A kind of salicylic acid nanoemulsion with sustained and controlled release effect and research on its anti-acne efficacy

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Abstract

Background: To develop a stable, low-irritant salicylic acid transporter for effective skin care benefits.

Objective: The purpose of this paper is to provide a salicylic acid nanoemulsion and its preparation method and application, and to explore salicylic acid nanoemulsion's in vitro release behavior, safety, effect on the activity of P. acnes and clinical acne-removing efficacy.

Methods: The salicylic acid nanoemulsion was prepared by ultra-high pressure nano-homogenization. In addition, the human body patch test was used to determine the safety of the salicylic acid nanoemulsion. The dialysis bag method was used to study the in vitro release of the salicylic acid nanoemulsion. Finally, the antibacterial diameter of salicylic acid nanoemulsion and free salicylic acid against P. acnes was measured by agar plate diffusion method (punching method).

Results: The salicylic acid nanoemulsion prepared in this paper had a uniform morphology, an average particle size of 21.4 nm, a PDI of 0.107, a drug loading of 30.0%, and an encapsulation efficiency of 83.7%. The results of the human patch test showed that none of the 21 subjects had any adverse reactions. In vitro sustained and controlled release experiments showed that salicylic acid nanoemulsion had significant sustained and controlled release characteristics; finally, by agar plate diffusion method, the results showed that the average size of the inhibition zone of free salicylic acid and salicylic acid nanoemulsion was 7.23, respectively. mm, 9.49 mm, indicating that the antibacterial effect of salicylic acid was

significantly improved after nano-encapsulation. The clinical results of acne treatment show that salicylic acid nanoemulsion has a significant effect of removing acne.

Conclusion: Compared with free salicylic acid, the nano-emulsion salicylic acid prepared in this study has lower skin irritation, higher safety, stronger antibacterial activity, and provided new insight into the topical application of skin care cosmetics, especially skin anti-acne products.

Keywords: Salicylic acid; Nanoemulsion; Release behavior; Safety; Anti-acne efficacy.

Introduction

Salicylic acid is a fat-soluble organic acid with the chemical formula C7H6O3. Existing in willow bark, white bead leaves and sweet birch in nature, it is an important fine chemical raw material and can be used in the preparation of aspirin and other drugs. It has been used in skin disorders for nearly 2,000 years [1]. Salicylic acid has the effect of exfoliating keratin, smoothing the skin, and lightening pigmentation. It mainly dissolves the adhesive between the stratum corneum [2], so that the old keratinocytes that fail to fall off normally will fall off, which will accelerate the fall of the cuticle and melanin. The role of metabolism can be used in whitening products. Salicylic acid can also be used to clear acne [3], mainly relying on its ability to penetrate deep into pores through sebaceous glands, dissolve oil, exfoliate keratin, clear blackheads, and improve pore blockage. The latest research shows [4] that salicylic acid can control oil by downregulating the AMPK/SREBP1 signaling pathway in sebocytes, and effectively treat acne vulgaris. In addition, salicylic acid has antibacterial and bactericidal effects on Propionibacterium acnes, Staphylococcus aureus, etc., which cause inflammation of hair follicles and cause skin purulence or infection, and can effectively improve and treat acne [5]. Based on the efficacy of salicylic acid described above, it can be seen that salicylic acid also has the characteristics of treating four major pathological characteristics of acne vulgaris, such as relieving excessive keratinization, regulating excessive secretion of sebum, blocking inflammatory response and inhibiting anaerobic acne propionic acid Bacillus growth. However, the solubility of salicylic acid is extremely low, so it is difficult to apply to conventional cosmetic formulations. In addition, high concentrations of salicylic acid are very irritating to the skin. Although the concentration of salicylic acid in skin care products is usually 0.5~2%, there are still many consumers who will experience

