Preparation of Blueberry Anthocyanin Nanoemulsion And Its Stability and Skin Safety Evaluation

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Abstract

BACKGROUND: Blueberry anthocyanins have strong antioxidant activity, but their instability and low bioavailability limit their use. Nanoemulsion is a new type of food and drug carrier with stable thermodynamic properties. Therefore, anthocyanins will be encapsulated with nanoemulsion to improve its stability and application value.

RESULTS: In this study, the best surfactants, oil phases and cosurfactants for the preparation of blueberry anthocyanin nanoemulsion 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 nanoemulsion was 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 nanoemulsion 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.

CONCLUSION: The blueberry anthocyanin nanoemulsion prepared in this study has good stability at room temperature and is safe for guinea pig hair removal skin. It provides a basis for improving the stability and bioavailability of blueberry anthocyanin, and provides a reference for the application of blueberry anthocyanin nanoemulsion in skin beauty and transdermal drug delivery.

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, Pelunidin and 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, 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. Nanoemulsion, or microemulsion, is thermodynamically stable, isotropic, transparent or

translucent, homogeneous dispersed system spontaneously formed from water, oil, surfactant, and cosurfactant (5). Nanoemulsion and microemulsion have different particle sizes, droplets typically have sizes of 20-500 nm in nanoemulsion and 10-100 nm in microemulsion (6). As a promising new drug carrier, nanoemulsion has incomparable advantages, such as stable thermodynamic properties, easy preparation and preservation, improved solubility and absorption rate of drugs, low viscosity, slow release and targeting.

In general, there are three types of nanoemulsion: water-in-oil, oil-in-water, and bi-continuous nanoemulsion. Nanoemulsion can be prepared by mixing oil, water, surfactant and co-surfactant in proportion. If each component is selected properly, the preparation of nanoemulsion is independent of the order of addition of each component.

Nanoemulsion presents a higher active surface area/volume ratio due to their small droplet size, thus enhancing the transport of active compounds through biological membranes (7).

Nanoemulsion system is a multi-component system, with at least three components, namely water, oil and surfactant, usually 4-5 components, that is, plus cosurfactant and salt. If mixed surfactants and mixed oils are added, the system becomes more complex. 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. For systems with four components and more than four components, the method of combining variables, such as fixing the ratio of two components to make the actual variables less than three, can still be represented by a triangular phase diagram. Such phase diagram is called a pseudo-ternary phase diagram, which is an effective method to select the formulation of nanoemulsion.

In this study, blueberry anthocyanins were prepared into nanoemulsion 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 nanoemulsion in skin beauty and transdermic drug delivery.

Materials & Methods

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(8), the extracted blueberry anthocyanins were turned into lyophilized powder by vacuum freeze-drying.

Selection of surfactants

Castor oil polyoxyethylene ether 40 (EL-40), castor oil polyoxyethylene ether 35 (EL-35), polyoxyethylene ether hydrogenated castor oil (RH40), Tween-80, Tween-20 and Span-80 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.

Selection of oil phase

Isopropyl myristic (IPM), isopropyl palmitate (IPP) and Caprylic acid capric acid triglyceride (GTCC) 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°C and -4°C, and the best surfactant and oil phase were determined.

Determination of cosurfactants and optimal Km value (mass ratio of surfactant to cosurfactant)

Glycerol, ethanol, 1, 2-propanediol and 1, 3-butanediol 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(9), 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.

Determination of blueberry anthocyanin nanoemulsion formulation

By using spontaneous emulsification (SE)(10), blueberry anthocyanin nanoemulsion 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.

Characterization of blueberry anthocyanin nanoemulsion

The characteristics of blueberry anthocyanin nanoemulsion 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 nanoemulsion 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 nanoemulsion 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.

Determination of anthocyanin content in nanoemulsion

The content of anthocyanin in blueberry anthocyanin nanoemulsion was 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 nanoemulsion 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.

Stability evaluation of blueberry anthocyanin nanoemulsion

Long - time retention sample at room temperature: The blueberry anthocyanin nanoemulsion samples were placed at room temperature (Autumn, room temperature 10-20°C, 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°C, 25°C and 50°C 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.

