Research Article

Nanoemulsion-Based Gel Formulation of Astaxanthin for Enhanced Permeability: Potential as a Transdermal Drug Delivery System

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ABSTRACT

The xanthophyll carotenoid compound, astaxanthin, is a natural red pigment component found in many microorganisms and marine animals. Astaxanthin is characterized by poor bioavailability as it is water-insoluble, is poorly absorbed and is systemically eliminated. In the present study, we have designed a nanotechnology (nanoemulsion) delivery system containing astaxanthin for transdermal delivery routes, which have implications for increasing bioavailability of astaxanthin in the body. The astaxanthin nanoemulsion (As-SNE) was prepared by using the self-nanoemulsifying (SNE) method. The formed nanoemulsion was formulated into the hydrogel system by hydration method. Evaluation of As-SNE gel was performed by physical, chemical characterization and freeze-thaw stability test. Topical permeation of astaxanthin through Python reticulatus skin was estimated using the Franz diffusion cell. The optimized gel formulations have a good stability and good characteristics that was proven by freeze-thaw evaluation test. Chemical characterization was performed by antioxidant activities test using DPPH method, and the results showed that As-SNE gel shows very strong antioxidant activity and were comparable to pure astaxanthin. The ex vivo skin permeation profile of optimized formulations was compared with that of pure astaxanthin. Significant increase permeability was observed in optimized nanoemulsion gel formulations consisting of 10% As-SNE (in system consisting of sunflower oil: Kolliphor® RH40: PEG 400 with ratio of 1:8:1). These results suggest that nanoemulsions can serve as potential vehicles for improved transdermal delivery of astaxanthin.

Keywords: astaxanthin, self-nanoemulsifying method, hydration method, DPPH method, bioavailability, transdermal delivery.

INTRODUCTION

Astaxanthin is a lipophilic xanthophyll compound with reddish color, which is found in various microorganisms and marine animals. Astaxanthin belongs to the xanthophyll group, which are oxygenated derivatives of carotenes[1]. The xanthophylls and carotenes are two major groups of carotenoids that possess a number of health benefits. The biological benefits of carotenoids may be due to their antioxidant properties attributed to their physical and chemical interactions with cell membranes. Astaxanthin had higher antioxidant activity when compared to various carotenoids such as lutein, lycopene, α-carotene and β-carotene[2]. In humans, the bioavailability of carotenoids is low and variable. It ranges from 10 to 50% of a given dose, due to low solubility in gastrointestinal tract juices, leading to poor absorption by the epithelial cells of the small intestine[3]. It has been reported that astaxanthin bioavailability in humans was enhanced by lipid based formulations; high amounts of carotenes solubilized into the oil phase of the food matrix can lead to greater bioavailability[4]. Barros *et al.*[5] found that administration astaxanthin with combination of fish oil promoted hypolipidemic/ hypocholesterolemic effects in plasma and its increased phagocytic activity of activated neutrophils when compared with astaxanthin and fish oil alone. Drug delivery through transdermal route is an alternative route that can be used for enhances bioavailability of the drug, maintains the plasma drug levels, and avoids first-pass metabolism[6]. Transdermal delivery systems are non-invasive and can be self-administered; they can provide release for long periods of time. Therefore, Nanoemulsion-Based Gel Formulation of Astaxanthin with a high degree of permeation could be useful to enhance bioavailability and deliver astaxanthin in the body. There are already two generations of transdermal delivery systems that are well known. The first-generation approach to transdermal delivery is limited primarily by the barrier posed by skin's outermost layer, while the second generation of transdermal delivery systems recognizes that skin permeability enhancement (using enhancer) is needed to expand the scope of transdermal drugs[7]. Many of the dermal vehicles contain chemical enhancers and solvents to achieve these goals[8, 9.] But use of these chemical enhancers may be harmful, especially on chronic application, as many of them are irritants. Therefore, it is desirable to develop a nanotechnology delivery system that does not require the use of chemical enhancers to facilitate drug permeation through the skin. One of the most promising nanotechnology techniques for enhancement of transdermal permeation of drugs are nanoemulsions gel. These are thermodynamically stable transparent dispersions of oil and water stabilized by surfactant and cosurfactant molecules and having a droplet size of less than 200 nm[10, 11]. This article describes the potential use of nanoemulsion-based gel systems for the transdermal delivery of astaxanthin using nonirritating, pharmaceutically acceptable ingredients

without using additional permeation enhancers, since the excipients themselves act as permeation enhancers[12].