erythema and stinging after use. etc. phenomenon. Therefore, in order to overcome the shortcomings of free salicylic acid and improve its bioavailability, it is urgent to research and develop a stable and low-stimulation salicylic acid transporter, so that it can effectively exert its anti-acne effect. The salicylic acid nano composition prepared in this study not only overcomes the problems of solubility and irritation of free salicylic acid, but also can be targeted and delivered to inflammatory sites such as acne, and released at the inflammatory site, significantly enhancing the anti-inflammatory and acne-removing effect. The mechanism is: on the one hand, the salicylic acid nanocomposite can penetrate into the stratum corneum through intercellular lipids, and as time goes on, the encapsulated high-concentration salicylic acid will diffuse out to achieve sustained release, thereby prolonging the salicylic acid. The action time of salicylic acid increases; in the inflammatory site such as acne, the vascular permeability is enhanced, and the gap between endothelial cells increases, so the nanocarrier can be transported to the lesion through the relaxed blood vessels in the inflammatory area and accumulate in a large amount at the lesion, realizing the effect of salicylic acid in inflammation. In addition, it is more critical that there are very active lipases on acne skin. Lipase is a special kind of esterase that can hydrolyze triglycerides into fatty acids, diglycerides and glycerol, etc. Therefore, In the salicylic acid nano-composition, triglyceride is selected as the liquid lipid for preparing the nano-composition, and the lipase in the inflammatory site can degrade the triglyceride nano-carrier matrix material to trigger the decomposition of the nano-carrier to release salicylic acid, and In inflammatory areas such as acne, the activity of lipase will be significantly increased, so the nanocarrier can focus on releasing salicylic acid in the inflammatory area, achieving rapid and controlled release, and significantly improving the effect of anti-acne and anti-inflammatory. The purpose of this paper is to study a salicylic acid nanoemulsion and its preparation method and application, and to explore its in vitro release behavior, safety, effect on the activity of P. acnes, and clinical efficacy of acne treatment.

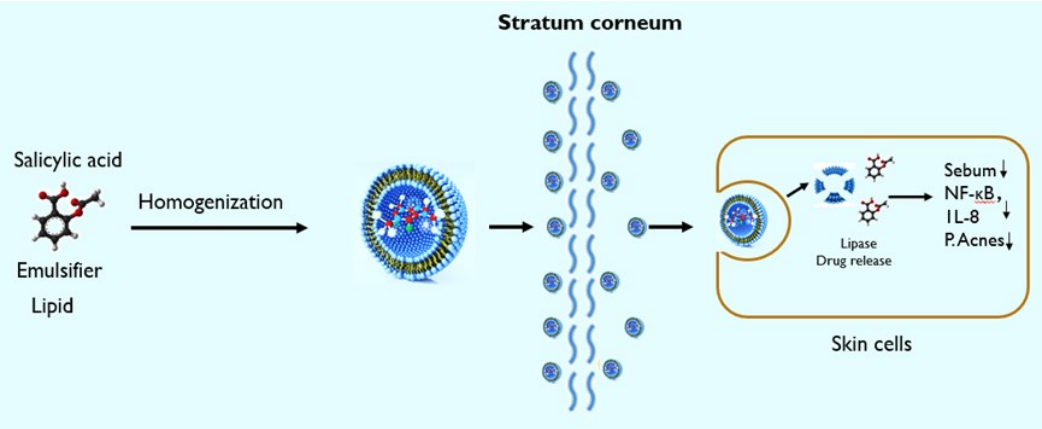

Fig.1 Schematic illustration of the mechanisms of co-delivering salicylic acid nanoemulsion

