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Original Article

FORMULATION AND CHARACTERIZATION OF ZEAXANTHIN NANOEMULSION RADIANCE SERUM AS ANTIOXIDANT

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ABSTRACT

Objective: This study aimed to increase the effectiveness of using zeaxanthin by developed nanoemulsion containing zeaxanthin which was then formulated into radiance serum for topical use.

Methods: Nanoemulsions are made using spontaneous nano emulsification methods/techniques, which are relatively simple technologically because they rely on the right combination of selected surfactants and co-surfactants. The optimization of the formula was carried out starting from determining the oil: surfactant: co-surfactant phase ratio and concentration of zeaxanthin. Nanoemulsion characterization in the form of physical characterization includes organoleptic tests, globule size and polydispersity index, zeta potential, pH and entrapment efficiency tests. The best results from nanoemulsion were then combined into serum preparations which were then tested for evaluation of the preparations, including organoleptic, homogeneity, viscosity, pH, spreadability, and antioxidant test.

Results: The results showed that the developed zeaxanthin nanoemulsion had a globule size in the range of 20-24 nm (with a normal globule size distribution curve), polydispersity index value of less than 0.3, zeta potential greater than (-20) mV and entrapment efficiency ranging from 80-85%.

Conclusion: The results of the evaluation showed that the serum radiance of zeaxanthin nanoemulsion had good physical, chemical and stability properties during storage with an IC_{50} value of zeaxanthin less than 50 ppm.

Keywords: Radiance serum, Zeaxanthin, Antioxidant, Nanoemulsion

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INTRODUCTION

Zeaxanthin is a compound (β,β-carotene-3R,3R'-diol) which has conjugated doubles and is a group of xanthophyll compounds that are lipophilic in the carotenoid group, which has the characteristics of being a strong antioxidant [1-4]. The bioavailability of some lipophilic carotenoids is very low, between 10-50%. The limited dissolution of gastrointestinal fluids is one of the causes of its low bioavailability. Another reason is that the saturated carotenoids enter the micelles formed by bile salts in the gastrointestinal tract at high doses [6]. Zeaxanthin in the lumen of the small intestine will be hydrolyzed by carboxyl ester lipase [7], Zeaxanthin in the lumen of the small intestine will be hydrolyzed by carboxyl ester lipase [8] Carotenoids that enter orally and are absorbed through the portal vein can be degraded by the first pass in the liver [9]. There are many benefits of consuming Zeaxanthin as an antioxidant, one of which is the protection of the skin against oxidative stress. Oxidative stress plays an important role in the process of skin aging and damage to the dermal layer in humans. Intrinsic (chronological) and extrinsic (aging photos) mechanisms occur, respectively, for the emergence of reactive oxygen species (ROS) through oxidative metabolism and as a result of exposure to ultraviolet (UV) rays from the sun. The formation of ROS can trigger skin aging [10-12]. One of the clinical manifestations that can occur due to skin aging is the occurrence of wrinkles on the skin, the appearance of dark spots due to skin pigmentation [13]. The development of a skin lightening serum (radiance serum) containing zeaxanthin nanoemulsion for topical use is one of the strategies used to increase the efficacy of the use of these carotenoid antioxidants. Through the administration of the topical route, it can eliminate the deficiencies that occur due to oral use. Then, by packaging zeaxanthin into a nanoemulsion, it maximizes its absorption into the dermal layer. The research began with the development of an astaxanthin nanoemulsion made from a nanoemulsion base that had been developed by Nurdianti, *et al.* 2017 [14]. Rdiance serum products based on natural ingredients with the development of nanoemulsion technology as lightening and anti-wrinkle. Physical and chemical characterization was carried out on the prepared nanoemulsions. Then zeaxanthin nanoemulsion was further formulated into radiance serum. The evaluation of the developed serum product includes organoleptic test, pH, viscosity, dispersion test, and antioxidant activity test.

MATERIALS AND METHODS

Materials

The materials used was zeaxanthin. Sunflower oil was purchased from Jan Dekker International (Netherland). Polyoxy-35-castor oil (Kolliphor® RH40) was purchased from BASF (Indonesia). Polyethylene glycol 400 (PEG 400) was purchased from Merck (Indonesia), niacinamid, EDTA, PEG-12 dimethicone, and sodium hyaluronate from thornhill. Canada, glycerin from Kemico Indonesia, xanthan gum from Doesen Biochemical (Ordos) China, vitamin E from ZMC China. All other chemicals used were of pharmaceutical grade.

