Pre-stability and functional tests of a leave-on hair emulsion developed with Aloe vera extract

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Abstract
Leave-on creams are cationic emulsions with lower active content than conditioners. They are a complement to the comb and contain agents that moisturize hair and return to it the effect of conditioners, facilitating hairstyle. The part of the plant which is used is the mucilage obtained from the leaves, as it has emollient and moisturizing properties. The purpose of this paper was to develop a leave-on emulsion containing Aloe vera extract, obtained by two different extraction methods, and one extract acquired commercially in order to evaluate its pre-stability and functionality in hair strands. The formulation of the leave-on emulsion was developed with Aloe vera extracts. These extracts were prepared with plant material (PM) plus 70% alcohol (Sample A) and PM, cereal alcohol and glycerin (Sample B). The pre-stability test was performed according to ANVISA Guide. We analysed tests on human hair strands to assess combability (after shampooing them with neutral shampoo, the standardized amount of the sample was spread on strands, which were combed to observe the slip point after 10 combing strokes) and appearance after drying (the strands were left hanging at 23°C and 33% humidity for 24 hours) and softness to the touch and volume (photographed data). In the pre-stability test, the samples were stable in color, odor, appearance and centrifugation test under all conditions, except in samples that were exposed to direct sunlight, when they were modified in color and odor. The pH remained stable in all samples and under all conditions. The spreadability went through which were not relevant to the formulation tested. For the tests on strands of hair, one of the samples was the base formulation without adding the plant extract, for comparison purposes. The average results of the strands test of all samples were 9.87 cm ± 0.6875 of sliding length, which were much greater when compared to the untreated sample that slid only 5 cm. After drying, the strands looked softer. There was softness to the touch, volume reduction and alignment of the hair strands, when compared to the untreated strands. We concluded that the base emulsion formulation, by its own, showed improvement in the hair strands for combing and appearance after drying. The formulations containing the extract showed no improvement in the tests on strands, when compared with the base formulation. The formulations developed have favorable results in the physicochemical pre-stability tests.

Keywords: Hair, Aloe vera, Leave on emulsion.

Introduction
Brazil is among the major world consumers of cosmetics. Hair is the number one factor when it comes to women's beauty care. They want to change color and style and, at the same time, they care about hair moisturizing and reconstructing.1

Biologically, hair works as a protection for the human body to stop scalp from being exposed to sun. Hair is often used as a symbol of femininity and beauty.2 A woman with damaged hair may have her self-esteem greatly affected, which makes her seek different products that appeal to hair moisturizing.3

Conditioners are cationic emulsions that improve hair appearance and manageability, providing volume, shine and softness. They make hair stronger, soften the cuticles and reduce friction during humid hair brushing, allowing the comb to slide through the hair, and reducing future mechanical damage. Washing hair on a daily basis contributes to dryness of strands. Hair conditioner is a great aid with recovering hair shine and volume.4

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Combing emulsions were created as conditioners with a lesser concentration of active agents. Today they are seen as complements to the comb, as moisturizing agents which give hair the same effect as conditioners. They also facilitate hairstyle, even hours after washing. Such cosmetic mechanisms tend to improve hair texture and softness. Its purpose is to enhance hair appearance after it is cleaned.5

The history of Aloe vera is ancient, and it is in the literature of several cultures. Its name probably derives from the Arabic word alloeh, which means a bitter and shiny substance. The first register of Aloe vera use was on a clay surface in Mesopotamia dated 2100 B.C. Known in ancient Egypt as “the plant of immortality”, it may have been used by Cleopatra for hair and skin care.6

In Brazil Aloe vera is popularly known as “babosa folha miúda”, “babosa folha grande” and “erva babosa”. The parts of the plant which are used are the dried latex of leaves and the mucilage obtained from them, known as “babosa gel”. It is used a lot in cosmetics for its emollient and moisturizing properties. For its high moisturizing properties, glycerin is added to Aloe vera to enhance its potential in cosmetic formulations.7 It is constituted mainly of water – about 96%-98%, and the rest of its composition includes complex molecules of carbohydrates, enzymes, proteins, amino acids, vitamins, minerals, among others.8

