High Performance Conditioning Shampoo with Hyaluronic Acid and Sustainable Surfactants

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Abstract: Recently, consumers have become invested in more natural and sustainable ingredients contained in personal care products. Unfortunately, cationic surfactants are still heavily relied on as primary conditioning agents in products such as conditioning shampoos because of their ability to cling well to the negatively charged surface of hair follicles. Additionally, sulfates are utilized as cleansing agents because they are highly effective and low cost. The objective of this study is to find a more sustainable formulation for a conditioning shampoo without compromising the desired wet combing, rheological, and surface activity properties. The systems which were investigated contained hyaluronic acid (HA) at a variety of molecular weights and concentrations, in combination with a surfactant, either acidic sophorolipid (ASL) or alkyl polyglucoside (APG), and varying the presence of sodium chloride. A Dia-stron was utilized to test the wet combing force, a rheometer recorded the viscosity at various shear rates, and a tensiometer measured the surface tension of the samples before a visual foaming study was conducted. Molecular weight and concentration seemed to have a large impact on wet combing force, as well as rheology, with the largest molecular weight and concentration producing the lowest friction coefficient and desired rheological profile. The addition of a surfactant significantly aids in the reduction in surface tension and increased foamability. Therefore, the optimal system to achieve the largest reduction in wet combing force, large viscosity with shear-thinning behavior, and relatively low surface tension with decent foaming is composed of 1% HA at 800 kDa, 10% ASL and 1% NaCl. This system shows a viable sulfate-free and silicone-free option that can achieve both conditioning and cleansing.

Keywords: hyaluronic acid; biosurfactants; biobased surfactant; lubrication; rheology; surface tension; foaming

1. Introduction

Consumers have continued to demand products that are used on their hair and skin be more sustainable and come from more natural sources. However, synthetic cationic surfactants are still heavily used in conditioners because of their ability to adhere well to the negative hair follicles and sulfates continue to be the surfactant of choice for shampoos because of their impeccable cleansing and foaming properties. To develop a more sustainable formulation for conditioning shampoos, the objective is to use more natural alternatives for the conditioning agent and surfactant choice. As previously mentioned, typical conditioning agents include cationic surfactants and silicone oil; however, cationic surfactants normally have poor lathering and cleansing abilities and do not pair well with anionic surfactants. Although Benhur, Diaz and Amin demonstrate that silicone oil is effective in conditioning and wet lubrication properties, the goal is to provide more differentiation in the industry in regard to the conditioning system while also moving in a more sustainable direction [1]. One conditioning agent heavily used in the skin care industry is hyaluronic acid (HA) because it is not only biocompatible, but it also has benefits such as hydration, skin rejuvenation to prevent signs of aging and skin plumping to minimize wrinkles [2]. HA is becoming a notable ingredient used in facial fillers due to these precise
benefits. The skin care industry is not the only industry that utilizes hyaluronic acid as its uses depend on its molecular weight. For example, Snetkov et al. completed an in-depth review on the structure and activity of HA in addition to explaining the possible biological uses for HA, such as its ability to act as an inducer of heat shock proteins or aid in the development of embryonic cells [3]. While it can accomplish both functions, they can only be achieved at a specific molecular weight [3]. This demonstrates how the molecular weight of hyaluronic acid can change the physical and physicochemical properties of the molecule. Additionally, Snetkov et al. review a few derivatives of hyaluronic acid, their properties, and their applications such as hyaluronic acid sodium salt (NaHA) [3]. NaHA does not differ significantly from HA, mainly in terms of their structures because HA is a linear heteropolysaccharide and NaHA forms a twin helix structure when in solution [3]. Overall, the properties, and therefore the applications, of both HA and NaHA are quite similar. While HA has been shown to have desirable lubricating and viscosity building properties, HA alone cannot serve as a basis for a conditioning shampoo because cleansing still needs to be achieved. Therefore, hyaluronic acid will be further explored both independently and in combination with surfactants which have a more sustainable advantage for a possible conditioning shampoo system.