MATERIALS AND METHODS

Astaxanthin (Astareal® L10) was obtained from Fuji Chemical Industries (Japan). Sunflower oil was purchased from Jan Dekker International (Netherland). Polyoxyethylene 20 sorbitan monooleate (polysorbate 80), poloxamer 407, polyoxy-35-castor oil (Kolliphor® RH40) were obtained from BASF (Indonesia). Polyethylene Glycol (400) was obtained from Merck, Tbk (Indonesia). Propylene Glycol was obtained from Dow Chemical Pacific Limited (Singapore). Carbomer (carbopol 934), glycerin, triethanolamine, DMDM hydantoin were obtained from PT. Brataco (Indonesia). *Python reticulatus* skin was obtained from Bandung Zoo (Indonesia). 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA). All other chemicals uses in the study were of analytical reagent grade.

Preparation of As-SNE gel

As-SNE (using different concentrations of surfactants and cosurfactants) were prepared by using self-nanoemulsifying (SNE) method which refers to previous study[13]. Optimized nanoemulsion formulations then incorporated into gel system by dispersing 10 % w/w of As-SNEs in a 1 % w/w of carbomer gel system (containing of 5 % glycerin, 1 % triethanolamine, 0.1 % DMDM hydantoin) slowly, followed by the slow addition of deionized water to adjust the final preparation to 100 % w/w. The composition of the various batches prepared is given in Table 1.

Table 1: Composition of various batches of As-SNE gels

Formula	Composition	A	B	C
Mix ratio	Sunflower oil	1	1	1
	Polysorbate 80	8	-	-
	Poloxamer 407	-	1	-
	Kolliphor® RH40	-	-	8
	Polyethylene Glycol 400	-	-	1
	Propylene Glycol	1	8	-
Carbomer		1 % (w/w)		
Glycerin		5 % (w/w)		
Triethanolamine		1 % (w/w)		
DMDM hydantoin		0.1 % (w/w)		
Deionized water		Adjust 100 % (w/w)		

Physical characterization of As-SNE gel

Organoleptic observation and pH determination

Organoleptic includes observation of color, odor, and clarity of As-SNE gel was observed. A pH of As-SNE gel was determined by using calibrated pH meter (Mettler Toledo).

Viscosity and spreadability test

Viscosity test of As-SNE gel was performed by using Viscometer (Brookfield®) with type spindle of 7 and shear rate 100 rpm (rotation per minute). Spreadability test was performed by putting ± 0.5 grams on glass 20x20 cm, then covered with mica plastic and given weight of 50 grams. After 1 minute, the formed diameter was measured.

All the physical characterization's test of As-SNE gel was observed until 28 days.

Freeze-thaw stability test

Freeze-thaw stability test of As-SNE gel was performed by putting the sample in the room temperature (± 30 °C) within 24 hours, the sample was transferred into the cold storage with temperature of ± 4 °C within 24 hours, and then the sample was transferred into the oven (Memmert®) with temperature of ± 40 °C within 24 hours. The conditions above were called one cycle. The sample was treated repeatedly over 6 cycles. After freeze-thaw stability test, organoleptic observation was performed.

Antioxidant activity determination

Antioxidant activity test of As-SNE gel was determined by adding 1 mL sampel solution (0.005 %, w/v) in a 2 mL DPPH solution (0.005 %, w/v), then mixed solution was incubated. The absorbance of mixed solution was measured using UV-Visible Spectrophotometer (Genesys 10S) with range of absorbance of 400-800 nm. Absorbance measurements were carried out until a stable absorbance was obtained. Antioxidant activity of As-SNE gel was compared to pure astaxanthin.

