**Original Research Article**

**FORMULATION AND CHARACTERIZATION OF ASTAXANTHIN SELF NANO EMULSIFYING DRUG DELIVERY SYSTEM (SNEDDS)**

**ABSTRACT**

SNEDDS (Self Nano Emulsifying Drug Delivery System) is an isotropic mixture of oil, surfactant, and co-surfactant which forms nanoemulsions spontaneously when in contact with gastric fluid thereby increasing the solubility of the active substance. One of the active substances that have low solubility is astaxanthin. This study aims to formulate and characterize Astaxanthin SNEDDS. This research is a laboratory experimental research. Astaxanthin SNEDDS made in 3 formulas by using the ratio of surfactants and co-surfactants that were characterized to produce a transmittance value of F1 91%, F2 90%, and F3 95%, with a particle size of F1 183.75 nm with a PDI 0.272, F2 195.25 nm with a PDI 0.341, and F3 105.75 nm with a PDI 0.392. So that the entrapment efficiency of astaxanthine SNEDDS of F1, F2, and F3 were 94.62%, 94.35%, and 95.57% respectively. The results showed that F3 with a surfactant concentration of 72% and co-surfactant 18% was the best formula in forming SNEDDS. It can be concluded that the higher the surfactant concentration, the greater its ability to reduce the interfacial tension of the oil droplets so as to obtain small particle sizes and high entrapment efficiency values.

**Keywords:** SNEEDS, astaxanthine, entrapment effieciency, surfactant

**INTRODUCTION**

In general, SNEDDS is a method of drug delivery through the manufacture of isotropic mixtures of oils, surfactants, cosurfactants, and drugs that spontaneously form nanoemulsions. Oil in water undergoes mild agitation when it comes into contact with the aqueous phase in the gastrointestinal tract and produces nanometer-sized droplets1.

The SNEDDS method has advantages including increasing the bioavailability of the active drug substance through oral use, increasing the dissolution rate and absorption of the active substance in the body particularly drug compounds having low solubility in water or lipophilic drugs such as drugs belonging to the BCS (Biopharmaceutical Drug Classification System) class II which these drugs have high permeability but low solubility so that it can reduce drug bioavailability2.

One of the active substances belonging to the BCS class 2 group is astaxanthin which is the main carotenoid found in aquatic organisms or animals that live in water such as shrimp, crab, salmon, and lobster as well as the microalgae *Haematococcus puvialis*. In several studies mentioned that astaxanthin is a super antioxidant, one of them is experimental research in vivo which states that astaxanthin is 14 to 60 times stronger than other antioxidants3. For that, there are many health benefits of astaxanthin, one of which can improve the immune system by increasing the production of immunoglobulins in response to polychronal stimuli with a daily dose of 4 mg / day which acts as an antioxidant that is useful for increasing the immune system and counteracting free radicals4,5.

In increasing the bioavailability of astaxanthin, many has been developed from lipid-based formulations to nano-emulsions, one of the previous studies developing bioavailability of astaxanthin, but this research, astaxanthin is made in self nanoemulsifying or spontaneous emulsion formation with a carrier oil component. Oleic acid was characterized with the results obtained in accordance with the requirements6.

Based on the background above, the research was conducted to formulate and characterize Liquid Self Nano Emulsifying Drug Delivery System of Astaxanthin that meet SNEDDS requirements.

**MATERIALS AND METHODS**

**Materials**

Astaxanthin powder was purchased from Sigma Aldrich, Singapore. Methanol, oleic acid, polyethyleneglycol 400, propyleneglycol and tween 20 were also purchased from Sigma Aldrich, Singapore.

**Methods**

**The making standard solution of astaxanthin**

Astaxanthin stock solution was prepared by dissolving 10 mg of astaxanthin into 10 mL methanol then dilution was carried out to make standard solutions with various concentration, namely, 10 ppm, 15 ppm, 20 ppm, 25 ppm and 30 ppm. After that, the absorbance was read using a UV-Vis Spectrophotometer with a wavelength of astaxanthin 470 nm.

