test.

The temperatures that are used in the freeze-thaw cycle test were -20°C and 25°C, whereas the temperatures that are used in the heating-cooling cycle test were 4°C and 40°C.

Table 4 indicates that there was no phase separation in all replications of the formula that were used to conduct the freeze-thaw cycle test.

Table 4 Result of the freeze-thaw cycle test

Replication	Phase behavior
1	No phase separation
2	No phase separation
3	No phase separation

Endurance test

The test is conducted to observe the character similarity of the nanoemulsion through various level of dilutions. The test is also can be used to ensure the similarity of drug release and to ensure that the drug would not form a sedimentation in higher level of dilution that could lower the absorption of drug (Date & Nagarsengker, 2007). The result of the endurance test was shown in table 5.

Table 5 Result of the endurance test

Dilution	Particle size (nm) [a] ± SD	PDI [a] ± SD	Transmittance (%) [a] ± SD
25x	100,5±0,02	0,32±0,01	97,26±0,01
50x	100,56±0,01	0,36±0,01	96,18±0,06
100x	100,73±0,06	0,30±0,08	96,04±0,01
250x	101,4±2,33	0,43±0,04	96,37 %±0,01

[a] Data is shown as average ± SD (n = 3)

% transmittance test is conducted to observe the ability of the sample solution to transmit the light that is fired from UV spectrophotometer, whereas % transmittance value of a formula describes the emulsification process of a surfactant (Anton & Vandamma, 2011). The higher value of the % transmittance indicates the better ability of surfactant that is used in the emulsification process. Based on table 5, the result of % transmittance test was considered good due the values were close to 100%. However, the value of % transmittance could not describe the particle size of the nanoemulsion that is formed in the emulsification process. Further analysis was conducted using the Particle Size Analyzer (PSA) to observe the particle size and the Polydispersity Index (PDI) value of GVT-0 SNEDDS.

PDI value indicates the measured particle size uniformity of an emulsion. The ideal particle size distribution is between the range of 0,20-0,70. The PDI value that greater than 0,40 indicates wider particle size distribution, in other words, the lower particle size uniformity of an emulsion (Mao, *et al*, 2009). A formula is considered stable if the measured particle size is ≤ 200 nm and the PDI value is between 0,20-0,70 with resistance to process of dilution (Makadia, *et al*, 2013; Mao, *et al*, 2009). One of the factors that affect the particle size is the homogenization process. The heat

that is generated by a friction and high pressure during the process of homogenization could affect the particle size of emulsion. The ionic surfactant that is used in an oil in water (o/w) type emulsion possesses molecular geometry that susceptible in the high temperature. The hydrophilic part of the surfactant molecule is experiencing dehydration when the temperature is rising. This causes a susceptibility to the particle of nanoemulsion to experience a coalescence so that the particle size of an emulsion is getting bigger (Tan & Nakajima, 2005; McClements, *et al*, 2007).

Based on table 5, the measured PDI and particle size were considered good, which were between 0,20-0,70 for the PDI and ≤ 200 nm for the measured particle size. It can be considered that the GVT-0 SNEDDS is stable against various level of dilutions, which are 25, 50, 100, and 250 times.

Accelerated storage stability test

Figure 4 Result of the accelerated storage stability test

The test is conducted to evaluate the short-term effect outside of the storage condition that is figured on the label (Anonymous, 2013). Based on table 6, the result indicates that the GVT-0 SNEDDS is stable against the accelerated storage. The particle size was ≤ 200 nm, the PDI value was between 0,20-0,70, and the % transmittance was close to 100% at weeks 0, 1, 2, 3, and 4.

Table6 Result of the accelerated storage stability test

Week	Particle size[a] ± SD	PDI[a] ± SD	% Transmittance[a] ± SD
0	100,41± 0,40	0,32± 0,12	97,76± 0,02
1	100,76± 1,45	0,46 ± 0,08	97,43± 0,01
2	101,05± 0,31	0,41 ± 0,04	96,58± 0,01
3	100,36± 1,89	0,32 ± 0,04	97,28± 0,01
4	100,59± 1,41	0,28± 0,17	97,34 ± 0,01

a Data is shown as average ± SD (n = 3)

CONCLUSION

GVT-0 SNEDDS possesses good stability that can be seen from the conducted stability study, which is no phase separation occurs in the centrifugation test, heating-cooling cycle test, and freeze-thaw cycle test. Result of the endurance test and accelerated storage stability test are considered good with the particle size ≤ 200nm, the PDI between 0,20-0,70, and the % transmittance value close to 100%.

Acknowledgment

We would like to acknowledge Curcumin Research Centre, Faculty of Pharmacy, Gadjah Mada University for providing GVT-0 in this study and Pharmacy Nano-Science, Pharmaceutical Technology Laboratory, Department of Pharmacy, Islamic University of Indonesia for providing laboratory facilites in this study. We also would like to address a gratitude to Penelitian Disertasi Doktor Kemeristekdikti for the funding to conduct a significant part of the research. Authors declare no conflict of interest.

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