Materials and Methods

Materials and equipment

Salicylic acid, Merck; 1,2-Pentanediol, Korea B&B Corporation; Caprylic/Capric Triglyceride, Croda Chemicals, UK; PEG-40 Hydrogenated Castor Oil, BASF, Germany; Tocopheryl Acetic Acid Esters, BASF, Germany; Shea Butter, JARCHEM; Jojoba Oil, Vantage Special Materials, USA; Triglyceride (Ethylhexanoate), Sunlight Dongcheng Chemical Co., Ltd.; Porcine Pancreatic Lipase, A Latin Reagent (Shanghai) Co., Ltd.; BHI medium, Gibco; agar, Shanghai Yuanye Biotechnology Co., Ltd.; other reagents, Sinopharm Chemical Reagent Co., Ltd.; Propionibacterium acnes, China Industrial Microorganism Collection; BOCL101 solution Phase chromatograph, Shimadzu Corporation, Japan; Zetasizer/Nano-ZS90 Nano Potential Particle Size Analyzer, Malvern Company, UK; AH-BASIC Ultra-High Pressure Nano Homogenizer, ATS Nano Technology (Suzhou) Co., Ltd., Sebumeter (Derma Unit SSC3), Minolta chromateter (CM 700d) produced by Minolta, Japan

The preparation of salicylic acid nanoemulsion

Weigh Salicylic Acid, 1,2-Pentanediol, Caprylic/Capric Triglyceride, PEG-40 Hydrogenated Castor Oil, Tocopheryl Acetate, Tris(ethylhexanoate) Glycerin, Shea Butter according to the prescription amount Add fat and jojoba seed oil into a beaker as the oil phase, heat and stir at 50°C for 20 minutes, and dissolve completely; take the distilled water of the recipe amount

as the water phase, heat to 50°C, stir until completely dissolved, and cool to 30-40°C; Add the oil phase to the water phase, keep stirring to obtain colostrum, carry out ultra-high pressure nano-homogenization of the colostrum, homogenize the colostrum at a pressure of 1000 bar, homogenize 3 times, and place it to cool to obtain the salicylic acid nanoemulsion

The characterization of physical and chemical properties

Particle Size and PDI

Take an appropriate amount of salicylic acid nanoemulsion and dilute it with ultrapure water to make the average light intensity of the sample solution 200-300. The particle size and PDI were measured with Zetasizer/Nano-ZS90 nano potential particle size analyzer, the particle size measurement angle was 90°, and the test temperature was 25°C.

Drug Loading and Encapsulation Efficiency

The drug loading and encapsulation efficiency of salicylic acid nanoemulsion were determined by ultrafiltration centrifugation method [9]. Take 0.5 mL of the prepared salicylic acid nanoemulsion and put it in an ultrafiltration centrifuge tube (molecular weight cut-off of 3.5 kDa). Acid content, chromatographic conditions: Agilent ZORBAX SB-C18 (4.6 × 250 mm, 5 μm) chromatographic column; mobile phase: methanol: water: glacial acetic acid solution (volume ratio: 60:39:1); flow rate: 1.0 mL /min, column temperature: 35 °C, injection volume 10 μL; the detection wavelength of the UV detector is 254 nm; determination of the total amount of salicylic acid in the salicylic acid nanoemulsion: take the same amount of salicylic acid nanoemulsion above, add an appropriate amount of The demulsification of ethanol until the solution is clear and transparent, the volumetric flask is fixed to volume and then filtered with a filter membrane, and the total drug content of salicylic acid is detected by HPLC; the drug load is calculated according to the following formula:

$$\text{Drug loading (\%)} = \frac{Wt - Wf}{Ws} * 100\%$$

In the formula, Wt is the total amount of salicylic acid, Wf is the content of free salicylic acid, and Ws is the total amount of carrier.

The Stability investigation of salicylic acid nanoemulsion

The prepared salicylic acid nanoemulsion was stored at room temperature, low temperature (4 °C), high temperature (45 °C) and high and low temperature cycles (-20 °C, room temperature, 45 °C), and was taken out at 7d, 15d and 30d, respectively. Measure and investigate the change of particle size and PDI.