Preparation of zeaxanthin nanoemulsion

The zeaxanthin nanoemulsion was prepared using the self-nano emulsifying (SNE) method by optimizing the ratio of the oil phase, surfactant and co-surfactant. The best ratio used is the result of a previous study (14) with a ratio of 1:8:1 (Sunflower oil): surfactant (Kolliphor® RH40): and co-surfactant (PEG 400). What was optimized in this study was the amount of zeaxanthin that was added to the nanoemulsion, namely 1,2,3 and 4 mg/1g of SNE. The mixture was stirred at 100 rpm for 30 min using a magnetic stirrer (IKA® C-MAG HS7), then sonicated for 1 hour and nanoemulsions were formed after adding deionized water with light stirring [14].

Physical and chemical characterization of zeaxanthin nanoemulsion

Includes organoleptic tests, pH, globule size, particle size distribution, visual appearance, zeta potential, and entrapment efficiency.

Preparation of radiance serum nanoemulsion zeaxanthin

The type of radiance serum that will be formulated is a liquid serum form which mostly consists of an aqueous phase. Optimization of the amount of emollients, humectants, and thickeners is done to get a serum that is soft and smooth and easy to apply on the skin. There are four formulas of radiance serum made with a concentration of 0.5% from each ratio of the amount of zeaxanthin that has been made. Here is one prototype of the radiance serum that wants to be further developed (table 1).

Table 1: Prototype formulation of radiance serum

Material	Material function	Concentration
Zeaxanthin-NE	Nanoemulsion Active Substance	0.05%
Niacinamide	Moisturizer	0.5%
Disodium etilendiamin tetraasetat (Disodium EDTA)	Chelating agent	0.2%
PEG-12 dimethicone	Emollient	2.0%
Rose hip oil (*Rosa moschata seed oil*)	Emollient	1.5%
PEG 400 caprylic/capric glycerides	Emulsifying agent	5.0%
DMDM Hydantoin	Antimicrobial	0.1%
Sodium hyaluronate	Humectants	1.0%
Glycerin	Humectants	3.0%
Xanthan gum	Thickener	0.1%
dl-alpha tocopheryl acetat	Antioxidant	1.0%
Natural rose fragrance	Fragrance	0.05%
Purified Water	Carrier	Ad 100%

Physical and chemical characterization of zeaxanthin serum radiance

Organoleptic and pH test

Organoleptic testing was carried out by visually observing the radiance of the zeaxanthin nanoemulsion serum produced, including color, odor, clarity. Then the pH test of the serum radiance was carried out by measuring the pH using a calibrated pH meter [21].

Viscosity and spreadability test

The radiance viscosity of the serum zeaxanthin nanoemulsion was determined using a Brookfield Viscometer with an appropriate spindle type and at a shear rate. The spreadability test was carried out by placing a sample of±0.5 g on a 20x20 cm glass, then the glass was covered with mica plastic and given a weight of±50 g. After 1 minute, the diameter of the distribution formed was measured [23].

Stability test

The stability test was carried out using the cycling test method with 6 test cycles where the organoleptic parameters, pH and dispersion were measured [24].

Antioxidant activity test

Zeaxanthin antioxidant activity test was determined by adding 1 ml of the sample solution to 2 ml of DPPH solution (0.005%, w/v), then the mixture was incubated for 5 min. The absorbance of the mixture was then measured using a UV-Visible Spectrophotometer with an absorbance range of 400-800 nm. Absorbance measurements were carried out until a stable absorbance was obtained [25].

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine significant intergroup differences of each parameter. A p-value<0.05 was considered statistically significant.

RESULTS

Zeaxanthin nanoemulsions were prepared using the spontaneous nano emulsification method in which an isotropic mixture of oil, surfactant, and drug will spontaneously form a nanoemulsion (oil in water) when it encounters the aqueous phase under mild agitation conditions. This mixture can form an emulsion spontaneously if the change in entropy for the dispersion system is greater than the energy required to increase the surface area of the dispersion. SNE also requires cosurfactants to facilitate the nano emulsification process or enhance drug incorporation in the nanoemulsion [15]. The results showed that the developed zeaxanthin nanoemulsion had a globule size in the range of 20-24 nm (with a normal globule size distribution curve), polydispersity index value of less than 0.3, zeta potential greater than (-20) mV and entrapment efficiency ranging from 80-85% (fig. 1).

