Preparation of Blueberry Anthocyanin Nanoemulsions and Their Stability and Skin Safety Evaluation

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Abstract: Blueberry anthocyanins have strong antioxidant activity, but their instability and low bioavailability limit their use. Nanoemulsions are new type of food and drug carrier with stable thermodynamic properties. Therefore, anthocyanins will be encapsulated with nanoemulsions to improve their stability and application value. In this study, the best surfactants, oil phases and cosurfactants for the preparation of blueberry anthocyanin nanoemulsions were screened by pseudo-ternary phase diagram method and the solubility of blueberry anthocyanin nanoemulsions were taken into consideration. Deep purplish red, clear and transparent anthocyanin nanoemulsions were prepared by simple and cheap low-energy emulsification method according to the formula. Normal and high temperature test and high speed centrifugal test proved that the blueberry anthocyanin nanoemulsions had good stability. Guinea pig skin irritation test and sensitization test showed that there was no irritation and sensitization to guinea pig hair removal skin. In conclusion, the blueberry anthocyanin nanoemulsions prepared in this study have good stability at room temperature and are safe for guinea pig hair removal skin. It provides a reference for the application of blueberry anthocyanins in skin beauty and transdermal drug delivery.

Keywords: Blueberry Anthocyanins; Nanoemulsions; Pseudo-ternary Phase Diagram; Stability; Skin Safety

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1 Introduction

Anthocyanins are pigments found in the flowers, leaves or fruits of plants, which give them red, blue or purple color. They have a polyphenolic structure and belong to the flavonoid group. Anthocyanins usually form glycosides by glycosidic bonds with one or more glucose, rhamnose, galactose, arabinose, etc. At present, there are mainly 6 anthocyanins in edible parts of plants, including Cyanidin, Pelargonidin, Peonidin, Delphinidin, Pelunidinand Malvidin.

Anthocyanins have gathered the attention of the scientific community mostly due to their vastrange of possible applications. Anthocyanins have strong antioxidant and free radical scavenging activities, and protect eyesight, lower blood glucose, blood pressure, cardiovascular system, nervous system, inhibit tumor cell proliferation, affect intestinal flora, anti-liver damage,

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anti-radiation and many other physiological functions [1-4]. As a good source of anthocyanins, blueberry has been widely used in food, medical treatment, cosmetics and other fields due to its high anthocyanin content and good functionality. However, the stability of blueberry anthocyanins is poor, and the processing conditions such as pH, temperature, light, metal ions and additives will affect the stability of blueberry anthocyanins, hinder the application of anthocyanins, resulting in more losses in the production process. How to maximize the retention of anthocyanins and their physiological activity is an urgent problem to be solved.

Therefore, we considered using carrier to encapsulate blueberry anthocyanins, which might improve its stability.

Nanoemulsions are thermodynamically stable, isotropic, transparent or translucent, homogeneous dispersed system spontaneously formed from water, oil, surfactant, and cosurfactant [5]. Nanoemulsions droplet sizes are generally in the range of 20 ~ 200 nm, and the common type are oil-in-water (O/W) and water-in-oil (W/O). They are prepared by a single step process. Due to the small droplet size, nanoemulsions have the stability of resisting precipitation or emulsification, but ostwald maturation is the main mechanism of the nanoemulsions rupture [6].

Many studies have produced 100-600nm nanodroplets, and the widely used methods include high pressure homogenization, ultrasonication, phase inversion, micro fluidizationu ltrasound and spontaneous emulsification [7]. The spontaneous emulsification process is low energy nanoemulsions preparation. The method is performed at room temperature and does not require any special equipment. Add water to oil and surfactant step by step and stir gently to form nanoemulsions.

Nanoemulsions have wide applications in the field of medicine, skincare, agriculture, food and various other fields as they provide an effective and protective means or vehicle [8]. Nanoemulsions have a wide range of applications in dermal drug delivery systems, such as a potential ophthalmic drug delivery system [9], the preparation of drugs for atopic eczema, thereby enabling a higher capacity to load lipophilic drugs and improving drug bioavailability [10]. Nanoemulsions also have a wide range of applications in cosmetics, such as those for dry skin, which can increase the ability of cosmetics to penetrate the skin [11].

Nanoemulsions in skin care due to the effective penetration of nanodroplets on the skin surface, pleasing appearance and stability of cosmetic ingredients, make the skin more hydrated [12]. Nanoemulsions can also increase the efficacy and safety of cosmetics. Due to the small particle size of nanoemulsions, they can penetrate into the skin through hair follicles and pores, causing less skin irritation and not affecting healthy tissues [13].