The second formulation adjustment is based on selecting a more sustainable surfactant choice when compared with the usual sulfates. A more natural surfactant is becoming more desirable over sulfates because sulfates have the ability to irritate the skin or hair. However, these anionic surfactants are the preferred benchmark surfactants because they have exceptional cleansing parameters such as low surface tension, high foamability and the ability to form wormlike micelles in dual-surfactant systems, resulting in a gel-like viscosity. Amin et al. discusses these parameters, including the physical and chemical properties of two sulfates, sodium lauryl ether sulfate (SLES) and sodium lauryl sulfate (SLS) [4]. These surfactants are typically compared with more natural alternatives to determine whether the properties exhibited are competitive enough to make the switch to the natural approach without losing product performance properties. Two viable options to replace sulfates include the use of biosurfactants or biobased surfactants. Biosurfactants are synthesized from yeast, bacteria and other microorganisms [5–8]. Drakontis and Amin provide an overview different biosurfactant applications and various performance properties [6]. Studies have been conducted on the optimization of surface activity and rheological properties of various biosurfactants [6–10]. Biobased surfactants, on the other hand, are manufactured from biobased sources such as starches or sugars. There is very limited optimization and application research on biobased surfactants to our knowledge. The two different surfactants that will be analyzed in this experiment are the acidic form of sophorolipid (ASL) and alkyl polyglycoside (APG), which are a biosurfactant and a biobased surfactant, respectively. These surfactants have been chosen because since they are both sustainable alternatives, this study can look into the potential differences in results due to the use of a biosurfactant compared against a biobased surfactant. Additionally, these two surfactants have been commercially scaled up and are sold at a reasonable cost. This study will optimize the addition of hyaluronic acid to each of these two natural surfactants in an attempt to find an efficient cleansing shampoo with hydrating properties.

The specific physico-chemical properties that are going to be tested to determine the product performance of the samples are wet combing force, rheology and surface tension with some foaming. First, focusing on wet combing force, Newman, Cohen and Hayes describe the method to quantitate and compare the effect of different cosmetic products on the required force to comb through a hair tress [11]. This technique is then used to calculate the reduction in the required force to determine the lubrication efficacy of a product. As previously mentioned, hyaluronic acid can be used in a variety of industries, for example, as an effective lubricating agent in the medical field. Kawai et al. study the effect of hyaluronic acid lubricating a joint [12]. The experiment concluded that hyaluronic acid decreases the friction coefficient, which proves its ability to increase lubrication between joints. In the personal care industry, Babgi et al. observed the effects of applying hyaluronic acid to
a healthy root surface as a conditioning agent [13]. In this study, the roots treated with HA resulted in higher cell viability compared to other samples. In a separate experiment conducted by Mueller et al., HA was able to increase the surface roughness of the root, which resulted in enhanced cell attachment and spreading onto the surface [14]. To our knowledge, there has not yet been research conducted on the conditioning effects of APG or acidic sophorolipid. The desired outcome is that hyaluronic acid, in combination with APG or ASL, will increase the reduction in wet combing force, therefore showing a decrease in the friction coefficient.

Rheology is critical for wet combing performance because it has the ability to influence lubrication properties. It is typically studied for personal care products because rheology correlates with various product attributes such as storage, removal from the container, and the rubbing into the hair or skin. Falcone, Palmeri and Berg conduct an experiment focused on studying the rheological properties of hyaluronic acid at different molecular weights and concentrations [15]. They found that at higher molecular weights, HA acts as an entangled polymer in solution that significantly differs from lower-molecular-weight HA. Additionally, the viscosity significantly depends on the concentration and molecular weight of HA. For both properties, viscosity will increase as molecular weight increases or as concentration increases. Pisarcik, Bakos and Ceppan address the typical flow curve of hyaluronic acid, expressing how solutions containing HA are non-Newtonian with shear-thinning behavior [16]. After analyzing the importance of each parameter, the objective is to develop a solution combining hyaluronic acid and alkyl polyglucoside or acidic sophorolipid to minimize surface tension, increasing foamability and the reduction in the wet combing force as a shear-thinning, non-Newtonian sample. Pingali, Benhur and Amin study the rheological response of sophorolipid in combination with chitosan [17]. They were able to show that the viscosity of sophorolipid alone is quite low and therefore demonstrates Newtonian behavior. When chitosan is added to the system, the repulsive interactions allow for entanglements to occur and cause the viscosity to increase. The rheological properties of APG were observed by Moore et al. and it was discovered that both longer APG chains and higher concentrations were able to produce higher-viscosity samples [18]. It is also important to note, even if original samples do not demonstrate high viscosities, the ability to build viscosity in systems is a desirable property. Rheology has the potential to influence the texture of the sample as well as the lubrication properties of the systems.