Table 2: Zeaxanthin nanoemulsion pH

Sample	Replication	pH	Average±SD
Formula 1	I	5.40	5.27±0.1078
	II	5.23	
	III	5.20	
Formula 2	I	4.75	4.76±0.01
	II	4.76	
	III	4.77	
Formula 3	I	4.59	4.58±0.0057
	II	4.58	
	III	4.58	
Formula 4	I	4.57	4.55±0.0115
	II	4.55	
	III	4.55	

Table 3: Physical and chemical properties of radiance serum nanoemulsion zeaxanthin

Parameters	F1	F2	F3	F4
Visual properties serum	Transparent yellow	Transparant yellow	Transparant yellow	Transparant yellow
pH of serum	5.25±0.023	5.09±0.0264	5.13±0.02	5.01±0.01
Viscosity value (cps)	3429±12.22	3720±16	3784±8.00	3872±8.00
Homogeneity test	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Spreadability test (cm)	5.13±0.2081	5.00±0.1	4.67±0.1527	4.57±0.1527

Values are given as mean±standard deviation (n=3). The results of the antioxidant activity test of zeaxanthin using DPPH and compared with standard vitamin C showed a very strong activity of zeaxanthin with an IC_{50} value of 9.044 ppm and a vitamin C standard of 2.4667 ppm.

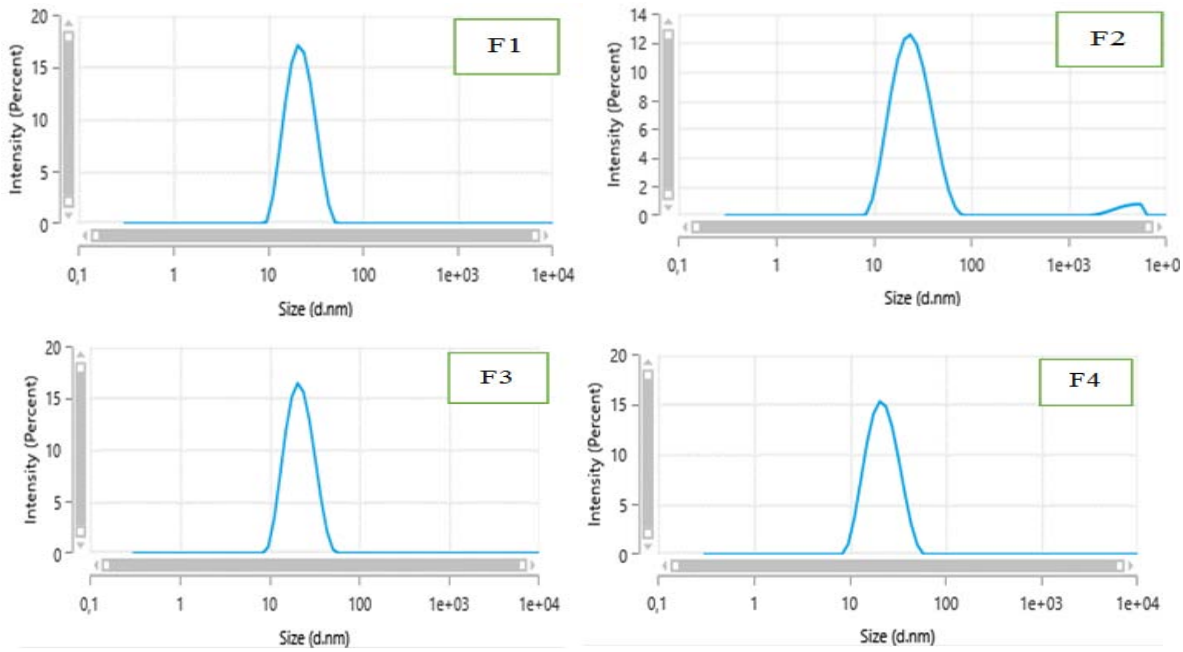

Fig. 1: **Zeaxanthin nanoemulsion globule size distribution curve. (F1) with Zeaxanthin concentration 1 mg/g SNE, (F2) with Zeaxanthin concentration 2 mg/g SNE, (F3) with Zeaxanthin concentration 3 mg/g SNE, (F4) with Zeaxanthin concentration 4 mg/g NE**