Under isothermal and isobaric conditions, the phase form of the three-component system can be represented by a plane triangle, which is called a ternary phase diagram. This is an effective method to select the formulation of nanoemulsions.

In this study, blueberry anthocyanins were prepared into nanoemulsions by pseudo-ternary phase diagram and referring to the solubility of blueberry anthocyanins, and its stability and skin safety were evaluated, in order to provide reference for the promotion and application of blueberry anthocyanins nanoemulsions in skin beauty and transdermic drug delivery.

2 Materials & Methods

2.1 Preparation of Anthocyanins

The organic blueberries were purchased from the blueberry plantation in Majang County, Guizhou (China), and the license for blueberry collection has been obtained. All the plant experiments were in compliance with relevant institutional, national, and international guidelines and legislation. Anthocyanins were extracted from organic blueberries produced in Majang County, Guizhou (China). The research group had completed this work in the early stage, and the content of anthocyanins in blueberries was 2.56 mg/g [14], the extracted blueberry anthocyanins were turned into lyophilized powder by vacuum freeze-drying.

2.2 Selection of Surfactants

Castor oil polyoxyethylene ether 40 (EL-40) (Shanghai Yuanye, China), castor oil polyoxyethylene ether 35 (EL-35) (O.BASF, Germany), polyoxyethylene ether hydrogenated castor oil (RH40) (Shanghai Yuanye, China), Tween-80 (Tianjin Rgent, China), Tween-20 (Tianjin Rgent, China) and Span-80 (Kermel, China) were used as the screening objects of surfactants. Appropriate amounts of blueberry anthocyanins were weighed and added to the above six surfactants respectively, and dropped into ultra-pure water while stirring. The color change and precipitation were observed to determine the dissolution of blueberry anthocyanins, and the appropriate surfactant was initially screened.

2.3 Selection of oil Phase

Isopropyl myristic (IPM) (Shanghai Yuanye, China), isopropyl palmitate (IPP) (TCI, Japan) and Caprylic acid capric acid triglyceride (GTCC) (Shanghai Yuanye, China) were selected as the screening objects of oil phase. Appropriate amounts of blueberry anthocyanins were added to the above four oil phases respectively, and ultra pure water was dropped while stirring. The color change and precipitation were observed to judge the dissolution of blueberry anthocyanins, and the appropriate oil phase were initially screened.

The preliminary screened surfactant and oil phase were combined, and then a certain amount of blueberry anthocyanins were added respectively. The emulsion transparency was observed after 1 week of storage at room temperature, 60 ℃ and -4 ℃, and the best surfactant and oil phase were determined.

2.4 Determination of Cosurfactants and Optimal Km value (Mass Ratio of Surfactant to Cosurfactant)

Glycerol (Solarbio, China), ethanol, 1, 2-propanediol (Tianjin Guangfu, China) and 1, 3-butanediol (Macklin, China) were selected as cosurfactants. The optimal surfactant and oil phase determined above were mixed into the four cosurfactants, then blueberry anthocyanins were added, and ultra pure water was dropped while stirring, and the amount of water was recorded. For reference [15], the ratios of 2:1, 3:1, 3:2 and 4:1 were taken as alternative Km values, the pseudo-ternary phase diagram was drawn with surfactant/cosurfactant, oil phase and water phase as three vertices to determine the best cosurfactant.

2.5 Determination of Blueberry Anthocyanin Nanoemulsions Formulation

By using spontaneous emulsification (SE) [16], blueberry anthocyanin nanoemulsions can be prepared by weighing a certain amount of blueberry anthocyanin, the best surfactant, cosurfactant and oil phase in a certain proportion, fully mixing, magnetic stirring and adding the prescription amount of ultra-pure water drop by drop.

2.6 Characterization of Blueberry Anthocyanin Nanoemulsions

The characteristics of blueberry anthocyanin nanoemulsions were observed by naked eye, and it was determined as oil-in-water (O/W) type or water-in-oil (W/O) type by staining method. The morphology and particle size of the nanoemulsions were observed by transmission electron microscope (Hitachi H-7650, Hitachi High-tech Shanghai international Trading Co., LTD), and the viscosity was determined by the flow rate of the nanoemulsions in the glass straw. The pH value was measured by pH meter, and the Zeta potential was measured by Zeta potential analyzer (Becaman coulter, Delsa Nano C). All values were measured three times and averaged.