Once the properties correlating to the conditioning aspect of the samples have been optimized, the next step is to optimize surface tension. Surface tension is a quantitative measurement demonstrating a solution’s ability to remove dirt, which directly correlates with cleansing efficacy [10]. This property is critical for products such as shampoo and body washes because their primary function is to clean the skin or hair. While this parameter is not crucial for the conditioning aspect of this study, it can aid in further optimization. Krause, Bellomo and Colby observed the surface tension of HA solutions and they discovered that solutions with higher concentrations of at least 0.35% have larger surface tension due to the significant amount of molecules at the air–water interface, hindering rapid absorption [19]. However, when concentrations were below this amount, hyaluronic acid had the ability to lower surface tension. This is confirmed with another study conducted by Knepper et al., who observed the surface tension after varying both molecular weight and concentration of HA solutions [20]. When looking at the properties of sophorolipid, the lactonic form may have a better ability at lowering surface tension; however, the acidic form produces better foam and has better solubility [21]. Ma, Li and Song analyzed the surface and biological activity of sophorolipid and determined that since the acidic form of the sophorolipid produced a lower critical micellar concentration, this had better surface properties than the lactonic form [22]. Lastly, the effects of adding APG to water at different chain lengths was studied by Sulek [23]. It was determined that overall, APG significantly lowers the surface tension from the original water value, but specifically, as chain length
increased, the surface tension decreased. Overall, the optimal results for surface tension of any HA-surfactant system would show the smallest value.

In addition to surface tension, foaming is another parameter that is important to consumers; however, not necessarily in terms of product performance but sensory performance. Foamability is typically governed by the surface tension, more specifically, surface adsorption [24]. There have only been a few studies conducted on the foaming abilities of hyaluronic acid. For example, Liu et al. mixed HA with an amphiphilic molecule to observe the foaming qualities [25]. They determined that the viscous amphiphilic solution could be utilized as a decent foaming agent and under intense stirring, can produce self-foaming solutions. Another study investigated how hyaluronic acid can affect foam stability, where Chen et al. discovered that HA can help improve a solution’s foaming half-life and therefore stabilize the foam more [26]. As previously mentioned, the acidic sophorolipid typically has better foaming properties when compared with the lactonic form. However, Hirata et al. conducted an experiment showing that when compared against other surfactants, sophorolipids typically have low foaming properties [27]. Zhou et al. analyzed the foaming abilities of APG and discovered that as chain length increases, a larger foam volume, which was also more stable, formed [28]. This is valid considering that Sulek showed that the longer chain lengths produced a lower surface tension value and both parameters do correlate with one another [23]. The objective of this study is to optimize the wet combing force, rheology, and surface activity of a solution containing hyaluronic acid and a natural surfactant and develop a successful sulfate- and-silicone-free conditioning shampoo.

2. Materials and Methods

2.1. Materials

The sodium salt of hyaluronic acid at three different molecular weights (8, 90, and 130 kDa) was obtained from Sigma Aldrich. Hyaluronic acid (HA) at a different molecular weight (800 kDa) was obtained from BOS Essential, supplied by Amazon. DI water was used to turn samples into solutions and then citric acid (20%) was added to adjust the pH of the samples. Alkyl polyglucoside (APG, 50%) was donated by BASF and the acidic form of sophorolipid (ASL, 50.54%) was provided by NatSurFact. Sodium chloride, purchased from Fisher Scientific, was used to test the counterion effect on surface activity and rheology.

2.1.1. Effect of Hyaluronic Acid Molecular Weight

The effect of the hyaluronic acid molecular weight in solution was observed by creating four different samples, all at 0.5 wt. % HA and 99.5 wt. % DI water. The four molecular weights observed were 8, 90, 130 and 800 kDa. All samples were mixed on a stir plate at 1000 rpm for 4 h, the pH was adjusted to approximately 6.5 with 20 wt. % citric acid and then set to rest for 24 h in 17 °C. Samples were then brought to room temperature before tests were conducted.