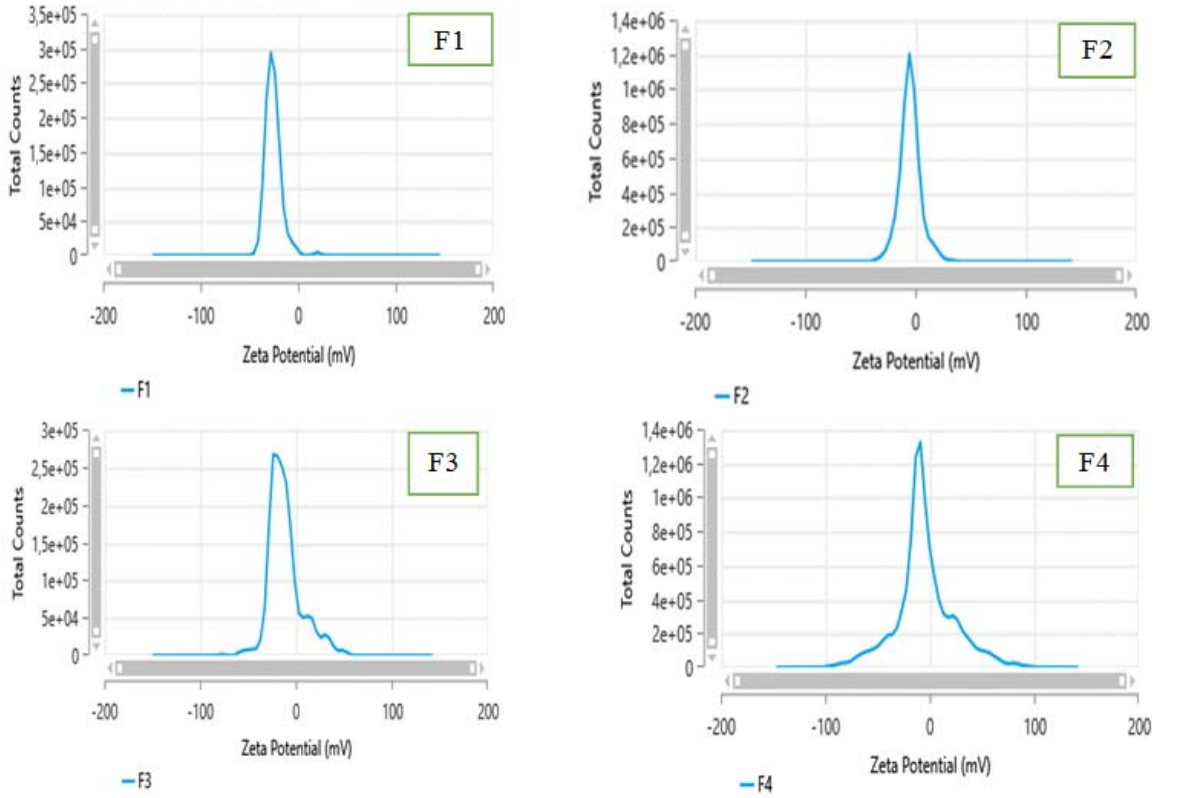

Fig. 2: Zeta potential value curve of zeaxanthin nanoemulsion. (F1) with Zeaxanthin concentration 1 mg/g SNE, (F2) with Zeaxanthin concentration 2 mg/g SNE, (F3) with Zeaxanthin concentration 3 mg/g SNE, (F4) with Zeaxanthin concentration 4 mg/g SNE

Table 4: Results of the antioxidant activity test of zeaxanthin

Sample	IC_{50}	Intensity value IC_{50}
Vitamin C	2.4667	Very strong
Zeaxanthin	9.44	Very strong

DISCUSSION

Nanoemulsion technology is widely known to increase the bioavailability of drugs that have low solubility in water [14]. The mechanism of increasing bioavailability is through increasing drug solubility, protecting the drug against hydrolytic enzymes, increasing the specific surface area of the globule, and changing the permeability induced by surfactant [16]. Spontaneous nano emulsification formation process is influenced by the specific properties of oil, surfactant, and cosurfactant, the concentration and ratio of oil-surfactant-cosurfactant and the temperature at which spontaneous nanoemulsion is formed. Therefore, only certain combinations of excipients can form a spontaneous nanoemulsion system [17].