In vitro release behavior studies

The research on slow release behavior

The dialysis bag method was used to study the release behavior of nanocomposites to salicylic acid in vitro. According to the guidance of the Pharmacopoeia, an aqueous solution containing 20% propylene glycol meeting the sink conditions was selected as the release medium. The dialysis bag (molecular weight cut-off of 3500 Da) was boiled with ultrapure water for 5 min and soaked in 50% ethanol solution overnight before use. Take free salicylic acid, salicylic acid-407 hydrogel (commercially available salicylic acid inclusions), and 5 g of the dispersion of salicylic acid nanoemulsion composition and place it in a dialysis bag (dilute each sample with water to salicylic acid) The acid concentration is the same), both ends are sealed with dialysis bag clips, put into a blue bottle containing 80 mL of release medium, and the blue bottle is placed in a shaker, shaken at 37 ° C, 100 rpm for 12 h, respectively at 1, 2 , 4, 6, 8, 10, and 12 h sampled 1 mL, and then replenished with the same volume of release medium. The concentration of salicylic acid in the samples at different time points was determined by HPLC, and the cumulative release percentage was calculated. The experimental results are shown in Figure 3.

The research on Controlled Release Behavior

The dialysis bag method was used to study the in vitro release behavior of lipase on salicylic acid nanocomposites and the effect of liquid lipids on the controlled release behavior of nanocomposites. According to the guidance of the Pharmacopoeia, an aqueous solution containing 20% propylene glycol meeting the sink conditions was selected as the release medium. The dialysis bag (molecular weight cut-off of 3500 Da) was boiled with ultrapure water for 5 min and soaked in 50% ethanol solution overnight before use. Take the salicylic acid-407 hydrogel containing 0.1% pancreatic lipase (common salicylic acid inclusions in the market) and 5 g of the salicylic acid nanoemulsion composition dispersion and place them in a dialysis bag respectively, and use a dialysis bag at both ends. The clips were sealed, put into a blue bottle containing 80 mL of release medium, and the blue bottle was placed in a

shaker for 12 hours at 37°C and 100 rpm for 1, 2, 4, 6, 8, 10, and 12 hours, respectively. 1 mL was sampled and subsequently replenished with the same volume of release medium. The concentration of salicylic acid in the samples at different time points was determined by HPLC, and the cumulative release percentage was calculated. The experimental results are shown in Figure 4.

The detection of anti-bacterial activity

Plate dilution method was used to detect the bacteriostatic effect of free salicylic acid and salicylic acid nanoemulsion. Select the modified medium and add agar to prepare solid plate medium. Spread 2ml of bacterial liquid evenly on the medium plate, punch a hole with a diameter of 6.00mm on the plate with a sterile hole punch, add the diluted samples of free salicylic acid and salicylic acid nanoemulsion into the hole, and set at the same time. Blank control group containing only medium. Incubate in a constant temperature incubator for 72 hours, and measure the diameter of the inhibition zone with a Vernier caliper.

The safety test-Human Patch Test

Twenty-one subjects were selected, and the reactions of the subjects' skin were recorded. Put the filter paper of 2% salicylic acid nano-cream cream into the spot tester, and the normal hole is blank cream. Both the sample and the blank essence were attached to the curvaceous side of the subject's forearm, and they were evenly applied to the skin by light pressure with the palm of the hand for 24 hours. Observe the skin reaction again after 48 hours after removing the plaque [6].

Clinical research

The study is based on the selection of 33 volunteers, aged 18-35 years, 3 males and 30 females, with mild to moderate acne on the face, who used an anti-acne gel mask (containing 6.4% salicylic Acid nanoemulsion, namely 2% free salicylic acid), after staying for 30 minutes, rinse with warm water and use it continuously for 4 weeks, and then use its own random parallel control instrument detection and clinical evaluation method to evaluate the improvement of acne after using the product.

Instrumental measurement: The measurement indicators include skin oil, chromaticity L* value, VISIA-CR image acquisition and target red zone area analysis.

For skin oil measurement, Submeter (Derma Unit SSC3) produced by COURAGE+KHAZAKA (CK) company in Germany was used to measure the skin oil of the target site of the subjects during the follow-up, and each site was measured only once. The

oil-controlling efficacy of the product was evaluated by comparing the change in skin oil before and after the product was used and, on the test, and control sides.