2.1.2. Effect of Hyaluronic Acid Concentration

In order to interpret the effect of hyaluronic acid concentration, four samples were created, varying the concentration, all using the hyaluronic acid with a molecular weight of 800 kDa. The four concentrations measured were 0.25, 0.5, 0.75 and 1 wt. %. The samples were then bought up to mark using DI water. All samples were mixed on a stir plate at 1000 rpm for 4 h, the pH was adjusted to approximately 6.5 with 20 wt. % citric acid and then set to rest for 24 h in 17 °C. Samples were then brought to room temperature before tests were conducted.

2.1.3. Effect of Hyaluronic Acid on Acidic Form of Sophorolipid

The effect of hyaluronic acid on ASL was observed by formulating 3 samples, all containing 10 wt. % of ASL, but varying the concentration of hyaluronic acid present. One sample had 0 wt. % HA and 90 wt. % DI water, the second sample had 0.5 wt. % HA and
89.5 wt. % DI water and the last sample had 1 wt. % HA and 89 wt. % DI water. All samples were mixed on a stir plate at 1000 rpm for 4 h, the pH was adjusted to approximately 6.5 with 20 wt. % citric acid and then set to rest for 24 h in 17 °C. Samples were then brought to room temperature before tests were conducted.

2.1.4. Effect of Hyaluronic Acid on Alkyl Polyglucoside

The effect of hyaluronic acid on APG was measured by formulating 3 samples, all containing 10 wt. % of APG, but varying the concentration of hyaluronic acid present. One sample had 0 wt. % HA and 90 wt. % DI water, the second sample had 0.5 wt. % HA and 89.5 wt. % DI water and the last sample had 1 wt. % HA and 89 wt. % DI water. All samples were mixed on a stir plate at 1000 rpm for 4 h, the pH was adjusted to approximately 6.5 with 20 wt. % citric acid and then set to rest for 24 h in 17 °C. Samples were then brought to room temperature before tests were conducted.

2.1.5. Effect of Salt Addition

In order to analyze the effect of counterion addition, 2 samples were created, both containing 1 wt. % NaCl, 1 wt. % HA and 88 wt. % DI water but the first being 10 wt. % ASL and the second being 10 wt. % APG. These samples were then compared with the two samples without NaCl, containing 1 wt. % HA, 89 wt. % DI water and 10 wt. % ASL or APG, respectively. All samples were mixed on a stir plate at 1000 rpm for 4 h, the pH was adjusted to approximately 6.5 with 20 wt. % citric acid and then set to rest for 24 h in 17 °C. Samples were then brought to room temperature before tests were conducted.

2.2. Sample Preparation

The 15 mL samples, which varied in hyaluronic acid molecular weight, concentration, surfactant addition and salt addition, were formulated in 30 mL glass vials. No buffer solution was used to dilute the mentioned ingredients, and therefore the only solvent was DI water. Citric acid was added as needed to adjust the pH of each sample to approximately 6.5. The glass vials were placed on a hot plate and set to stir at 1000 rpm for 4 h at room temperature. Some samples exhibited large viscosity as the hyaluronic acid dissolved and were continue to stir manually using a spoonula until homogeneous. Afterwards, the samples were placed in a refrigerator, set to 17 °C, and left to rest for 24 h. Prior to testing, samples were allowed to be brought to room temperature. The wet combing force, viscosity and surface tension of samples were measured without dilution. Select samples were diluted to 1 wt. % to observe foamability.

2.2.1. Wet Combing Force

To calculate the reduction in wet combing force of a hair tress, the force needed to comb through a wet hair tress was measured three different times on the Dia-stron MTT175 flexible miniature tensile tester. The hair tresses were virgin dark brown Caucasian hair which were 15 cm long, sealed with a 1” wide flex swatch and weighed approximately 2 g. The first measurement was taken from a wet hair tress, prior to the sample application. The second measurement was taken after 1 mL of sample was applied to the wet hair tress. The third measurement was recorded after the sample had been rinsed off of the hair tress. The combing force required pre and post treatment was compared to determine the percent reduction in wet combing force.