**The solubility test**

Each ingredient was measured as much as 1 mL of oil (oleic acid, olive oil, and VCO oil), surfactant (tween 20 and tween 80) and co-surfactant (propylene glycol and PEG 400) then put into an eppendorf tube, and added 10 mg Astaxanthin into each ingredient, vortexed for 15 minutes every day for 72 hours. After that, the sample was centrifuged for 26 minutes at a speed of 6000 rpm at room temperature. The supernatant was taken and analyzed by UV-Vis spectrophotometer to determine the concentration and solubility of astaxanthin. Based on these results, the material to be determined as the oil phase, surfactant and co-surfactant was selected.

**The optimization of astaxanthin SNEDDS**

Based on the solubility results, oleic acid was used as the oil phase, tween 20 and tween 80 as surfactants, and propyleneglycol as co-surfactants then done the optimization and formulation with ratio of oil : surfactant mix (1:9) and mixed surfactant ratio (surfactant : co-surfactant) between tween 20 : propyleneglycol and tween 80 : propyleneglycol were made ratio of 1:1, 2:1, 3:1, and 3.2 :0.8 respectively. Preparation was done by mixing the components of surfactant and co-surfactant then added the oil component with using a magnetic stirrer for 30 minutes, sonicated for 10 minutes. The results of the mixing were allowed to stand for 24 hours at room temperature to see the homogeneity.

**The characterization of astaxanthin SNEDDS**

**Transmittance test (%)**

The method of measuring the transmittance value is as much as 100 µL of the astaxanthin SNEDDS formulas which were added aquadest until the final volume 5 mL then vortexed for 1 minute. After that, all formulas were measured the value of transmittance using spectrophotometry at a wavelength of 650 nm with a blank of distilled water to determine the level of clarity. The transmittance value parameter, namely the absorbance value that is close to 100%, indicating the droplet size of the dispersion produced by SNEDDS which has reached the nanometer size, which can be seen visually from the transparency of the system formed7.

**The measurement of particle size of astaxanthine SNEEDS**

Measurement of the particle size average and polydispersity index (PDI) of astaxanthin SNEDDS was carried out using a Particle Size Analyzer (PSA).

**The measurement of entrapment efficiency**

Determination of entrapment efficiency serves to determine the amount of Astaxanthin that is entraped in SNEDDS. A total of 200 mg of SNEDDS astaxanthin formula was centrifuged at 3000 rpm for 15 minutes. Free astaxanthin will precipitate, so that the entangled astaxanthin can be analyzed using UV-VIS spectrophotometer at a wavelength of 470 nm4.

**RESULTS AND DISCUSSION**

**The measurement of astaxanthin standard solution**

Based on the results of the assessment of Astaxanthin absorbance (Table 1) with 5 concentrations, namely 10, 15, 20, 25, and 30 ppm using astaxanthin wavelength of 470 nm, the equation of the line y = 0.0257x - 0.1136 with r2 = 0.9965.

**Table 1: The results of astaxanthine absorbance in various concentrations**

Concentrations (ppm) absorbance (λ=470 nm)

 10 0.152

 15 0.267

 20 0.396

 25 0.512

 30 0.671

The absorbance data produced are classified as good because all the series of levels from the smallest to the largest have an absorbance value of 0.1 - 0.7, while the absorbance value is 0.1 - 0.7. The correlation cofficient obtained is 0.9965 which meets the requirements is more than 0.9770 or almost close to 1 so that the results obtained are linear between concentration and absorbance8.

**The solubility test**

Table 2 showed that the solubility results of the components of SNEDDS which have the highest solubility with astxanthin, namely oleic acid as the oil phase, tween 20 as surfactant and propylene glycol as co-surfactant.

**Table 2: The results of solubility test with astaxanthin**

Materials function solubility (mg/mL)

Oleic acid oil phase 198.91

Olive oil oil phase 182.10

VCO oil phase 172.22

Tween 20 surfactant 172.45

Tween 80 surfactant 169.33

Propylenglycol co-surfactant 174.78

PEG400 co-surfactant 157.97

Oleic acid as the oil phase has the highest solubility in dissolving astaxanthin, this is because oleic acid has a partition coefficient value more than 6.5 so that oleic acid is easily bind to lipophilic groups of other compounds. In addition, tween 20 has a higher solubility than tween 80 because tween 20 has an HLB value 16.7 which tends to be hydrophilic and nonionic, making tween 20 have a good ability to dissolve astaxanthin. As for the co-surfactant, propyleneglycol has a higher solubility than PEG 400, this indicates that propyleneglycol has the same polarity as astaxanthin.

