IFSCC 2025 full paper (IFSCC2025-1122)

Mechanism study on sclareolide deposition from rinse-off products

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1. Introduction

In recent years, consumers are paying more and more attention to scalp health, not only because it is the foundation of healthy hair [1], but also because many people are suffering from scalp problems, such as oily scalp, dry scalp, sensitive scalp, or itchy scalp with dandruff. Scalp looks vastly different from other skin areas of the body, because it has large hair follicle with terminal hair and more sebaceous glands and sweat glands but is anatomically similar to the rest of our skin with extra density and made up of five different layers [2,3]. The sophisticated ingredients that have been traditionally used in skin care formulas are increasingly used in scalp care products, such as vitamins, hyaluronic acid, collagen, botanical extracts and so on. The actual efficacy of these ingredients on scalp has been proven in practice [4].

As primary function, shampoo was developed for cleaning the dirt and sebum from hair and scalp. Then 2 in 1 shampoo was developed for hair conditioning which gained significant importance as regular practice in our daily life. New developments for shampoo in the market are focusing on scalp care. The efficacy of scalp care shampoo not only depends on having more beneficial ingredients but also how to ensure the deposition of beneficial ingredients from shampoo onto human scalp during short contact time, instead of getting washed off with water. Scalp care ingredients deposition after shampooing is the key factor to success of one scalp care shampoo formula.

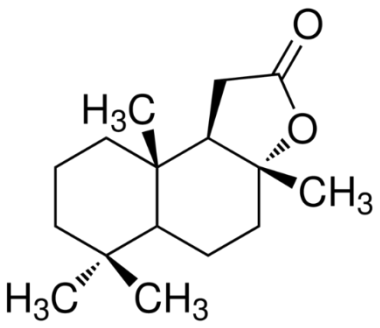

Figure 1. chemical structure of sclareolide.

Sclareolide (Figure 1) is naturally found in a variety of plants. It has unique aroma characteristics and unique flavor fixing ability, such as sweet taste, woody and herbal fragrance. It has extensive applications in cosmetics, spice essence, chemicals, medicine, food and other fields. With the increasing market awareness, its multiple values have been increasingly discovered. It has antibacterial, anti-inflammatory, antioxidant, anti-dandruff, anti-itching, soothing and nourishing effects on human skin [5]. The double-blind, placebo-controlled clinical study showed that scalp serum having sclareolide could prevent dandruff obviously and decrease scalp sensitivity, reinforce scalp barrier function [6].

The objective of this work is to develop *in vitro* test methods to measure sclareolide deposition from shampoo, on hair tresses and artificial skin (VITRO-SKIN® N-19) from IMS Inc. Artificial skin is a synthetic skin substrate that effectively mimics the surface properties of human skin [7] and has been applied as a test substrate for SPF measurement, UVA and UVB protection, emollient and spreading etc. [8-10], which has both optimized protein and lipid components and is designed to have topography, pH, critical surface tension and ionic strength. In this study, sclareolide deposited on hair or VITRO-SKIN was extracted with ethanol after washing with shampoo. The extraction was analyzed by HPLC to determine the content of sclareolide deposited on substrate.

2. Materials and Methods

Guar Hydroxypropyltrimonium Chloride (N-hance™ CCG45, Ashland); Diazolidinyl Urea (and) Iodopropynyl Butylcarbamate (and) Propylene Glycol (Liquid Germall™ Plus, Ashland); Sodium Laureth Sulfate (Texpon® N70, BASF); Sclareolide (Sclareance™, Ashland); Cocamidopropyl Betaine (TEGO® BETAIN F 50, EVONIK); Cocamide MEA (Comperlan® 100C ,BASF); Ethanol, Acetonitrile and Water (HPLC grade, Aladdin); Sodium Chloride, Sodium Hydroxide and Citric Acid from local suppliers, were used. Artificial Skin (VITRO-SKIN® N-19, IMS, Inc.) was hydrated following the manufacturer's recommendations, 18 hours at 90-95% relative humidity at room temperature. Bleached Asian hair was purchased from International Hair Importers & Products. Each hair tress was 3 cm wide, 3.5 g in weight and 25 cm in length. Clear shampoo prototypes evaluated for sclareolide deposition were listed in Table 1, having 0.1%, 0.2%, 0.3% or 0.5% sclareolide, total four samples for sclareolide deposition evaluation tests.