2.2.2. Mechanical Rheology

TA instrument DHR-3 rheometer (TA Instruments, New Castle, DE, USA) was used to measure the viscosity of the samples as well as produce a flow curve. A 40 mm parallel plate was used for each experiment with a Peltier plate controlling the temperature at 25 °C and the gap at 100 μm. The viscosity was taken over a range of shear rates from 0.1 s⁻¹ to 5000 s⁻¹.
2.2.3. Surface Tension

Surface tension at the air–water interface was tested at 20 °C using the Du Noüy ring method on the Attension Sigma 701 Tensiometer (Biolin Scientific, Gothenburg, Sweden). A small vessel was used for each test with 15 mL of sample.

2.2.4. Foaming

The foam test was performed by diluting the samples to 1 wt. % of original sample, then holding the samples together and shaking uniformly, 8 times. The foam build-up was recorded at T = 0, 15 s, 30 s, 1 min and 5 min.

3. Results

Given that the objective of this study is to optimize various performance properties, the data pertaining to the wet combing force, rheology, surface tension and foaming will be explored.

3.1. Wet Combing Force

The reduction in wet combing force can illustrate how effective each sample is at lubrication and therefore how well it can decrease the friction coefficient of the hair. The percent reduction was measured for a variety of samples outlined below.

3.1.1. Effect of Hyaluronic Acid Molecular Weight

Various formulations were prepped, each consisting of 0.5 wt. % hyaluronic acid of a different molecular weight, with no sodium chloride or surfactant present. Table 1 shows the corresponding percent reduction in wet combing force for each molecular weight of hyaluronic acid.

| HA MW (kDa) | Wet Combing Reduction Force % |
|------------|-------------------------------|
| 8          | 85.0                          |
| 90         | 79.5                          |
| 130        | 49.2                          |
| 800        | 54.2                          |

Observing the table above, it can be seen that the lowest molecular weight studied was 8 kDa, resulting in an 85% reduction, which is the largest observed reduction in wet combing force. The larger the percent reduction in wet combing force, the more effective the sample is at conditioning the hair, which is the desired result. As the molecular weight increased to 90 and 130 kDa, the reduction dropped to 79.5% and 49.2%, respectively. The largest hyaluronic acid molecular weight utilized was 800 kDa, which produced a slightly larger reduction of 54.2% in wet combing force when compared to the 130 kDa sample.

3.1.2. Effect of Hyaluronic Acid Concentration

Four samples were prepared using the 800 kDa hyaluronic acid in four different concentrations and used to test the wet combing reduction force. Table 2 outlines the resulting reductions with the concentration of hyaluronic acid present.

| HA (wt. %) | Wet Combing Reduction Force % |
|------------|-------------------------------|
| 0.25       | 43.3                          |
| 0.5        | 54.2                          |
| 0.75       | 59.5                          |
| 1.0        | 60.8                          |
As expected, the sample with the highest concentration of hyaluronic acid produced the largest reduction in wet combining force at 60.8%. As the concentration decreases to 0.75%, 0.5% and 0.25%, the percent reduction decreases as well to 59.5%, 54.2% and 43.3%, respectively.

3.1.3. Effect of Hyaluronic Acid and ASL

The reduction in wet combing force was recorded for samples containing different concentrations of 800 kDa hyaluronic acid and 10 wt. % ASL. The results can be found in Table 3.

Table 3. Effect of HA Concentration with ASL on Wet Combing Reduction Force.

| HA (wt. %) | Wet Combing Reduction Force % |
|------------|-------------------------------|
| 0          | 54.4                          |
| 0.5        | 66.7                          |
| 1.0        | 71.9                          |

Similar to the results in the previous section, as the concentration of hyaluronic acid increases, the percent reduction in wet combing force also increases. Here, the addition of ASL, a biosurfactant, has led the overall percent reduction to increase, with the highest reduction being 71.9% at 1 wt. % HA. The sample which contains no hyaluronic acid and only 10 wt. % ASL illustrates a percent reduction of 54.4% and the sample with 0.5 wt. % HA falls in between the two results at 66.7%.

3.1.4. Effect of Hyaluronic Acid and APG

Similar formulations varying the hyaluronic acid concentration were prepared replacing the 10 wt. % of ASL with 10 wt. % APG. Table 4 below gives an overview of the concentration of HA in each sample and the resulting reduction in wet combing force.