The confirmation of the efficacy of cosmetics is very important for consumers, who want to see the appealing results to which they were drawn, when they purchased the product. Thus, new methodologies to evaluate such products have been continuously developed, with focus on scientific confirmation of the real benefits proposed. Besides testing products, it is extremely important to assess their effects on hair, in order to observe performance.5

The test on strands of hair shows how the product acts in human hair, and predicts the result that can be expected. A lot of factors affect the way each type of hair reacts to the product. After the test, the product is directed to applicability.4

In the phase of product development, along with the test on strands of hair, quality control trials should be conducted, in order to evaluate the physicochemical and microbiological characteristics of the product.5,10

The product’s stability is the period of time during which the product maintained – within the specified limits and throughout the storage and use period – the same properties and characteristics it had during manipulation. The pre-stability test is carried out as a preliminary test for the formulation continuance or improvement, depending on the results. There is concern with stability since the beginning, in ingredient selection.11

Based on the already known moisturizing characteristics of the Aloe vera plant, and with the increasing market for hair products and the appeal of natural products, it is interesting to research and test different products with Aloe vera extracts, and then define the product for the best hair result.

The purpose of this paper was to develop a leave-on emulsion containing Aloe vera extract, obtained by two different extraction methods, and one extract acquired commercially. We also aimed at evaluating the formulation pre-stability and activity in hair strands.

Materials and Methods

The Aloe vera plant was collected at Farmácia Viva of the Pharmacy Department at Centro Universitário Cesmac, located in the town of Marechal Deodoro – Alagoas, in August 2018. The leaves were cut near the base of the plant.

The Aloe vera extracts were prepared on the same day they were collected. After that, the extracts were incorporated into the base formulation of the leave-on emulsion.

After the Aloe vera plant was collected, the leaves were opened for extracting the gel. The extracts were prepared by maceration; 70% alcohol was added to extract A with 300 g Aloe vera mucilage. To extract B with 300 Aloe vera, 270 mL glycerin and 30 mL cereal alcohol were added. They were left in room temperature for 8 days, in hermetically sealed containers, protected from light. After maceration, the extracts were filtered and stored in PET amber containers, in room temperature, for addition to formulation later.

The raw materials for the formulation of the leave-on emulsion were acquired directly at a prescription pharmacy and donated by qualified suppliers. The emulsion (Table 1) was prepared according to Good Practices of Manipulation.11

The sample (leave-on base formulation) was divided into 4 equal parts (250 g). The parts were named A, B, C, D. Emulsion A had no extract; 5% extract A was added to emulsion B; 5% extract B was added to emulsion C. Emulsion D was incorporated into 5% commercial extract acquired in a prescription pharmacy. All samples were stored in room temperature, in PET containers.

Physicochemical Analyses

The following tests were conducted: organoleptic characteristics – color, odor and aspect; pH direct reading

| Ingredients (INCI name) | %   | Function        |
|------------------------|-----|-----------------|
| Behetrimonium chloride | 4.00| Conditioning agent |
| Phenoxytoanol          | 1.00| Preservant      |
| Butylated hydroxytoluene (BHT) | 0.05 | Antioxidant |
| Ceteareth              | 5.00| Emulsifer       |
| Coconut oil (Cocos nucifera) | 1.00 | Emollient |
| Disodium EDTA          | 0.20| Chelating agent |
| Citric acid            | 1.00| Acidifier       |
| Propylene glycol       | 3.00| Wetting agent   |
| Aqua                   | 100.00| Vehicle         |
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at 10% solution; and centrifugation 300 rpm for 30 minutes.\textsuperscript{10,12}

Spreadability is defined as the expansion of a semi-solid formulation on a surface, after a certain period of time.\textsuperscript{13}

Pre-stability Test
The purpose of the pre-stability test\textsuperscript{14} is to analyze the period of time during which the product maintained – throughout the storage and use period – the same properties and characteristics it had during manipulation.

Twenty grams of each sample were weighed and stored in 4 different containers, totaling 16 containers. The storage conditions were: refrigerator (5ºC), heated chamber (45ºC), exposure to sunlight, and room temperature (25ºC).

After 15 days, the aforementioned physicochemical analyses were repeated.