Table 1. clear shampoo prototypes for deposition evaluations

INCI name	Sample 1-4
Aqua	To 100%
Guar Hydroxypropyltrimounium Chloride	0.2%
Sodium Laureth Sulfate	12%
Cocamidopropyl Betaine	2%
Cocamide MEA	1.5%
Sclareolide	0.1%, 0.2%, 0.3% or 0.5%
Preservative	0.4%
Sodium Chloride	1.0%
Sodium Hydroxide	q.s.
Citric Acid	q.s.
pH 6.0	

2.1. HPLC method for sclareolide

Sclareolide analysis was performed using HPLC 1260 Infinity II from Agilent and a reverse phase C18 column (Agilent Poroshell 120 EC-C18, 4.6 × 150 mm, 4 µm). The mobile phase was acetonitrile and water at a ratio of 60:40 (v/v). The column temperature, mobile phase flow rate and injection volume were set at 35 ℃, 1 mL/min and 10 µL, respectively. The optimum detection wavelength was determined by UV absorption and set to 214 nm.

2.2. Extraction of sclareolide from artificial skin

0.5% sclareolide was solubilized in the surfactant solution, 100 mg of which was spread on each piece of artificial skin (3x3.7 cm). After drying, artificial skin was extracted by ethanol under sonication for various times (30 min, 60 min, 120 min or 180 min). The ethanol extraction was measured by HPLC and the extraction efficiency of sclareolide for various times was compared to getting the best time to extract sclareolide from artificial skin.

2.3. Determination of sclareolide on artificial skin during washing

Shampoo is generally used by applying it to wet hair and scalp, massaging the product into the hair and scalp, and then rinsing it out thoroughly. When applied to wet hair and scalp, the shampoo is diluted 5-10 times by the water on wet hair and scalp. In the current method, the procedure of washing artificial skin simulated the daily shampooing process. Firstly, the hydrated artificial skin was treated with 20% shampoo dilution, which is similar with applying shampoo to wet hair and scalp, then after dry, extract the artificial skin with ethanol under sonication to get the sclareolide amount on artificial skin before rinsed by water, the pre-rinse amount. Another piece of artificial skin after treated with 20% shampoo dilution was further rinsed with water under agitation at 150 rpm for 5 minutes (Shaker, JULABO SW20) to simulate the process of rinsing during daily shampooing. After drying, this piece of artificial skin was also extracted by ethanol under sonication to get the sclareolide amount after rinsing, the post-rinse amount.

2.4. Determination of sclareolide on hair tress after washing

Bleached Asian hair was supplied as 25 cm tresses from International Hair Importers. Tresses were washed three times with a 12% (w/w) sodium laureth sulfate solution followed by extensive water rinsing to remove any surface contamination. Previously cleaned tress was wetted under running water at 35 ℃ with a controlled flow rate of 4 L/min. The excess water was removed by running a thumb and forefinger along the length of the swatch. Place hair tress into large weigh boat. Apply 0.6 g shampoo prototype (with 0.2% sclareolide inside) along the length of the tress and massage shampoo into tress for 30 seconds. Then the tress was rinsed under the running water for 30 seconds. Air dry and equilibrate at 25℃ with a relative humidity of 55% overnight. Then the tress was cut into short fiber snippets and extracted in ethanol under sonication for 3 hours. The ethanol extraction was analyzed by HPLC to get the sclareolide amount.