Table 4. Effect of HA Concentration with APG on Wet Combing Reduction Force.

| HA (wt. %) | Wet Combing Reduction Force % |
|------------|-------------------------------|
| 0          | 30.2                          |
| 0.5        | 42.4                          |
| 1.0        | 48.7                          |

As previous trends are further confirmed, the samples with the lower concentration of hyaluronic acid are not as efficient in terms of reducing the wet combing force for a hair tress. The sample containing only APG and no HA resulted in a wet combing reduction force of 30.2%, which increased to 42.4% and 48.7% as HA concentration increased to 0.5 wt. % and 1 wt. %, respectively.

3.1.5. Effect of Salt Addition

Four different formulations were utilized to study the effect of salt addition on systems containing hyaluronic acid and a surfactant. Tables 5 and 6 below show the reduction in wet combing force for systems containing 1% HA, 10% surfactant and either 0% or 1% NaCl.

Table 5. Effect of HA Concentration with ASL on Wet Combing Reduction Force.

| HA (wt. %) | ASL (wt. %) | NaCl (wt. %) | Wet Combing Reduction Force % |
|------------|-------------|--------------|-------------------------------|
| 1.0        | 10          | 0            | 71.9                          |
| 1.0        | 10          | 1.0          | 78.1                          |
Table 6. Effect of HA Concentration with APG on Wet Combing Reduction Force.

| HA (wt. %) | APG (wt. %) | NaCl (wt. %) | Wet Combing Reduction Force % |
|------------|-------------|--------------|------------------------------|
| 1.0        | 10          | 0            | 48.7                         |
| 1.0        | 10          | 1.0          | 45.0                         |

Observing the effect of salt addition on the system containing the acidic sophorolipid, at 0% NaCl, the reduction in wet combing force is at 71.9%; and at 1% NaCl, there is a significant increase to 78.1%, which is the largest reduction in wet combing force out of all the systems containing HA and a surfactant. Salt addition had a reverse effect on the system containing APG, where the reduction in the wet combing force decreased from 48.7% to 45% as salt increased from 0% to 1%.

3.2. Rheological Properties

Rheology was used to analyze several parameters of the sample which can lead to predictions in the texture of a system, how a system will act under different shear conditions and can connect viscosity to tribological performance. The rheological profiles of different studies are depicted below.

3.2.1. Effect of Hyaluronic Acid Molecular Weight

The formulations previously prepared, each consisting of 0.5 wt.% hyaluronic acid of a different molecular weight, with no sodium chloride or surfactant present, were used for further characterization. Figure 1 shows the corresponding flow curve for each molecular weight of hyaluronic acid.

![Figure 1. Effect of HA molecular weight on viscosity vs. shear rate.](image)

As seen in the graph above, the hyaluronic acid with the 800 kDa molecular weight can be classified as non-Newtonian with shear-thinning behavior, which are the desired rheological effects. The other three lower-molecular-weight hyaluronic acids were Newtonian and decreased in viscosity as the molecular weight decreased.

3.2.2. Effect of Hyaluronic Acid Concentration

The four samples prepared with the 800 kDa hyaluronic acid at different concentrations were used again to measure the viscosity as shear rate increases. Figure 2 outlines the resulting flow curves as the concentration of hyaluronic acid varies.
After analyzing the figure above, all concentrations of the 800 kDa hyaluronic acid are non-Newtonian, demonstrating shear-thinning properties. It can also be seen that 1 wt. % HA has the highest overall viscosity, and decreases as concentrations decreases, resulting in 0.25 wt. % HA to have the lowest overall viscosity.

### 3.2.3. Effect of Hyaluronic Acid with ASL

The viscosity vs. shear rate was recorded of samples containing different concentrations of 800 kDa hyaluronic acid and 10 wt. % ASL. The results can be found in Figure 3.

The samples containing 10 wt. % ASL and either 1 wt. % HA or 0.5 wt. % HA continue to be non-Newtonian with shear-thinning behavior as seen previously. It can also be observed the sample with 1 wt. % HA has the highest overall viscosity. The sample containing 10 wt. % ASL without hyaluronic acid is Newtonian and has the lowest overall viscosity.