Permeation studies

Diffusion profile test was carried out by using Franz diffusion cell with *Python reticulatus* skin as membrane barrier. ± 0.5 gram of As-SNE gel was putted onto the ±

2.8 cm^2 of skin as a donor compartment. The receptor compartment contains of 50 mL of phosphate buffer pH 7.4. While the Franz diffusion cell operates, the temperature was set constant of 37° C ± 0.5 °C with water jacket. Sampling was carried out of 1 mL from receptor compartment at 5, 10, 15, 30, 45, 60, 75, 90, 105, and 120 minutes using a micro pipette and immediately replaced with phosphate buffer pH 7.4 of the same volume. The sample was putted into a 5 mL volumetric flask and the volume was adjusted to 5 mL and shaken homogeneously. Then the absorbance was measured by UV-Visible Spectrophotometer (Genesys 10S) at the maximum wavelength of 417 nm. Astaxanthin levels which penetrated the receptor fluid every time sampling was calculated[14].

RESULTS AND DISCUSSION

Preparation of As-SNE gel

As-SNE was prepared by using self-nanoemulsifying (SNE) method which refers to previous study. Nurdianti *et al.*[12] reported that there were 3 optimized mixes of oil phase, surfactant, and cosurfactant which had a good physical characterization. All the compositions of A, B, and C As-SNE had droplets in the nano-range (10-20 nm) with entrapment efficiency value range of 80%. Polydispersity index (PI) indicates the uniformity of globules size within the formulation, higher the PI, lower the uniformity of the globules size in the formulation[15]. All of compositions had a PI were less than 0.5.

Carbopol is very useful as a major component of drug delivery gel systems for buccal, ocular, rectal, and nasal, especially for transdermal applications[16]. In gel formation, when neutralized with TEA, the swelled microgel particles form a closely packed structure thus forming an elastic network[17, 18]. The results showed that all of the formed gel with content of A, B, and C mixes were clear and transparent with a reddish color (figure 1).

Physical characterization of As-SNE gel

Organoleptic observation, pH determination, viscosity, and spreadability test were carried out until 28 days in room temperature (± 30 ºC). The results of physical characterization of As-SNE gel are shown in table 2.

Table 2: Physical characterization of As-SNE gel

Formula	Physical characterization's type	Day			
		0	7	14	28
A	Organoleptic - Color - Odor - Clarity	- Orange - Odorless - Clear	- Orange - Odorless - Clear	- Orange - Odorless - Clear	- Orange - Odorless - Clear
	pH	4.9	5.1	5.4	5.8
	Viscosity (cPs)	5,870	5,900	6,200	7,460
	Spreadability (cm^2)	4.9	5.1	5.4	5.8
B	Organoleptic - Color - Odor - Clarity	- Orange - Odorless - Clear	- Orange - Odorless - Clear	- Orange - Odorless - Clear	- Orange - Odorless - Clear
	pH	7.2	7.2	7.2	7.1
	Viscosity (cPs)	5,650	5,600	5,540	5,423
	Spreadability (cm^2)	5.0	5.1	5.1	5.4
C	Organoleptic - Color - Odor - Clarity	- Orange - Odorless - Clear	- Orange - Odorless - Clear	- Orange - Odorless - Clear	- Orange - Odorless - Clear
	pH	6.3	6.3	6.3	6.3
	Viscosity (cPs)	12,760	12,560	12,480	9,680
	Spreadability (cm^2)	5.3	5.5	5.1	5.7

Based on the results above from day 0 to day 28, it was seen that the As-SNE gel had good physical stability, there was no significant change between the 3 mixtures above. Spreadability test was done by using parallel-plate method to determine the ability of gel to spread on the skin (topically). A gel is preferred if it can spread easily on the skin, because its use is easier and more comfortable. Based on the results of above for 28 days, the gel had a wide spreadability of about 5 cm means that gel had a good spreadability[19]. Viscosity modification is an important part of semi-solid formulations and may impact skin retention of the dosage form and drug

delivery/penetration via the skin. Generally, the viscosity of gel formulations reflects consistency. Formula C had a higher consistency than formulas A and B, this is due to the composition of the As-SNE, which in formula C contains the high ratio of surfactant with semisolid form. Overall, the As-SNE gel had good properties as a semisolid dosage form.