2.7 Determination of Anthocyanin Content in Nanoemulsions

The content of anthocyanin in blueberry anthocyanin nanoemulsions were determined by UV-vis spectrophotometer. The anthocyanin standard reserve solution (Cyanidin 3-O-glucoside) with the concentration of 500μg/mL was prepared and diluted into 30, 50, 100, 150, 200, 250, 300, 350μg/mL, respectively. The absorbance value was measured by UV-vis spectrophotometer, and the standard curve was prepared. Blueberry anthocyanin standard and anthocyanin nanoemulsions were placed on UV-vis spectrophotometer for spectral scanning to determine the maximum absorption peak. The content of anthocyanins in the samples was determined according to the maximum absorption peak and standard curve.

2.8 Stability Evaluation of Blueberry Anthocyanin Nanoemulsions

Long - time retention sample at room temperature: The blueberry anthocyanin nanoemulsion samples were placed at room temperature (Autumn, room temperature 10-20 ℃, relative humidity 75%) for 1 and 2 weeks to observe whether there were stratification, turbidity or precipitation.

High temperature experiment: The anthocyanin nanoemulsion samples were placed at 4 ℃, 25 ℃ and 50 ℃ for 1 week to observe whether there was stratification, turbidity or precipitation.

Dilution stability test: The anthocyanin nanoemulsion samples were diluted with ultra-pure water by 100, 200, 500, 1000 times to observe whether there was stratification, turbidity or precipitation.

High speed centrifugal test: The anthocyanin nanoemulsion samples were centrifuged at 3000r/min for 20min to observe whether there was stratification, turbidity or precipitation.

2.9 Skin Safety Evaluation of Blueberry Anthocyanin Nanoemulsions

2.9.1 Skin Irritation Test

Experimental animals: The ethics Review Committee of Guizhou Medical University approved this study (NO. 1900075), all animal testing methods were performed in accordance with the relevant guidelines and regulations. 24 healthy guinea pigs (350-450g) were selected, with half male and half female. It was randomly divided into single stimulation test and multiple stimulation test with 12 guinea pigs in each test. And each test was randomly divided into 3 groups: normal saline group, blank nanoemulsions group and anthocyanin nanoemulsions group, with 4 guinea pigs in each group. 24h before the experiment, guinea pigs were treated with hair removal, about 3cm×3cm on each side of the back.

Table 1 Scoring criteria of skinirritation and sensitization reaction

Reaction	Score
Erythema	
No erythema	0
Mild erythema (barely visible)	1
Moderate erythema (visible)	2
Severe erythema	3
Purplish red erythema to mild eschar formation	4
Edema	
No edema	0
Mild edema (barely visible)	1
Moderate edema (marked swelling)	2
Severe edema (skin swelling about 1mm, well defined)	3
Severe edema (skin swelling more than 1mm and enlarged)	4

Note: Average score = (total erythema score + total edema score)/total number of animals

Single dose skin irritation test: The left and right sides of guinea pigs were self-controlled. The hair removal area on the left side was coated with 0.5ml blueberry anthocyanin nanoemulsions, covered with gauze and fixed with bandage for at least 4h. The right side was coated with distilled water as blank control. At 1h, 24h, 48h, 72h after removing the patch, observe whether there was erythema and edema at the application site, and determine the intensity of stimulus response (Table 1 and Table 2) [17].

Multiple dose skin irritation tests: The application and fixation method of anthocyanin nanoemulsions were the same as the single skin stimulation test. The difference was that the left depilation area was regularly applied once a day for 7 consecutive days. At 1h, 24h, 48h, 72h after removing the patch, observation was performed as the single skin irritation test.

Table 2 Evaluation standard of skin irritation intensity

Average score	Reaction intensity classification
0~	No irritation
0.5~	Mild irritation
3.0~	Moderate irritation
6.0~8.0	Severe irritation

2.9.2 Skin Sensitization Test

Refer to the Buehler test for minor modifications.

Experimental animals: 12 healthy guinea pigs (350-450g) were selected, with half male and half female. They were randomly divided into 3 groups with 4 animals in each group: anthocyanin nanoemulsions group, blank nanoemulsions group and positive control group (2, 4-dinitrochlorobenzene). Guinea pigs hair removal were treated as skin irritation test.