Measurement of skin L* value: Minolta chromameter (CM 700d) produced by Japan Minolta Company was used to measure the skin L* value of the target site of the subjects during the follow-up, and a total of three measurements were taken, and the average value was taken. The skin-lightening efficacy of the product was evaluated by comparing the change in L* value before and after use of the product and between the test and control sides.

VISIA-CR facial image acquisition and target skin red area analysis, by comparing the changes in the skin red area before and after using the product, as well as the test side and the control side, to assist in evaluating the product's acne-removing effect; at the same time, it is used to assist researchers. Before and after comparison assessment.

Investigator evaluation, mainly by two professional dermatologists, evaluation indicators include: the number of non-inflammatory acne on the face (the number of whiteheads + the number of blackheads) and the number of inflammatory acne (the number of red papules + the number of pustules), The anti-acne efficacy of the product is evaluated by the degree of change of each index before and after the use of the product, the test side and the control side; for safety assessment, all adverse skin reactions such as skin erythema, stinging, itching, burning, etc., that occur during the study period should be recorded , and report serious adverse reactions in a timely manner.

Statistical analysis

Statistical processing was performed using SPSS 20.0 software. Data were compared mainly using the paired Student's t-test(two-tailed) or Tukey's test. Statistical significance was established at $p < 0.05$. Data are expressed as the mean±SD.

Results and Discussion

The characterization of physical and chemical properties

Particle Size and PDI

The high-concentration salicylic acid nanoemulsion prepared in this paper is a light yellow transparent and clear liquid. The particle size measured by the nano potential particle size analyzer is 21.4 nm, the PDI is 0.107, the drug loading is 30.0%, and the encapsulation

efficiency is 83.7 %. The particle size distribution of salicylic acid nanoemulsion is shown in Figure 2.

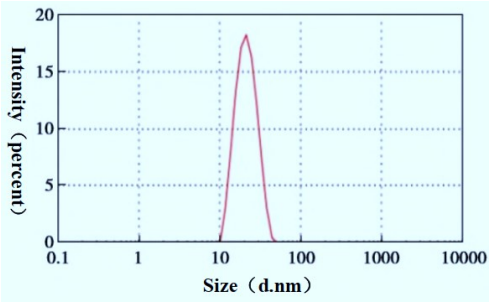

Fig. 2 Particle size distribution of salicylic acid nanoemulsion

The Stability investigation of salicylic acid nanoemulsion

The stability test results of salicylic acid nanoemulsion are shown in Table 1.

Table 1 Stability investigation results of salicylic acid nanoemulsion

time /d	RT		4 ℃		45℃	
	PDI	size/d. nm	PDI	size/d. nm	PDI	size/d. nm
7	0. 197	22. 4	0. 122	23. 2	0. 258	23. 6
15	0. 134	23. 1	0. 139	22. 7	0. 247	25. 2
30	0. 154	21. 1	0. 151	21. 6	0. 275	26. 1

It can be seen from Table 1 that the particle size and PDI of the salicylic acid nanoemulsion are basically stable at low temperature and normal temperature after being placed under the conditions of room temperature, low temperature (4 °C) and high temperature (45 °C) for 30 days. The nanoemulsion remained transparent, no delamination and demulsification were found, and the stability was good. After three high and low temperature cycles, the salicylic acid nanoemulsion has a particle size of 23.1 nm and a PDI of 0.214. The appearance remains transparent and the stability is good.

In vitro release behavior studies

The research on slow release behavior

The in vitro release behaviors of free salicylic acid, salicylic acid-407 gel (common salicylic acid inclusions in the market) and salicylic acid nanoemulsion are shown in Figure 3.