Tests on Strands of Hair
The tests were adapted from protocols of companies that carry out security and efficiency tests in cosmetics. The hair strands were weighed (approximately 16 g each) and divided into 5 equal parts.\textsuperscript{15}

Later all strands were shampooed with a standardized amount of 2 mL neutral shampoo for 2 minutes and rinsed for 1 minute. For removing excess water, the strands were dried with 2 paper towels.

They were named as Control (shampooed only), and samples A (leave on base; B: leave on with extract A; C: leave on with extract B; D:leave on with Aloe vera commercial extract); 0.50 g of base formulation was added to samples A, B, C and D.

Combability Test
After the samples were applied to hair, a fine-tooth comb was slid through hair length in order to observe the slip point. The distance covered by the comb was measured in centimeters, after 10 combing strokes. The procedure was conducted by the same person, to eliminate interferences.

Appearance Test on Hair Strands
After the combability test, the strands were detangled and placed in controlled temperature and relative humidity, 23ºC and 33%, respectively, for 24 hours. After that the appearance of hair was analyzed. The results were photographed.

Results and Discussion
The samples, 24 hours after manipulation and the pre-stability test were analyzed in association and presented the following results: as shown in Table 2 for sample A (base emulsion); in Table 3 for sample B (emulsion with extract A), Table 4 for sample C (emulsion with extract B); and Table 5 for sample D (emulsion with commercial extract). The white color in the base formulation was maintained in the three samples with the added extract. When the pre-stability test was applied, the color was slightly changed – a little yellowish in all samples exposed to direct sunlight.

### Table 2. Physicochemical analyses results of sample A (base emulsion)

|         | T0         | 5°C | 25°C | 45°C | Sunlight |
|---------|------------|-----|------|------|----------|
| Color (NL) | White | NC  | NC   | NC   | Modified |
| Color (AL) | White | NC  | NC   | NC   | Modified |
| Aspect | Homo | Homo | Homo | Homo | Homo |
| Smell | Essence characteristic | NC | NC | NC | Modified |
| pH | 5.5 | 5.5 | 5.7 | 5.5 | 5.4 |
| Ei | 17.35 | 12.44 | 14.86 | 22.90 | 17.72 |
| Centrifugation | NC | NC | NC  | Changed | NC |

Color: Natural Light (NL); Artificial Light (AL); No Change (NC); Homo (Homogeneous); Hetero (Heterogeneous).

### Table 3. Physicochemical analyzes results of sample B (emulsion with extract A)

|         | T0         | 5°C | 25°C | 45°C | Sunlight |
|---------|------------|-----|------|------|----------|
| Color (NL) | White | NC  | NC   | NC   | Modified |
| Color (AL) | White | NC  | NC   | NC   | Modified |
| Aspect | Homo | Homo | Homo | Homo | Homo |
| Smell | Essence characteristic | NC | NC | NC | Modified |
| pH | 5.5 | 5.5 | 5.6 | 5.5 | 5.5 |
| Ei | 18.47 | 12.13 | 25.07 | 22.73 | 22.90 |
| Centrifugation | NC | NC | NC  | Changed | NC |

Color: Natural Light (NL); Artificial Light (AL); No Change (NC); Homo (Homogeneous); Hetero (Heterogeneous).
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There was a possible problem with the antioxidant used in the formulation (BHT). A supplementary antioxidant agent could have been added to the formulation, such as vitamin E.

The odor is characteristic of the essence in the base formulation, and there was no alteration when the extracts were added. In the pre-stability test, there was alteration in all samples under direct sunlight, specially sample A (only the base formulation), where the odor was intensely modified, which is typical of ammonia. When in the heated chamber (45°C), sample B was slightly altered. The antioxidant agent did not support the stress, possibly. We confirmed that the antioxidant system should be reviewed for this formulation.

As for appearance, the samples presented homogeneous aspect in their base formulation, and after the extracts were added. After the pre-stability test, the samples showed some alterations, and their spreadability was increased after exposure to heat and direct light. The formulation had physical problems when it was subjected to some kind of stress, and, therefore, its spreadability was also altered.

The centrifugation test is used to assess the physical stability of emulsions. Hence, when subjected to centrifugation, the components are likely to be separated, in case the emulsion does not have good stability. It is one of the mandatory tests for emulsions, after preparation. In centrifugation there was no phase separation in the manipulated samples. In the pre-stability test, there was no separation in the samples kept in refrigerator, nor in the ones exposed to direct light and in room temperature. The phases were separated (coalescence) in the samples kept in the heated chamber (45°C), which demonstrates problems in their physicochemical stability.