The results of observations of the zeaxanthin nanoemulsion made are yellow transparent, have no odor, and are clear (table 2). The formation of nanoemulsions is a description of the clear transparent mixture due to the dispersion of oil globules with homogeneous and nano-sized drugs in solution [14]). In this study, the optimum ratio of oil phase: surfactant: co-surfactant from the warp study [14] was a ratio of 1:1:8, which consisted of sunflower oil as the oil phase, Cremophor RH 40 as a surfactant and PEG 400 as a co-surfactant. surfactant. The optimization of this research is only the concentration of zeaxanthin in every g of SNE. From the four optimization mixtures above, it produces globule sizes ranging from 20-24 nm (with a normal globule size distribution curve), polydispersity index value of less than 0.3, zeta potential greater than (-20) mV and entrapment efficiency ranging from 80-85 %.10-20 nm. This polydispersity index provides information about the physical stability of a dispersion system that is formed, which is more stable in the long term [18, 19].

The zeta potential value can indicate the stability of a system containing dispersed globules through the repulsion between particles of the same charge when they are close together. A zeta potential value greater than (+30) mV or less than (-30) mV will be electrostatically stable, while a zeta potential value greater than (+20) mV or less than (-20) mV will be stable. sterically [14]. Zeaxanthin nanoemulsions with zeta potential values close to±20 mV are sterically stabilized by the presence of non-ionic surfactant polymer chains in micelles [20]. The value of the entrapment efficiency of the active substance is an important parameter in the formulation related to the active substance that can be entangled in the nanoemulsion dosage formula. The entrapment efficiency values of all formulas are around 80-85%. This indicates that the entrapment of Zeaxanthin in the nanoemulsion globules is quite efficient.

The results of the characterization of the zeaxanthin nanoemulsion were good, so they were combined in the form of radiance serum to optimize the efficacy of the zeaxanthin nanoemulsion as an antioxidant. Radiance Serum is a product that contains high concentrations of protein, vitamins, or antioxidants based on oil or water. Serums or concentrates contain approximately 10 times more biologically active substances than creams or other preparations. Therefore, serum works to solve cosmetic problems faster and more effectively.

The results of the radiance evaluation of zeaxanthin nanoemulsion serum seen from organoleptic tests include color, odor and form in the form of a transparent yellow liquid preparation that is odorless so that from an aesthetic point of view, it is very attractive to be produced on an industrial scale. Likewise, from the results of homogeneity to see the mixing of solid materials that are combined so that it becomes a homogeneous mixture with no microscopic fine solid particles visible [21].

The pH test is carried out of knowing the level of acidity or alkalinity of a preparation, where if the preparation is too acidic it will cause irritation to the skin and will cause a burning sensation on the skin so the pH of the preparation is expected to be in accordance with the pH of the skin, which is in the range of 4-7 [21].

The dispersion test was carried out to determine the speed of dispersion or even distribution of the radiance serum preparation. The test results show that the radiance dispersion of serum is in the range of 4.57-5.13 cm and this meets the requirements of the required value of 5-7 cm [21]. The value of dispersion is strongly influenced by the value of viscosity, where preparations that have a low viscosity value will have greater dispersion and vice versa. Viscosity test aims to see the viscosity of a preparation. The measurement results show that the viscosity value of the serum radiance is in the range of 3429-3872 cps. The range of viscosity values of good preparation is in the range of 2000-4000 cps [22].

The results of the antioxidant activity test were carried out using the DPPH (1,1-diphenyl-2-pycrilihydrazyl) method, where DPPH is a fairly stable free radical compound. In addition, the DPPH method has the advantage that the analysis is simple, fast, easy and sensitive to samples with small concentrations (Karadag, *et al.*, 2009). Based on the results of the antioxidant activity test, it is stated that zeaxanthin is a very strong antioxidant because it is in the IC50 range<50 ppm (23).

CONCLUSION

In this study, a nanoemulsion-based radiance serum formulation for topical administration was successfully developed to deliver lipophilic agent such as Zeaxanthin. The Zeaxanthin nanoemulsions had good physical and chemical characteristics and its antioxidant activity is very strong and with an IC50 value of less than 50 ppm. the results of the evaluation of the zeaxanthin nanoemulsion serum radiance preparation meet the test requirements.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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