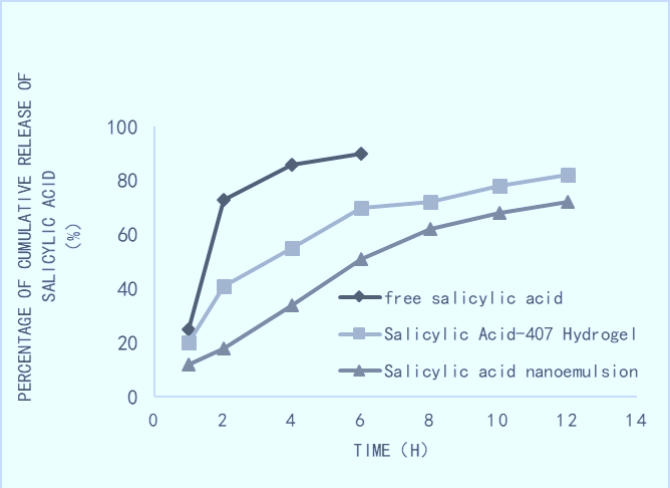

Fig. 3 Influence of the release behavior of salicylic acid nanoemulsion in vitro

It can be seen from Figure 3 that the free salicylic acid solution is rapidly released within 0 to 2 h. Compared with free salicylic acid, the release rate of salicylic acid nanoemulsion after being encapsulated by nanocarriers is significantly slower, indicating that salicylic acid exhibits a sustained release effect after nano-encapsulation, and salicylic acid nanocomposites can pass through cells. The interfacial lipids penetrate into the stratum corneum, and over time, the encapsulated high-concentration salicylic acid will diffuse out to achieve sustained release, thereby prolonging the action time of salicylic acid. Compared with free salicylic acid, the release time of salicylic acid nanoemulsion is extended by 5 times; as can be seen from Figure 3, compared with the commercially available salicylic acid-407 hydrogel, the sustained release effect of the salicylic acid nanoemulsion composition is more significant, Sustained release performance is better.

The research on Controlled Release Behavior

The in vitro release behavior of lipase to salicylic acid nanoemulsion is shown in Figure 4

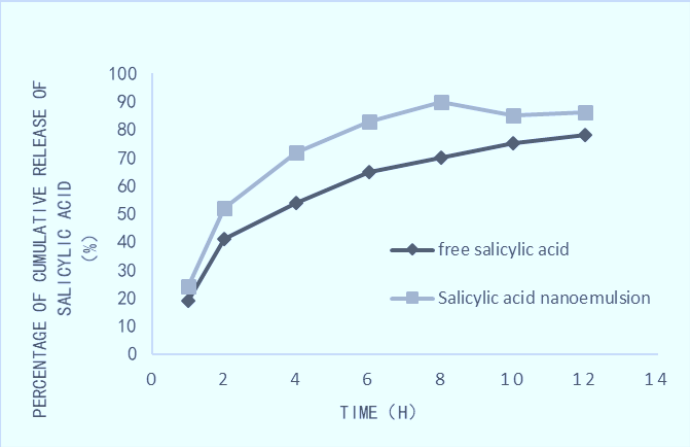

Fig. 4 The effect of lipase on the release behavior of salicylic acid nanoemulsion in vitro

It can be seen from Figure 4 that after adding lipase to the receiving medium to simulate an inflammatory environment, the release rate of salicylic acid from the salicylic acid nanocomposite is significantly accelerated. In the presence of lipase, the release of salicylic acid increased from 19% to 52% at 2 h, which significantly accelerated the release of salicylic acid. When the release time was 8 h, the release medium containing 0.1% of lipase accumulated salicylic acid. The release percentage was 91.1%, and the release was basically complete. In the sustained-release test shown in Figure 3, only 72% was released at 12h. It can be seen that the presence of lipase can increase the release efficiency of salicylic acid nanoemulsion. In a simulated inflammatory environment, the salicylic acid nanocarriers provided herein release salicylic acid faster than the commercial product salicylic acid-407 hydrogel. It shows that in the presence of lipase, the commercially available salicylic acid-407 hydrogel has no controlled release effect.