Induction exposure: A suitable patch was soaked in blueberry anthocyanin nanoemulsions and applied to the left depilation area on day 1, 7 and 14 of the experiment, respectively, and was fixed with bandage for 6 hours.

Challenge exposure: On the 14th day after the last induction exposure, anthocyanin nanoemulsions were applied to the right depilation area of guinea pigs in the same way. After 6h, the application was removed, and skin erythema and edema were observed at 1h, 24h, 48h and 72h after stimulated contact (Table 1 and Table 3).

Table 3 Evaluation standard of skin sensitization intensity

Sensitization rate (%)	Classification	Intensity of sensitization
0~	I	Weak sensitization
11~	II	Mild sensitization
31~	III	Moderate sensitization
61~	IV	Strong sensitization
81~100	V	Extreme sensitization

Note: Sensitization rate (%)=number of animals with erythema or edema/total number of animals in the group

3 Results

3.1 Screening of Optimum Conditions for Preparation of Blueberry Anthocyanin Nanoemulsions

3.1.1 Determination of Surfactants

Blueberry anthocyanins were dissolved in different surfactants (Figure 1A), Tween-80 is a yellow solution, but when mixed with blueberry anthocyanins, it turns dark, nearly dark-purple. The color of blueberry anthocyanins was lighter and stratified after Tween-20. EL-35 has good solubility, suitable solution color and less precipitation. Stirring in RH-40 is uneven, with a little stratification; But in EL-40 and Span-80, the solution color is lighter and there is a large amount of precipitation. Therefore, EL-35 was chosen as the surfactant.

3.1.2 Determination of Oil Phase

Blueberry anthocyanins were soluble in IPM and GTCC, and stratified in IPP (Figure 1B). Therefore, IPM and GTCC were used as alternative oil phases.

Then, the oil phase (IPM and GTCC) was combined with the surfactant (EL-35) and the combination of surfactant (EL-35/RH40, EL-35/Span80), and blueberry anthocyanins were added. It was found that only the combination of EL-35 and IPM could be stable and transparent at room temperature, 60 ℃ and -4 ℃ for 1 week. Therefore, EL-35 was chosen as the best surfactant and IPM as the best oil phase (Table 4).

Table 4 Determination of optimum surfactant and oil phase

Oil phase	**room temperature**	**60 ℃**	**-4 ℃**	**final results**
IPP/EL-35	turbid	turbid	turbid	-
IPM/EL-35	transparent	transparent	transparent	+
GTCC/EL-35	turbid	turbid	turbid	-
GTCC/EL-35/RH40	turbid	turbid	turbid	-
GTCC/EL-35/Span80	turbid	turbid	turbid	-
IPM/EL-35/RH40	turbid	turbid	turbid	-
IPM/EL-35/Span80	semitransparent	turbid	turbid	-

Note: "+" is suitable for preparing nanoemulsions; "-" is not suitable for the preparation of nanoemulsions

3.1.3 Determination of Cosurfactant and Optimum Km Value

Glycerol, ethanol, 1, 2-propanediol and 1, 3-butanediol were selected as screening objects of cosurfactant, and the ratios of 2:1, 3:1, 3:2 and 4:1 were selected as alternative Km values. Pseudo-ternary phase diagrams were drawn with three vertices of surfactant/cosurfactant, oil phase and water phase. When glycerol was used as cosurfactant, the nanometer emulsion area was the largest, so glycerol was chosen as cosurfactant (Figure 1C). The optimal Km value was 4:1 (Figure 1D).

3.2 Preparation of Blueberry Anthocyanin Nanoemulsions

According to the above determination results, the blueberry anthocyanin nanoemulsions formulation was determined, the mass ratio of blueberry anthocyanin, EL-35, IPM, glycerin and double steaming water was 2:16:5:4:40. At room temperature, EL-35 was thoroughly mixed with glycerin and IPM, then blueberry anthocyanin was added and stirred with magnetic force at the speed of 800 rpm. The blueberry anthocyanin nanoemulsions were prepared by slowly dropping the prescribed amount of ultra-pure water while stirring.