Skin safety evaluation of blueberry anthocyanin nanoemulsion

Skin irritation test

Experimental animals: The ethics Review Committee of Guizhou Medical University approved this study (NO.1900250), 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 nanoemulsion group and anthocyanin nanoemulsion 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.

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 nanoemulsion, 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(tab.1and tab.2) (11).

Multiple dose skin irritation tests: The application and fixation method of anthocyanin nanoemulsion was 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 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	

Table2 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

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 nanoemulsion group, blank nanoemulsion 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 nanoemulsion 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 nanoemulsion was 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 (tab.1 and tab.3).

Table3 Evaluation standard of skin sensitization intensity

Sensitization rate (%)	Classification	Intensity of sensitization
0~	□	Weak sensitization
11~	□	Mild sensitization
31~	□	Moderate sensitization
61~	□	Strong sensitization
81~100	□	Extreme sensitization
Note: Sensitization rate (%)=number of animals with erythema or edema/total number of animals in the group		

Results

Screening of optimum conditions for preparation of blueberry anthocyanin nanoemulsion Determination of surfactants

Blueberry anthocyanins were dissolved in different surfactants (Fig. 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.

Determination of oil phase

Blueberry anthocyanins were soluble in IPM and GTCC, and stratified in IPP (Fig. 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°C and -4°C for 1 week. Therefore, EL-35 was chosen as the best surfactant and IPM as the best oil phase (tab.4).

Table4 Determination of optimum surfactant and oil phase

Oil phase	room temperature	60°C	-4°C	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 nanoemulsion; “-” is not suitable for the preparation of nanoemulsion				

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(Fig. 1C).The optimal Km value was 4:1(Fig. 1D).

Preparation of blueberry anthocyanin nanoemulsion

According to the above determination results, the blueberry anthocyanin nanoemulsion 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 nanoemulsion was prepared by slowly dropping the prescribed amount of ultra-pure water while stirring.

Characterization of blueberry anthocyanin nanoemulsion

Blueberry anthocyanin nanoemulsion is dark purple-red, clear and transparent under sunlight, with uniform texture and certain fluidity (Fig. 1E). It was found that the diffusion rate of methylene blue in the nanoemulsion was significantly faster than that of Sudan $\square$, so blueberry anthocyanin nanoemulsion was

determined to be O/W type. Transmission electron microscopy showed that blueberry anthocyanin nanoemulsion was a spherical polydisperse system with a particle size of 10-600nm, and no adhesion or agglomeration was observed (Fig. 1F). The viscosity was 2.0-2.5s, pH was 6.89, and Zeta potential was 4.42.

Determination of anthocyanin content in blueberry anthocyanin

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 (Fig. 1G), and the anthocyanin content in blueberry anthocyanin samples was 608.89µg/mL.

Stability evaluation of blueberry anthocyanin nanoemulsion

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

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

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

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

Safety evaluation of blueberry anthocyanin nanoemulsion

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 administration of blueberry anthocyanin nanoemulsion. Anthocyanin nano emulsion showed no irritation to the skin (Tab.5-6; Fig. 3A-B).

Results of skin sensitization test

After the skin sensitization test of blueberry anthocyanin nanoemulsion, 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 (Tab.7; Fig. 3C).

Table 5 Single dose skin irritation test results of blueberry anthocyanin nanoemulsion (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	
Anthocyanin nanoemulsion 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 nanoemulsion(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	

Table7 Skin sensitization test results of blueberry anthocyanin nanoemulsion(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	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	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	Extreme sensitization
	24	0	0	5.25	100	
	48	0	0	6.25	100	
	72	0	0	5.00	100	

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 (12). 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 (13).

Nanoemulsion are characterized by small droplet size, good stability, transparent appearance and rheological properties. These properties make nanoemulsion an attractive candidate for food, cosmetic, pharmaceutical industries and drug delivery applications (14). And they can be produced in different forms, such as foam, liquid, cream and spray (15).

In this study, based on the solubility of blueberry anthocyanins and pseudo-terpolymer phase diagram method, the best formula for the preparation of nanoemulsion 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 nanoemulsion.

