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RESEARCH ARTICLE

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

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Abstract

Aim and Objective: 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. Astaxanthin is one of the active substances having low solubility so it suits well with this nanoformulation. This study aims to formulate and characterize Astaxanthin SNEDDS.
Methods: This research is a laboratory experimental research using spontaneous emulsification method.
Results: Astaxanthin SNEDDS was made in 3 formulations 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. The entrapment efficiency (%EE) of Astaxanthine SNEDDS was found to be as follows; F1, F2, and F3 had 94.62, 94.35, 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.
Conclusion: 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: Astaxanthin, entrapment effieciency, surfactant, SNEEDS.

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 droplets[1]. 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 bioavailability[2]. One of the active substances belonging to the BCS class II 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*. Several studies have mentioned that Astaxanthin is a super antioxidant, one of these studies had performed *in vivo* tests stating that Astaxanthin is 14 to 60 times stronger than other antioxidants i.e. Quercetin[3]. 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 radicals[4,5].

In increasing the bioavailability of Astaxanthin, many researches has been developed from lipid-based formulations to nano-emulsions, but in the current research, Astaxanthin is made in self nanoemulsifying system. The oil phase used is oleic acid which has given results in accordance with the requirements[6].

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

Astaxanthin powder, methanol, oleic acid, olive oil, VCO, polyethylene glycol 400, propylene glycol, tween 20, and tween 80 are all purchased from Sigma Aldrich, Singapore.

Preparation of 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 serial dilutions with various concentration, namely, 10, 15, 20, 25, and 30 ppm. After that, the absorbance was read using a UV-Vis Spectrophotometer at the wavelength 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), 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 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 propylene glycol 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: propylene glycol and tween 80 : propylene glycol were made ratio of 1:1, 2:1, 3:1, and 3.2:0.8 respectively based on Astaxanthin solubility. 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

Measurement of % Transmittance

The method of measuring the transmittance value to determine the level of clarity is as follows; an amount of 100 µL of the Astaxanthin loaded SNEDDS was added with water until the final volume reach 5 mL then vortexed for 1 minute. All formulations were then measured using UV spectrophotometer to check the value of transmittance 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 formed system[7].

Measurement of particle size of Astaxanthine loaded SNEEDS

Measurement of average article size and polydispersity index (PDI) of Astaxanthin loaded SNEDDS was carried out using a Particle Size Analyzer (PSA) SZ-100, Horiba.

Measurement of % entrapment efficiency (%EE)

A total of 200 mg of SNEDDS loaded with Astaxanthin was centrifuged at 3000 rpm for 15 minutes. Free Astaxanthin will precipitate and supernatan was measured, so that the entangled Astaxanthin can be analyzed using UV-VIS spectrophotometer at a wavelength of 470 nm[4].

Statistical analysis

Data analysis in the evaluation of the preparation used a statistical test with using paired T-Test on viscosity test and emulsification time test.

RESULTS AND DISCUSSION

Based on the measurements of Astaxanthin's absorbance with 5 concentrations, namely 10, 15, 20, 25, and 30 ppm at λ_{max} 470 nm, with R^2=0.9965. 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 absorbance[8].

Table 1: The results of solubility test with Astaxanthin.

Materials	Function	Solubility (mg/mL)
Oleic acid	oil phase	182.10
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

Table 1 shows that the components of SNEDDS having the highest solubility for Astaxanthin were, namely; oleic acid as the oil phase, tween 20 as surfactant and propylene glycol as co-surfactant. 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 can 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 means it is more hydrophilic and enables tween 20 to dissolve Astaxanthin.

As for the co-surfactant, propylene glycol, it has higher solubility than PEG 400, this indicates that propylene glycol has more similar polarity as Astaxanthin. Table 2 showed that the results of the optimization of the SNEDDS base 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.

Table 2: The optimization of SNEDDS base.

Formula	Ratio		Clarity
	Oil: surfactant mix	Surfactant: co-surfactant	
		Tween 20 : Propylene glycol	
A	1 : 9	1 : 1	cloudy
B		2 : 1	clear
C		3 : 1	clear
D		3.2 : 0.8	clear
		Tween 80 : Propylene glycol	
E		3.2 : 0.8	cloudy
F		3 : 1	cloudy
G		2 : 1	cloudy
H		1 : 1	cloudy

The results obtained that formulas B, C and D produce a clear physical appearance of SNEDDS. 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 acid compared to the use of tween 80 with propylene glycol. According to literature tween 20 and propylene glycol have a lower molecular weight and viscosity and a short chain structure 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 soluble[9,10].

Table 3: % Transmittance, size, PDI, % entrapment efficiency measurement.

Formula	% Transmittance	Size (nm)	PDI	% EE
F1	91	183.75	0.272	94.62
F2	90	195.25	0.341	94.35
F3	95	105.75	0.392	95.57

F1: oleic acid: Tween 20: propylene glycol= 10%: 72%: 18%
F2: 10%: 67.5%: 22.5%; F3: 10%: 59%: 31%

Table 3 showed that the measured transmittance of Astaxanthin SNEDDS using a UV-VIS Spectrophotometer was above 90% for all formulations. The Astaxanthin SNEDDS transmittance percent of the three formulas ranged from 90-95% and produces a clear dispersion. Based on the results presented in Table 4, the percent transmittance obtained by formula 3 was the highest compared 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 percentage[7]. Table 4 also showed that the results of measuring the diameter of the Astaxanthin SNEDDS using the particle size analyzer showed that all formulations had of particle size<200 nm and acceptable polydispersity index indicating uniformity of size distribution. Based on Table 4, 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 which is greater than formula 1 and 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 particle size. The particle sizes of all formulations were in the range of 105-195 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 uniformity[2,11]. 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 entrap the active substance so that the drug was high which can improve the drug delivery system to the target. The greater the value of the entrapment efficiency, the higher the drug concentration present in the carrier of emulsion[12,13].

Limitations

The limitations of the present work are represented in characterization of samples particularly to figure out particle size using TEM due to limited resources and equipment as a result of the economic circumstances in Indonesia.

CONCLUSIONS

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%.

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AUTHOR'S CONTRIBUTIONS

Tungadi R: writing original draft, literature survey. **Jusuf H:** investigation, data interpretation. Both authors read and approved the final manuscript for publication.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

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

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