3.3 Characterization of Blueberry Anthocyanin Nanoemulsions

Blueberry anthocyanin nanoemulsions were dark purple-red, clear and transparent under sunlight, with uniform texture and certain fluidity (Figure 1E). It was found that the diffusion rate of methylene blue in the nanoemulsions were significantly faster than that of Sudan Ⅲ, so blueberry anthocyanin nanoemulsions were determined to be O/W type. Transmission electron microscopy showed that blueberry anthocyanin nanoemulsions were spherical polydisperse system with particles size of 10-600nm, and no adhesion or agglomeration were observed (Figure 1F). The viscosity was 2.0-2.5s, pH was 6.89, and Zeta potential was 4.42.

3.4 Determination of Anthocyanin Content in Nanoemulsions

After spectral scanning by UV-vis spectrophotometer, it was found that the maximum absorption peak was at 550nm. The absorbance value was measured at this wavelength to make a standard curve (Figure 1G), and the anthocyanin content in nanoemulsion samples were 608.89μg/mL.

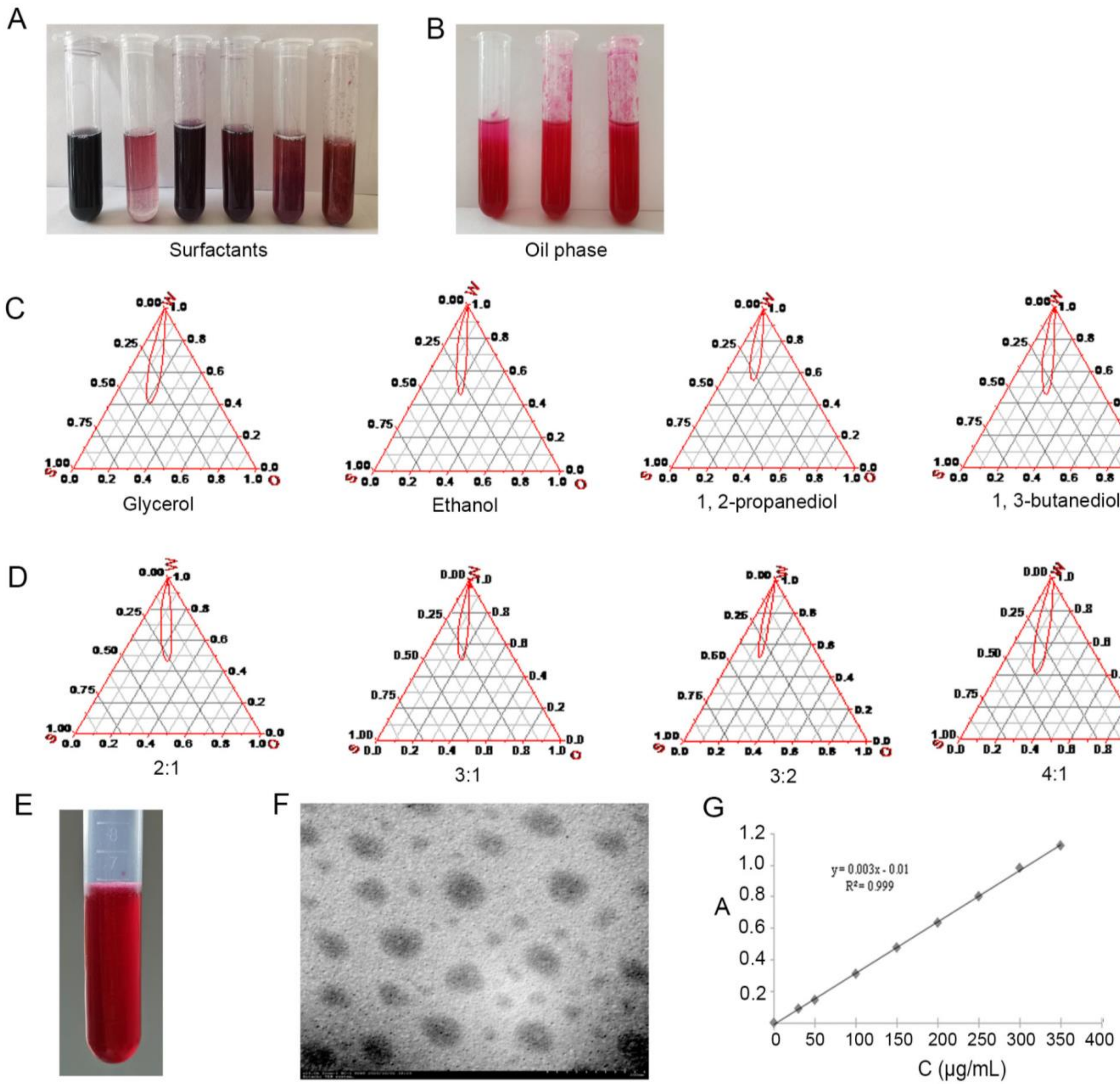

Figure 1 Preparation of blueberry anthocyanin nanoemulsions. A Comparison of different surfactants. The order from left to right was tween-80, Tween-20, EL-35, RH-40, EL-40, and SPAN-80; B Comparison of different oil phases. The order from left to right was IPP, GTCC and IPM; C Effects of different cosurfactants on the area of pseudo-ternary phase diagram nanoemulsions; D Effects of different Km values on the area of pseudo-ternary diagram nanoemulsions; E Blueberry anthocyanin nanoemulsions prepared according to the formulation (under sunlight); F Represent picture of the ultrastructure of blueberry anthocyanin nanoemulsions observed by TEM. Scar bar = 200 nm; G Standard curve of anthocyanin nanoemulsions