**The optimization of astaxanthin SNEDDS**

Table 3 showed that the results of the optimization of the SNEDDS base with the ratio of oil, surfactant, and co-surfactant, this base optimization is carried out by varying the use of surfactants such as tween 20 and tween 80 and the ratio of mix surfactant to produce a SNEDDS base with a clear physical appearance. The results obtained that formulas B, C and D produce a clear physical appearance of SNEDDS.

**Table 3: The optimization of SNEDDS base**

Formula Ratio Ratio Evaluation of

oil : surfactant mix surfactant : co-surfactant clarity

 Tween 20 : Propyleneglycol

A 1 : 1 cloudy

B 2 : 1 clear

C 3 : 1 clear

D 3.2 : 0.8 clear

 1 : 9 Tween 80 : Propyleneglycol

E 3.2 : 0.8 cloudy

F 3 : 1 cloudy

G 2 : 1 cloudy

H 1 : 1 cloudy

Formulas B, C and D using surfactant tween 20 and co-surfactant propylene glycol were more capable of producing a homogeneous and clear mixture with the addition of oleic acid compared to the use of tween 80 with propylene glycol. According to the literature tween 20 and propylene glycol have a lower molecular weight and viscosity and a better structure simpler than tween 80 and propylene glycol, so it can interact more easily with astaxanthin. The presence of free hydroxyl groups and free oxygen in astaxanthin interacting with SNEDDS and will form hydrogen bonds which make astaxanthin more soluble9,10.

**The characterization of astaxanthin SNEDDS**

**Transmittance test (%)**

Table 4 showed that the results of measuring the transmittance of astaxanthin SNEDDS using a UV-VIS Spectrophotometer produce all formulations above 90%.

**Table 4: Transmittance percent measurement**

Formula % transmittance

 1 91

 2 90

 3 95

Based on the results presented in table 4, the percent transmittance test obtained by formula 3 has a higher transmittance percent than formulas 1 and 2 because the surfactant composition in formula 3 is more than formula 1 and 2. The larger surfactant composition can affect the droplet size of the emulsion. It means that the smaller the size produced, the clearer the SNEDDS obtained, the greater the transmittance percentage. The astaxanthin SNEDDS transmittance percent of the three formulas ranged from 90% - 95% and produces a clear dispersion7.

**Table 5: Measurement of particle size using PSA**

Formula Particle size (nm) Polydispersity index

 1 183.75 0.272

 2 195.25 0.341

 3 105.75 0.392

Table 5 showed that the results of measuring the diameter of the astaxanthin SNEDDS using the particle size analyzer result all formulas of particle size < 200 nm and polydispersity index showed the uniformity of size distribution.

Based on table 5, the results of particle size measurements show that formula 3 produces a smaller particle size than formulas 1 and 2. This is influenced by The surfactant concentration used in formula 3 is greater than formula 1 and formula 2. According to the literature the use of a large surfactant concentration can reduce interfacial tension because the surfactant will surround the oil droplets when emulsified in water so that it will form a nanometer size. The particle sizes of all formulations were in the range of 105 nm -195 nm which falls within the range of SNEDDS particle size with a polydispersity index of 0.272 – 0.392 stating that all formulas have particle size uniformity2,11.

**Table 6: The measurement of entrapment efficiency using spectrofotometry UV-VIS**

Formula % Entrapment efficiency

1. 94.62%
2. 94.35%
3. 95.57%

Table 6 showed that the measurement results of astaxanthin SNEDDS have entrapment efficiency above 90% i.e. the range of 94% -95% which can be said that the nanoemulsion system formed and is able to absorb the active substance so that the drug content contained in the nanoemulsion is high entrapment and can improve the drug delivery system to the target. The greater value of the entrapment efficiency is the higher the drug concentration present in the carrier of emulsion12,13.

