**Reviewer’s Comments**

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**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 comes in contact with gastric fluid thereby increasing the solubility of active substances. Astaxanthinis oneof the active substances having low solubility so it suits well with this nanoformulation.. This study aims to formulate and characterize Astaxanthin SNEDDS. This research is a laboratory experimental research. Astaxanthin SNEDDS was made in 3 formulationsby 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. The entrapment efficiency (%EE)of astaxanthine SNEDDS was found to be as follows;F1, F2, and F3 had94.62, 94.3, and 95.57%EE, 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 in water. Oil in water undergoes mild agitation 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 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 IIgroup is Astaxanthin which is the main carotenoid found in aquatic organisms or animals thatlive in water such as shrimp, crab, salmon, and lobster as well as the microalgae *Haematococcus puvialis*. Several studies have mentioned that Astaxanthin is a super antioxidant,one of these studieshad performed*in vivo*tests stated 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 researches has been developed from lipid-based formulations to nano-emulsions, but in the currentresearch,Astaxanthin is made in self nanoemulsifyingsystem.The oil phase used is oleic acidwhich has given risetoresults 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,methanol, oleic acid, polyethyleneglycol 400, propyleneglycol and tween 20 areallpurchased from Sigma Aldrich, Singapore.

**MethodsPreparation of 0….0standard 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 serial dilutions with various concentration, namely, 10, 15, 20, 25 and 30 ppm. After that, the absorbance was read using a UV-Vis Spectrophotometer atthewavelength of maximum absorbance of Astaxanthin; 470 nm.

**solubility study 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 3 days. 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 optimizationof 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 optimizationand 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 withusing 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**

**Measurementof %Transmittance**

The method of measuring the transmittance value to determine the level of clarityis as follows; an amount of 100 µL of the Astaxanthinloaded SNEDDS wasadded with water until the final volume reach 5 mL then vortexed for 1 minute. All formulations were then measuredusing UV spectrophotometer to check the value oftransmittance at a wavelength of 650 nm with a blank of distilled water. The transmittance value parameter, that is close to 100%, indicating the droplet size of the dispersion produced by SNEDDS has reached the nanometer size, which can be seen visually from the transparency of the formedsystem7.

**Measurement of particle size of Astaxanthine loaded SNEEDS**

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

**Measurement of % entrapment efficiency (%EE)**

Determination of %entrapment efficiency serves to determine the amount of Astaxanthin that is entraped in SNEDDS. A total of 200 mg of SNEDDS loadedwith Astaxanthin was centrifuged at 3000rpm 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.

**Statistical analysis**

**RESULTS AND DISCUSSION**

**Construction of Calibration curve of Astaxanthin**

Based on the measurements of Astaxanthin’s absorbance (Table 1) with 5 concentrations, namely 10,15,20,25, and 30 ppm at λmax470 nm, the equation of the line was y = 0.0257x - 0.1136 with R2= 0.9965.

**Table 1:Absorbance measurements of various concentrations ofAstaxanthine in ………**

Concentrations (ppm) Absorbance (λ=470 nm)

 10 0.152

 15 0.267

 20 0.396

 25 0.512

 30 0.671

The correlation coefficient obtained is 0.9965 which meets the requirements which 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 showsthat the componentsof SNEDDS having thehighest solubility of for Astxanthin werenamely; 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 caneasilybind 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 means it is more hydrophilic, which enablestween 20 to dissolve Astaxanthin. As for the co-surfactant, propyleneglycol, ithas higher solubility than PEG 400, this indicates that propyleneglycol has more similar polarity as Astaxanthin.

**The optimization of astaxanthin SNEDDS**

Table 3 showed that the results of the optimization of the SNEDDS with the ratio of oil, surfactant, and co-surfactant.This 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 showedthat formulations 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

Formulations 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 acidcompared to the use of tween 80 with propylene glycol. According to literature, tween 20 and propylene glycol havelower molecular weight and viscosity and a better structuresimpler 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.

**Characterization of Astaxanthinloaded SNEDDS**

**Measuring % Transmittance**

Table 4 showed that the measured transmittance of Astaxanthin SNEDDS using a UV-VIS Spectrophotometer was above 90%forall formulations.

**Table 4:Transmittance percent measurement**

Formula % transmittance

 1 91

 2 90

 3 95

The Astaxanthin SNEDDS transmittance percent of the three formulas ranged from90% - 95% and produces a clear dispersion.Based on the results presented in table 4, the percent transmittance obtained by formula 3 was the highestcompared to formulations 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 percentage7.

**Table 5: Measurement of particle size using particle size analyzer (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 showed thatall formulations had particle size < 200 nm andacceptable polydispersity index indicatinguniformity of size distribution.

Based on table 5, the results of particle size measurements show that formula 3 produces smaller particle size than formulas 1 and 2. This is influenced bythe surfactant concentration used in formula 3 which is greater than formula 1 and 2. According to the literature the use of a large surfactant concentration can reduceinterfacial tension because the surfactant will surround the oil droplets when emulsified in water so that it will form a nanometer particle size. The particle sizes of all formulations werein 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 haveparticle 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 % entrapment efficiency were all above 90% i.e. the range of 94% -95% which means that the nanoemulsion system is able to entrapthe active substanceso that the drug of drugwas high which can improve the drug delivery system to the target. The greater the value of the entrapment efficiency the higher the drug concentrationpresent 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 183.75nm; 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.

**LIMITATIONS OF THE STUDY**

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