2.5. Data analysis

Data was plotted using Microsoft Excel. The retention rate of sclareolide on artificial skin was calculated by Equation 1 and retention rate on human hair was calculated by Equation 2.

$$Retention\ rate - artifical\ skin = \frac{sclareolide\ amount\ on\ artificial\ skin\ (post - rinse)}{sclareolide\ amount\ on\ artificial\ skin\ (pre - rinse)} \times 100\% \quad \text{Equation 1}$$

$$Retention\ rate - hair = \frac{sclareolide\ amount\ on\ hair\ tress\ (after\ washing)}{sclareolide\ amount\ (in\ shampoo)\ applied\ on\ hair\ tress} \times 100\% \quad \text{Equation 2}$$

3. Results

3.1. Extraction efficiency of sclareolide from artificial skin

To get accurate extraction efficiency, it's important to ensure that the ingredient deposited on artificial skin is extracted completely. Sclareolide has extremely low water solubility but could be easily dissolved by ethanol. Artificial skin with known amount of sclareolide was extracted by ethanol under sonication for various times, 30 minutes to 3 hours. Figure 2 shows the extraction efficiency after different sonication times. After sonication 30 min – 60 min, less than 80% sclareolide on the artificial skin was extracted by ethanol. Almost 90% of the sclareolide was extracted after 2 hours' sonication. Complete extraction could be obtained after 3 hours' sonication, which was chosen as the extraction method for the following deposition analysis.

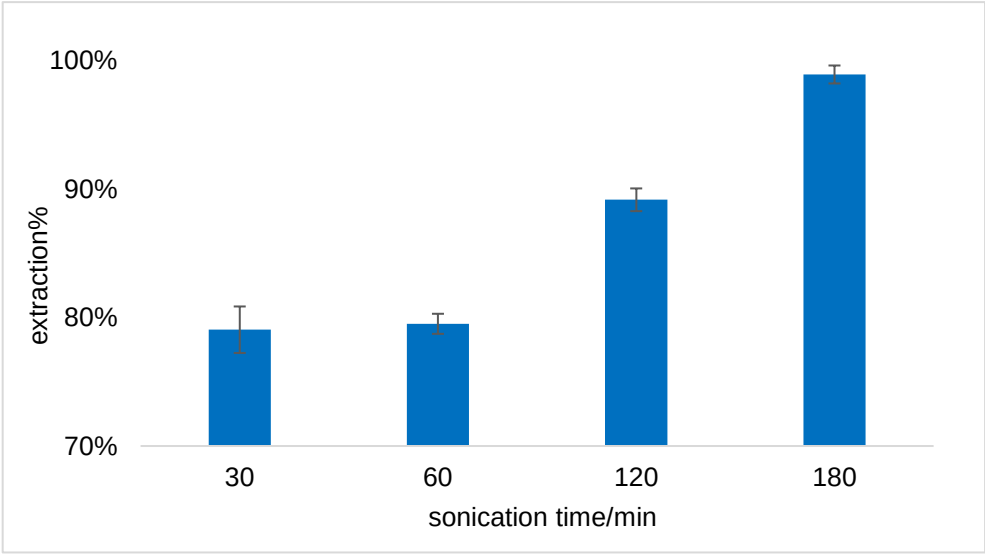

Figure 2. extraction efficiency of sclareolide from artificial skin

3.2. Retention of sclareolide on artificial skin

Clear shampoo prototypes formulated with different dosages of sclareolide were evaluated for deposition on artificial skin, and the results were shown in Figure 3. The deposited amount increases with sclareolide level in shampoo. After water rinsing, the retention rate of

sclareolide on artificial skin was calculated according to Equation 1, the results obtained varied from 84.8% (0.1% sclareolide in shampoo prototype) to 71.0% (0.5% sclareolide in shampoo prototype), averagely 73.5% sclareolide was kept on the artificial skin after rinsing with water for all the shampoo prototypes evaluated.