3.5 Stability Evaluation of Blueberry Anthocyanin Nanoemulsions

Long-term stability at room temperature: The anthocyanin nanoemulsions remained clear and transparent after being placed at room temperature (10-20 ℃ in autumn) for 1 and 2 weeks, and there was no stratification of oil and water, showing good stability (Figure 2A).

High temperature test: The anthocyanin nanoemulsions remained clear and transparent after being placed at 4 ℃ and 25 ℃ for 1 week, respectively. The color became lighter after being placed at 50 ℃ for 1 week, but it was still clear and transparent (Figure 2B).

Dilution stability test: The blueberry anthocyanin nanoemulsions remained clear and transparent after diluted to 100, 200, 500 and 1000 times. (Figure 2C).

High speed centrifugal test: After centrifugation at 3000r/min for 20 minutes, the anthocyanin nanoemulsions were still clear and no stratification occurred (Figure 2D).

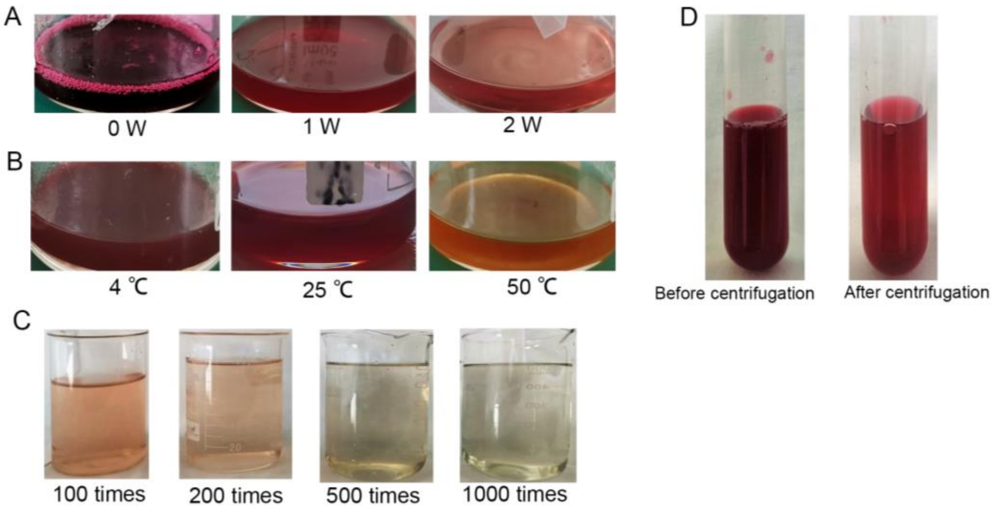

Figure 2 Stability evaluation of blueberry anthocyanin nanoemulsions. A Nanoemulsions were stored at room temperature for 0, 1 and 2 weeks; B The results of high temperature test; C The results of dilution stability test; D The results of high speed centrifugal test

3.6 Safety Evaluation of Blueberry Anthocyanin Nanoemulsions

3.6.1 Results of Skin Irritation Test

The left skin of guinea pigs in normal saline group, blank nano emulsion group and anthocyanin nano emulsion group was observed at 1h, 24h, 48h and 72h after the single and multiple dose of skin stimulation test of dministration of blueberry anthocyanin nanoemulsions. Anthocyanin nanoemulsions showed no irritation to the skin (Tables 5-6; Figure 3 A-B).