The stability of formulations is linked to product safety because toxic and irritating by-products can be generated.

In the results obtained from the combability test, as shown in Table 6, there was no significant difference in how much the comb slid in all strands containing Aloe vera extract, when compared to the strands to which only the leave-on base formulation was applied. When compared to the sample that was washed only with shampoo, there was considerable improvement. Sample "D" showed 100% improvement. The incorporation of Aloe vera extract

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**Table 4. Physicochemical analyses results of sample C (emulsion with extract B)**

| Time | 5°C | 25°C | 45°C | Sunlight |
|------|-----|------|------|----------|
| Color (NL) | White | NC | NC | NC | Modified |
| Color (AL) | White | NC | NC | NC | Modified |
| Aspect | Homo | Homo | Homo | Hetero | Homo |
| Smell | Essence characteristic | NC | Modified | NC | Modified |
| pH | 5.5 | 5.5 | 5.6 | 5.5 | 5.5 |
| Ei | 16.61 | 13.01 | 18.10 | 27.34 | 21.24 |
| Centrifugation | NC | NC | NC | Changed | NC |

**Table 5. Physicochemical analyses results of sample D (emulsion with commercial extract)**

| Time | 5°C | 25°C | 45°C | Sunlight |
|------|-----|------|------|----------|
| Color (NL) | White | NC | NC | NC | Modified |
| Color (AL) | White | NC | NC | NC | Modified |
| Aspect | Homo | Homo | Homo | Hetero | Homo |
| Smell | Essence characteristic | NC | Modified | NC | Modified |
| pH | 5.5 | 5.5 | 5.6 | 5.5 | 5.5 |
| Ei | 16.62 | 17.35 | 21.40 | 23.76 | 21.40 |
| Centrifugation | NC | NC | NC | Changed | NC |

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Color: Natural Light (NL); Artificial Light (AL); No Change (NC); Homo (Homogeneous); Hetero (Heterogeneous).
contributed very little to better hair combability.

After the strands were washed and the leave-on emulsion samples were applied, they were left for 24 hours in 23°C temperature and 33% relative humidity. After such period, the visual analysis of the strands was conducted, and photographs were taken (Figures 1 and 2).

The samples showed improvement in their visual aspect and softness to the touch, when compared to the strands washed only with shampoo. Nevertheless, there was no significant difference among the strands with base formulation and the strands with the added extracts. Therefore, a new study on extracts would be relevant, with a view to improving this aspect.

Conclusion
The results of the physicochemical tests just after the manipulation met the standards which were set, but on the physicochemical results in the pre-stability tests, all the samples that were exposed to direct sunlight demonstrated changes on the color and smell, and those exposed 15 days to 45°C presented coalescence (heterogeneous aspect and phases separation on the centrifugation test).

According to the data presented on the functional tests (strands of hair and combability), it was concluded that the base emulsion formulation showed relevant improvement when added to hair strands, when compared to the sample control. The extracts added on the base emulsion formulation did not show influence when the tests on hair strands were conducted, suggesting interference of the solvent polarity on the hair moisturizing action of Aloe vera already reported in the literature.

Therefore, the need for further studies is evident, so is the enhancement of the formulation used, either for altering it for improving the combability test or for the stability test, as such tests are important requirements.

Competing Interests
None

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| Sample | Maximum Slip Point (cm) |
|--------|-------------------------|
| Control | 5.5                     |
| A       | 10.0                    |
| B       | 10.0                    |
| C       | 8.5                     |
| D       | 11.0                    |

Table 6. Combing test shows the maximum slip point of the comb in the strands in centimeters.

Figure 1. Hair strands after shampooing and applying the samples. Control: shampoo; A: leave on base; B: leave on with extract A; C: leave on with extract B; D: leave on with Aloe vera commercial extract.

Figure 2. Hair damaged strands, with 0.50 g of each sample stored at 22°C and 33% relative humidity for 24 hours. Control: shampoo; A: leave on base; B: leave on with extract A; C: leave on with extract B; D: leave on with Aloe vera commercial extract.
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