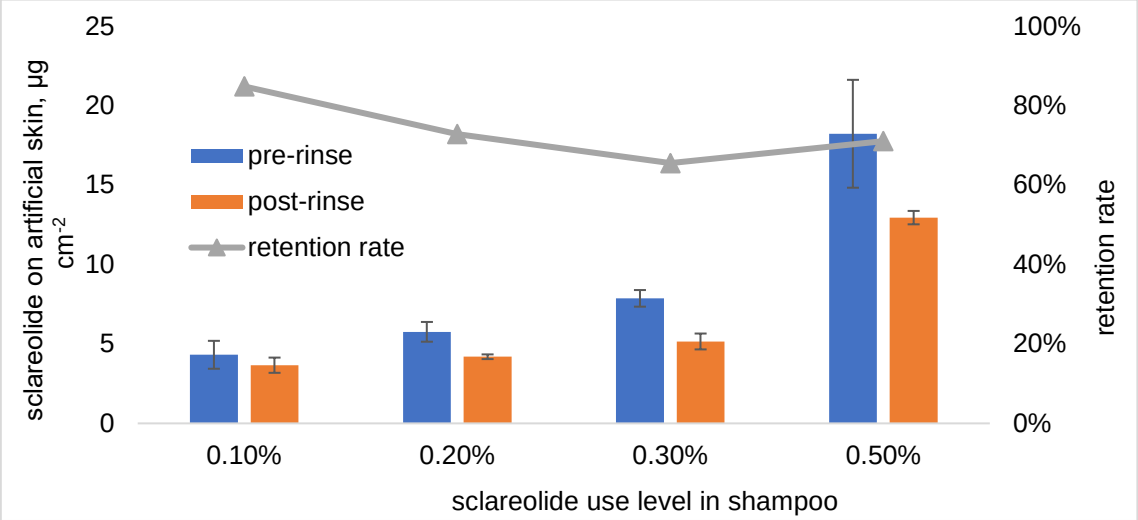

Figure 3. deposition of sclareolide onto artificial skin from shampoo.

3.3 Retention of sclareolide on human hair

Deposition of sclareolide through the clear shampoo prototype having 0.2% sclareolide was evaluated, as shown in Table 2. After regular shampooing, 37.1% of sclareolide was deposited onto hair tress after rinsing.

Table 2. deposition of sclareolide on hair tress after washing with shampoo prototype having 0.2% sclareolide

Parallel sample	Deposition of sclareolide on hair after washing, µg/g	Retention rate - hair	Average retention rate - hair
Hair tress 1	163.0	35.7%	37.1%
Hair tress 2	173.1	38.5%	

4. Discussion

Extracting ingredients from scalp is costly and labor consuming, and the extraction efficiency is relatively low in In vivo clinical study, while In vitro method using artificial skin to replace human skin and eliminate the need for human volunteers is faster and cheaper. Chen et al has evaluated deposition of climbazole on artificial skin and human scalp simultaneously and got consistent results [13]. Although the amount of sclareolide deposited on artificial skin may be different from the amount deposited on scalp during daily shampooing, the retention rate of sclareolide from calculating the sclareolide amount on artificial skin before and after rinsing, could exactly evaluate the ability of sclareolide resisted to rinsed off by water during shampooing [11]. The retention rate of piroctone olamine and salicylic acid were also evaluated. Piroctone olamine has a retention rate of around 80% while salicylic acid is only about 10% [12]. These results also explained why piroctone olamine is widely used in anti-dandruff shampoo for it has a great retention rate on scalp through shampooing and benefit scalp health in

daily life. Sclareolide has a retention rate of more than 70%, comparable with piroctone olamine, which shows its potential application in scalp care shampoo to deliver its benefits such as anti-dandruff, anti-itchy, oil control and soothing to scalp.

The evaluation of active depositions on hair is more convenient due to the usage of hair tresses. There are many studies related to deposition of beneficial ingredients on hair tresses, such as silicones, vegetable oils or cationic polymers to improve hair conditioning performance, hair shine and so on [14,15]. As the data shown, the retention rate of sclareolide is also high, about 37.1%, due to the hydrophobic nature.

5. Conclusion

No matter how many benefits the active ingredients could provide to scalp, effective delivery through shampooing is especially important for the success of one scalp care shampoo formula. In the present paper, the authors provide a method to evaluate the ability of active ingredients being resisted to be rinsed off by water during shampooing. It could be a valuable tool to screen scalp care ingredients that fit well in shampoo.

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