Freeze-thaw stability test

Freeze-thaw stability test aims to test the stability of the gel preparation, whether syneresis phenomenon occurs. Syneresis means contraction of gel upon standing and separation of water from the gel matrixes. Based on the results from day 0 to day 28, it was seen that the As-SNE gel had good physical stability after 6 cycles, there was no significant change between the 3 mixtures (no syneresis occurred) (Figure 1).

Antioxidant activity determination

Antioxidant activity test of As-SNE gel was performed by using DPPH's method. DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol[20]. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution. The use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry[20]. The results of antioxidant activity test can be seen in Table 3.

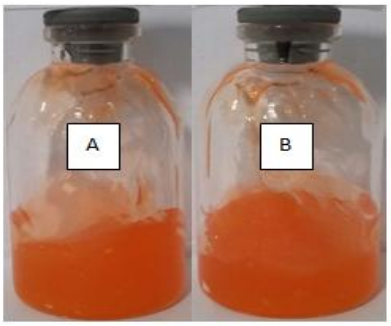

Figure 1: Organoleptic visualization of As-SNE gel after 6 cycles freeze-thaw test [(A) Day 0 and (B) Day 28].

Table 2: Antioxidant activity test of As-SNE gel

Results	Pure Astaxanthin	As-SNE gel			Antioxidant Activity
		Formula A	Formula B	Formula C	
IC_{50} values (µg/mL)	1.30	6.58	6.82	5.20	Very active

IC_{50} value is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color). The IC_{50} describes the activity of antioxidant, where the lower the value of IC_{50}, the higher the antioxidant activity[21]. Reynertson[22] has classified the IC_{50} value into 4 categories, namely IC_{50} less than 50 µg/mL which is categorized as very active, 50-100 µg/mL which is categorized as active, 100-200 µg/mL which is categorized as quite active and more than 200 µg/mL which is categorized as inactive as an antioxidant. Based on the results above it is indicated that all of the formulas of As-SNE gels are categorized as very active antioxidant, similar to pure astaxanthin.

Permeation studies

The ex vivo permeation profiles of As-SNE gel through *Python reticulatus* skin are shown in the graph (Figure 2). A steady increase of the concentration of astaxanthin with time is observed.

Statistical evaluation of the flux throughout the 120 minutes of study showed that among all the formulations exhibited significantly higher permeation compared to pure astaxanthin (0,00044 %/cm^2), while the cumulative permeation of formula C (1,565 %/cm^2) was higher than formula A (0,823 %/cm^2) and B (1,095 %/cm^2) at 120 minutes post-application. The difference of amount of astaxanthin penetrating the skin compared to pure astaxanthin occurs because in the As-SNE gel, there were chemical enhancers such as surfactant and cosurfactant that may disrupt the highly ordered bilayer structures of the intracellular lipids in the stratum corneum by inserting amphiphilic molecules into these bilayers[7].

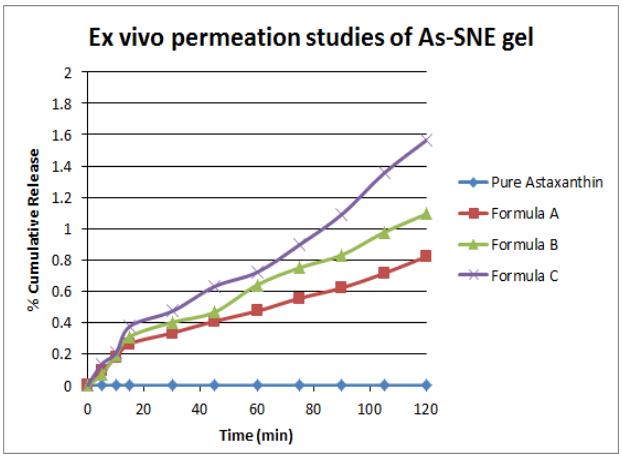

Figure 2: Ex vivo permeation studies on As-SNE gel [formula A, B, C] and pure astaxanthin.

CONCLUSION

In this work, a nanoemulsion-based gel formulation for topical administration was developed to deliver lipophilic agents such as astaxanthin. The astaxanthin nanoemulsions had good physical characteristics, good stability, and significantly higher permeation compared to pure astaxanthin. Thus, it can be concluded that the developed nanoemulsion-based gel has a great potential to be used for topical lipophilic drug delivery.

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