There are very active special enzymes in skin acne inflammation, such as lipase, lipase is a special kind of esterase, which can hydrolyze triacylglycerol into fatty acid, and its natural substrate is generally water-insoluble long-chain fatty acid acyl ester [7]. This study showed that in the presence of lipase, matrix materials such as caprylic acid/capric triglyceride and triglyceride (ethyl hexanoate) in salicylic acid nanoemulsion can be triggered to be hydrolyzed by lipase, thereby rapidly releasing water. Salic acid, which releases and enriches salicylic acid at high concentrations in the inflammatory site, enhancing its acne-removing effect.

The detection of anti-bacteria

The antibacterial diameter of different sample stock solutions against P. acnes was measured by agar plate diffusion method (punching method). The results are shown in Table 2.

Table 2 The effect of different salicylic acid samples on the inhibitory diameter of P. acnes

sample	Inhibition zone diameter／mm
blank	6．00±0．11
free salicylic acid	7．23±0．34**
Salicylic Acid Nanoemulsion	8．14±0．28**#

Note: compared with blank control, **P<0.01; compared with free salicylic acid, #P<0.05.

It can be seen from Table 2 that in the antibacterial activity experiment, after adding the samples of each group on the 6.00mm paper, the diameter of the bacteriostatic ring in the blank control group containing only the medium was 6.00mm, and there was no antibacterial phenomenon. After salicylic acid, the average size of the inhibition zone was 7.23 mm. After adding 5 μL of the same concentration of salicylic acid nanoemulsion, the average size of the inhibition zone was 8.14 mm. Compared with free salicylic acid, the diameter of the inhibition zone of salicylic acid nanoemulsion increased significantly, indicating that salicylic acid was encapsulated by nanocarriers, and the activity of inhibiting P. acnes was enhanced.

The safety test-Human Patch Test

The human patch test was conducted after compounding the salicylic acid nanoemulsion with the cream. The results showed that none of the 21 subjects had light erythema, erythema, edematous erythema, significant redness, infiltration or papules and associated papules. The salicylic acid nanoemulsion prepared in this experiment is not irritating to human skin.

Clinical research

33 volunteers highly recognized the acne-removing efficacy and mildness of the product (containing 6.4% salicylic acid nanoemulsion). During the 28-day test period, the test product did not have 1 case of skin adverse reactions of grade 1 or above, which proves that The product has good safety; in addition, the measurement results of the instrument show that the oil secretion is reduced by 10.78%, and there is a significant difference, which proves that

the product has oil control effect; the skin lightening value L* is increased by 4.32%, and there is a significant difference , which proves that the product has the effect of brightening the skin tone; VISIA-CA analyzes the red area of the skin target site, reducing 56.71%, and there is a significant difference, which proves that the product has the effect of lightening red acne marks; the researcher's clinical evaluation results show that this This product has a significant anti-acne effect. Among them, the number of non-inflammatory acne (the number of blackheads + the number of whiteheads) decreased by 30.04%, and the number of inflammatory acne (the number of red papules + the number of pustules) decreased by 20.71%. Comprehensive evaluation of the above test results shows that the product (containing 6.4% salicylic acid nanoemulsion) has good mildness, and also has good oil control, brightening skin tone, anti-acne and lightening red acne marks.

Conclusion

The salicylic acid nanoemulsion prepared in this study contains triglycerides. In inflammatory sites such as acne, lipase can degrade the triglyceride nanocarrier matrix material, trigger the decomposition of the nanocarriers to release salicylic acid, and realize the controlled release of target sites and enhance its Antibacterial efficacy. Compared with free salicylic acid, the nano-emulsion salicylic acid has lower skin irritation, higher safety and stronger bacteriostatic activity. Secondly, the anti-acne efficacy and safety of this nano-emulsion salicylic acid have been clinically proven. To sum up, nanoemulsion technology can realize targeted aggregation, controlled release, synergy and stimulation reduction of salicylic acid in the inflammatory site, and has a good application prospect in skin care cosmetics, especially anti-acne products.

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