**CONCLUSION**

Based on the results of this research, the astaxanthin SNEDDS preparation result in a good formula using oleic acid (oil), tween 20 (surfactant) and propylene glycol (co-surfactant) showing a transmittance value of F1 91%, F2 90%, and F3 95%, with a particle size of F1 183.75 nm; PDI 0.272, F2 195.25 nm; PDI 0.341, and F3 105.75 nm; PDI 0.392, and the calculation of the entrapment efficiency of F1 94.62%, F2 94.35%, and F3 95.57%.

**CONFLICT OF INTEREST**

The authors stated that they do not have any conflict of interest.

**AUTHOR’S CONTRIBUTIONS**

All the authors contributed in experimental and interpreting the results of the work. Every one writes the section that he works on it.

**REFERENCES**

1. Sahumena, M. H., Suryani, & Neni Rahmadan. Formulasi Self-Nanoemulsifiying Drug Delivery System (SNEDDS) Asam Mefenamat Menggunakan Vco Dengan Kombinasi Surfaktan Tween Dan Span. Journal Syifa Sciences And Clinical Research 2019; 37-46.
2. Date, A. A., Desai, N., Dixit, R., & Nagarsenker. Self Nano Emulsifying Drug Delivery Systems: Formulation Insights, Applications And Advances. Nanomedicine 2010; 1595–1616.
3. Borlongan, , C. K., Kanning, K., & Poulos, S. G. Free Radical Damage And Oxidative Stress In Huntington's Disease. J. Fla. Med.Assoc 1996; 335-341.
4. Jyonouchi, H., Sun , S., & Gross, M. Effect Of Carotenoids On In Vitro Immunoglobulin Production By Human Peripheral Blood Mononuclear Cells Astaxanthin, A Carotenoid Without Vitamin A Activity, Enhances In Vitro Immunoglobulin Production In Response To A T-Dependent Stimulant And Antigen. Nutr. Cancer 1995; 171–183.
5. Nurdianti, L., Aryani, R., & Indra. Formulasi Dan Karakterisasi Sne (Self Nanoemulsion) Astaxanthin Dari Haematococcus Pluvialis Sebagai Super Antioksidan Alami. Jurnal Sains Farmasi & Klinis 2017; 30-36.
6. Kaur, G., Pankaj, C., & Halikumar, S. L. Formulation Development Of Selfnanoemulsifying Drug Delivery System (Snedds) Of Celecoxib For Improvement Of Oral Bioavailability. Pharmacophore 2013; 120-133.
7. Huda, N., & Iis Wahyuningsih. Karakterisasi Self-Nanoemulsifying Drug Delivery System (Snedds) Minyak Buah Merah (Pandanus Conoideus Lam.). Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia 2016; 49-57.
8. Tulandi, G., Sri , S., & Widya, A. Validasi Metode Analisis Untuk Penetapan Kadar Parasetamol Dalam Sediaan Tablet Secara Spektrofotometri Ultraviolet. Manado: Pharmacon Jurnal Ilmiah Farmasi 2015.
9. Indriani, V., Novita Eka Kartab Putri Tobing, & Laode Rija. Formulasi SelfNanoemulsifying Drug Delivery System (Snedds) Ekstrak Biji Ramania (Bouea Macrophylla Griff) Dengan Asam Oleat Oleic Acid) Sebagai Minyak Pembawa. Mulawarman Pharmaceuticals Conferences 2018; 276-284.
10. Sharma , V., Pratiush Saxena, Lalit Singh, & Pooja Singh. Self Emulsifying Drug Delivery System; A Novel Approach. Journal of Pharmacy Research; 2012:5.
11. Sahumena, M. H., Suryani, & Neni Rahmadan. Formulasi Self-Nanoemulsifiying Drug Delivery System (Snedds) Asam Mefenamat Menggunakan Vco Dengan Kombinasi Surfaktan Tween Dan Span. Journal Syifa Sciences And Clinical Research 2019; 37-46.
12. Otarola, J., Lista, A. G., Fernández Band, & Garrido, M. Capillary Electrophoresis To Determine Entrapment Efficiency Of A Nanostructured Lipid Carrier Loaded With Piroxicam. Journal Of Pharmaceutical Analysis 2015; 70-73.
13. Wirnarti, Suwaldi, Matin, & Hakim. Formulation Of Insulin Self Nanoemulsifying Drug Delivery System And Its In Vitro-In Vivo Study. Indonesian J. Pharm. 2018; 29(3):158- 166