3.6.2 Results of Skin Sensitization Test

After the skin sensitization test of blueberry anthocyanin nanoemulsions, it was found that the blank nanoemulsion group and the anthocyanin nanoemulsion group showed weak skin sensitization, while the positive control group (2, 4-dinitrochlorobenzene) guinea pigs showed different degrees of erythema or edema, showing strong sensitization (Table 7; Figure 3 C).

Table 5 Single dose skin irritation test results of blueberry anthocyanin nanoemulsions (n=4)

Group	Observation time (h)	Erythema score	Edema scores	Average score	Intensity scale
Normal saline group	1	0	0	0	No irritation
	24	0	0	0	
	48	0	0	0	
	72	0	0	0	
Blank nano emulsion group	1	0	0	0	No irritation
	24	0	0	0	
	48	0	0	0	
	72	0	0	0	

Group	Observation time (h)	Erythema score	Edema scores	Average score	Intensity scale
Anthocyanin nanoemulsions group	1	0.25	0	0.25	No irritation
	24	0	0	0	
	48	0	0	0	
	72	0	0	0	

Table 6 Multiple dose skin irritation test results of blueberry anthocyanin nanoemulsions (n=4)

Group	Observation time (h)	Erythema score	Edema scores	Average score	Intensity scale
Normal saline group	1	0	0	0	No irritation
	24	0	0	0	
	48	0	0	0	
	72	0	0	0	
Blank nano emulsion group	1	0	0	0	No irritation
	24	0	0	0	
	48	0	0	0	
	72	0.25	0	0.25	
Anthocyanin nano emulsion group	1	0.25	0	0.25	No irritation
	24	0	0	0	
	48	0	0	0	
	72	0	0	0	

Table 7 Skin sensitization test results of blueberry anthocyanin nanoemulsions (n=4)

Group	Observation time (h)	Erythema score	Edema scores	Average score	Positive rate of sensitization (%)	Intensity scale
Blank nano emulsion group	1	0	0	0	0	I Weak sensitization
	24	0	0	0	0	
	48	0	0	0	0	
	72	0	0	0	0	
Anthocyanin nano emulsion group	1	0	0	0	0	I Weak sensitization
	24	0	0	0	0	
	48	0	0	0	0	
	72	0	0	0	0	
Positive control group	1	0.25	0	2.00	100	V Extreme sensitization
	24	0	0	5.25	100	
	48	0	0	6.25	100	
	72	0	0	5.00	100	

Figure 3 Safety evaluation of blueberry anthocyanin nanoemulsions. A Response to single dose skin irritation test of nanoemulsions; B Response to multiple dose skin irritation test of nanoemulsions; C Response to skin sensitization test of nanoemulsions

4 Discussion

Blueberry anthocyanins have strong antioxidant properties, so they have many physiological functions beneficial to human body, such as anti-inflammation, anti-cancer, reducing the incidence of cardiovascular diseases, controlling diabetes and alleviating obesity [18]. Therefore, blueberry anthocyanins are widely used in medicine, health food, cosmetics and other fields. But the stability of blueberry anthocyanin is poor and there is a lot of loss in the production process. How to retain anthocyanin and its physiological activity to the maximum extent is a problem that needs to be solved, and the bioavailability of anthocyanins in human gastrointestinal tract is low, however, encapsulation with nanotechnology can prevent its degradation and improve bioavailability and bioactivity [19].

Nanoemulsions are characterized by small droplet size, good stability, transparent appearance and rheological properties. These properties make nanoemulsions an attractive candidate for food, cosmetic, pharmaceutical industries and drug delivery applications [20]. And they can be produced in different forms, such as foam, liquid, cream and spray [21].

In this study, based on the solubility of blueberry anthocyanins and pseudo-terpolymer phase diagram method, the best formula for the preparation of nanoemulsions was selected, the mass ratio of blueberry anthocyanin, EL-35 (surfactant), IPM (oil phase), glycerin (cosurfactant), ultra-pure water was 2:16:5:4:40. Surfactants and cosurfactants play an important role in the formation and stability of nanoemulsions. Suitable compounds were identified as palmitic acid (lipid), Span 85, lecithin (W/O surfactant) and Pluronic F127 (aqueous stabilizer), with an average particle size of 455.2 nm, and ethanol was added as co-surfactant [22]. However, El-35, IPM and glycerol selected in this study are all raw materials for food or cosmetics, non-irritating, non-toxic, and without adding ethanol and other chemical agents.