Raheleh Ravanfar et al. optimized the preparation of red cabbage anthocyanin microemulsion by Plackett Burman and Box Behnken design. 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 (16). 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 nanoemulsion can be divided into two steps, the first step is to form macroemulsion, the second step is to transform into nanoemulsion. In the second step, emulsification methods are usually divided into high-energy and low-energy emulsification methods (14, 17, 18). 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 nanoemulsion, 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 nanoemulsion was 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 608.89 μ g/mL. The spherical polydisperse system of 10-600nm were observed under electron microscope. Because the size of nanoemulsion droplets is 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 (10, 19, 20).

The stability test results showed that blueberry anthocyanin nanoemulsion could maintain good stability after 2 weeks at room temperature. The high temperature test showed that the nanoemulsion had good stability when placed at 4°C and 25°C for 1 week, and the color became pale when placed at 50°C for 1 week, but they were still clear and transparent. The dilution test and high speed centrifugation test show that the stability was good.

Nanoemulsion is dynamically stable and relatively less sensitive to dilution and temperature changes than microemulsions. Mechanisms of nanoemulsion instability include flocculation, coalescence, Ostwald ripening and creaming/ sedimentation. During flocculation, the droplets come close to each other due to attractive interactions and move as a unit. In contrast, at coalescence, the droplets merge together to form one large droplet. DLVO theory predicts that when the maximum repulsion of droplet - droplet interaction potential is low, droplets get close to each other and fall into the primary minimum, the

irreversibly flocculated state. In this process, droplets tend to stick together when they come into direct contact. Therefore, it is difficult to distinguish flocculation from coalescence in emulsion (14). Typically, due to the adsorptive layer of the emulsifier on the droplet, steric hindrance interactions increase the maximum repulsion, thus stabilizing the flocculation and coalescence of the emulsion.

After single and multiple dose skin irritation test, the results showed that blueberry anthocyanin nanoemulsion had no irritation to guinea pig hair removal skin. The results of skin sensitization test displayed that anthocyanin nanoemulsion 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 nanoemulsion preparation of local medication can provide unique advantages, because nanoemulsion 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 nanoemulsion will be used for local drug delivery. Topical drugs formulated using nanoemulsion provide unique advantages because the dispersed phase of O/W nanoemulsion enhances the solubility of lipophilic drugs in the oil phase, while the continuous phase provides a mild, skin-friendly environment to dissolve biopolymers. Therefore, quite a few studies focused on using nanoemulsion as topical drug delivery (14).

Conclusions

In this study, the best surfactants, oil phases and cosurfactants for the preparation of blueberry anthocyanin nanoemulsion 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 nanoemulsion was 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 nanoemulsion 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. This nanoemulsion provides a basis for improving the stability and bioavailability of blueberry anthocyanin, and provides a reference for the application of blueberry anthocyanin nanoemulsion in skin beauty and transdermal drug delivery.

Declarations

Contributions

Mingyue YIN designed the study. Jieyu SONG, Xiaoling ZHOU, Wei ZHANG and Ao WU prepared nanoemulsion. Jieyu SONG and Xiaoling ZHOU evaluated the stability and skin safety of the nanoemulsion. 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.

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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Figures

Figure 1

Preparation of blueberry anthocyanin nanoemulsion. **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 nanoemulsion; **D** Effects of different Km values on the area of pseudo-ternary diagram nanoemulsion; **E** Blueberry anthocyanin nanoemulsion prepared according to the formulation (under sunlight); **F** Represent picture of the ultrastructure of blueberry anthocyanin nanoemulsion observed by TEM. Scar bar = 200 nm; **G** Standard curve of anthocyanin nanoemulsion.

Figure 2

Stability evaluation of blueberry anthocyanin nanoemulsion. **A** Nanoemulsion was 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.

Figure 3

Safety evaluation of blueberry anthocyanin nanoemulsion. **A** Response to single dose skin irritation test of nanoemulsion; **B** Response to multiple dose skin irritation test of nanoemulsion; **C** Response to skin sensitization test of nanoemulsion.