The preparation process of nanoemulsions can be divided into two steps, the first step is to form macroemulsion, the second step is to transform into nanoemulsions. In the second step, emulsification methods are usually divided into high-energy and low-energy emulsification methods [20, 23, 24]. High-energy emulsification methods are to use ultrasonic generator or high pressure homogenizer and other special equipment, through high energy to reduce the size of large droplets to small nano size; However, the low-energy emulsification methods are to use the chemical potential or environmental conditions of the components to form nanoemulsions, which only require simple stirring and little energy. They can be classified as spontaneous emulsification (SE), phase inversion composition (PIC) and phase inversion temperature (PIT).

Recently, the low-energy emulsification methods have become more and more popular because they do not require expensive equipment, save energy and are suitable for large-scale production. In this study, O/W type blueberry anthocyanin nanoemulsions were prepared by magnetic stirring through oil phase, water phase, surfactant and cosurfactant. They were dark purplish red in appearance (the color of anthocyanins), clear and transparent, uniform in texture, and had certain fluidity. Anthocyanin content was 608.89μg/mL. The spherical polydisperse system of 10-600nm were observed under electron microscope. Because the sizes of nanoemulsions droplets are smaller than the wavelength of visible light, they scatter only faint light waves, they are usually transparent or translucent in appearance. However, when the droplet size reaches the nanometer scale, the irregularity of the surface plays an important role in adhesion, and the increased surface area can show greater biological activity, thus improving the bioavailability of encapsulated materials [16, 25, 26].

The stability test results showed that blueberry anthocyanin nanoemulsions could maintain good stability after 2 weeks at room temperature. The high temperature test showed that the nanoemulsions had good stability when placed at 4℃ and 25℃ for 1 week, and the color became pale when placed at 50℃ for 1 week, but they were still clear and transparent. The dilution test and high speed centrifugation test show that the stability was good. The main cause of nanoemulsions instability is the Ostwald maturation phenomenon.

After single and multiple dose skin irritation test, the results showed that blueberry anthocyanin nanoemulsions had no irritation to guinea pig hair removal skin. The results of skin sensitization test displayed that anthocyanin nanoemulsions showed weak sensitization to guinea pig hair removal skin (Grade I).

While the skin protects us from our environment, it also acts as a transport barrier for drugs entering the body through the skin. Use nanoemulsions preparation of local medication can provide unique advantages, because nanoemulsions of O/W of dispersed phase enhanced lipotropy drug solubility in the oil phase, and the continuous phase provides a gentle, friendly environment to the skin, can dissolve biopolymers, therefore, considerable research nanoemulsions will be used for local drug delivery. Topical drugs formulated using nanoemulsions provide unique advantages because the dispersed phase of O/W nanoemulsions enhances the solubility of lipophilic drugs in the oil phase, while the continuous phase provides a mild, skin-friendly environment to dissolve drugs. Therefore, quite a few studies focused on using nanoemulsions as topical drug delivery [20].

5 Conclusions

In this study, the best surfactants, oil phases and cosurfactants for the preparation of blueberry anthocyanin nanoemulsions were screened by pseudo-ternary phase diagram method and the solubility of blueberry anthocyanin nanoemulsion was taken into consideration. Deep purplish red, clear and transparent anthocyanin nanoemulsions were prepared by simple and cheap low-energy emulsification method according to the formula. Normal temperature test, high temperature test and high speed centrifugal test proved that the blueberry anthocyanin nanoemulsions had good stability. Guinea pig skin irritation test and sensitization test showed that there was no irritation and sensitization to guinea pig hair removal skin. Therefore, the nanoemulsion provides a reference for improving the stability and bioavailability of blueberry anthocyanins. Combined with the high nutritional value of blueberry anthocyanins and the stability of nanoemulsion, the nanoemulsion can be considered for use in skin beauty products or transdermal drug delivery systems in the future.

Contributions

Mingyue YIN designed the study. Jieyu SONG, Xiaoling ZHOU, Wei ZHANG and Ao WU prepared nanoemulsions. Jieyu SONG and Xiaoling ZHOU evaluated the stability and skin safety of the nanoemulsions. Mingyue YIN, Jieyu SONG and Xiaoling ZHOU participated in data analysis and evaluation. Mingyue YIN wrote the first draft of the manuscript and finished the revision.

Declarations

Authors declare no competing financial interests or personal relationships with other people or organizations that could inappropriately influence (bias) our work.

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