### 3.2.4. Effect of Hyaluronic Acid with APG

Similar formulations varying the hyaluronic acid concentration, replacing the 10 wt. % of ASL with 10 wt. % APG, were characterized. Figure 4 below gives an overview of the concentration of HA in each sample and the resulting flow curve.
The viscosity vs. shear rate effects of HA concentrations with APG are very similar to the trends shown with HA and ASL. 1 wt. % HA and 10 wt. % APG have the highest overall viscosity, and show shear-thinning, non-Newtonian characteristics along with 0.5 wt. % HA and 10 wt. % APG. The sample containing only 10 wt. % APG has the lowest overall viscosity and is Newtonian.

3.2.5. Effect of Salt Addition

The four formulations that were utilized to study the effect of salt addition on systems containing hyaluronic acid and a surfactant were used to test rheological properties. Figures 5 and 6 below show the flow curves for systems containing 1% HA, 10% surfactant and either 0% or 1% NaCl.

Figure 4. Effect of HA concentration with APG on viscosity vs. shear rate.

Figure 5. Effect of NaCl concentration for HA + ASL on viscosity vs. shear rate.
Figure 6. Effect of NaCl concentration for HA + APG on viscosity vs. shear rate.

Observing the effect of salt addition on the system containing the acidic sophorolipid, both samples have a very similar flow curve; however, at lower shear rates, the viscosity of the sample containing 0% NaCl is slightly higher than the sample containing 1% NaCl. The opposite trend is seen in the system containing APG, where, at lower shear rates, the sample containing 1% NaCl has a slightly higher viscosity than in the sample without NaCl. All four samples remain to be seen as non-Newtonian with shear-thinning behavior.

3.3. Surface Activity: Surface Tension at the Air–Water Interface and Foaming

Surface tension enabled the system to undergo further optimization once the conditioning properties and rheological profiles had been established. Therefore, after analyzing the rheological profiles on the various molecular weight hyaluronic acids, the formulation composed of 800 kDa showed the desired rheological effects. To further the optimization of samples, surface tension values were only recorded for formulations containing the 800 kDa HA. The surface activity of various systems is illustrated below.

3.3.1. Effect of Hyaluronic Acid Concentration

Formulations varying the concentration of hyaluronic acid were used to measure surface tension. The results are shown in Figure 7.

Figure 7. Effect of HA concentration on surface tension.
Analyzing the graph above, it can be shown that as the concentration of hyaluronic acid increases, the surface tension increases as well. The lowest surface tension recorded was 58.717 at 0.25 wt. % HA and jumps to 68.267 at 0.5 wt. % HA. From there, the increase in surface tension is more gradual, increasing to 70.932 and culminating at 72.353 for 0.75 wt. % HA and 1 wt. % HA, respectively.

3.3.2. Effect of Hyaluronic Acid with ASL

The systems containing 10 wt. % ASL and a variety of hyaluronic acid concentrations were used for further characterization. The surface tension results are shown in Figure 8.

The addition of a surfactant drastically decreased the overall surface tension; however, a similar trend is observed where surface tension increases as the concentration of HA increases. The surface tension of pure ASL is 31.875, which increases to 32.119 at a concentration of 0.5 wt. % HA. At 1 wt. % HA, the resulting surface tension is 32.578.

3.3.3. Effect of Hyaluronic Acid with APG

Similarly, the effect of hyaluronic acid concentration on surface tension was measured for systems containing APG as its primary surfactant. The corresponding results are shown in Figure 9.
The figure above has kept on trend, increasing in surface tension as hyaluronic acid concentration is increased, but APG has decreased the overall surface tension measurements. The lowest surface tension value recorded was 29.189 at 0% HA. Surface tension then increased to 29.450 and 29.765 at 0.5 wt. % HA and 1 wt. % HA, respectively.

3.3.4. Effect of Salt Addition

The effect of salt was observed for two systems, both containing 1 wt. % HA but one system with 10 wt. % ASL and the other with 10 wt. % APG. The concentration of NaCl varied between 0 and 1 wt. %. The surface tension values of both systems are shown in Figure 10.

![Figure 10. Effect of NaCl concentration on surface tension.](image)

Observing the system containing the acidic sophorolipid, salt addition had a greater effect on changing the surface tension. At 0% NaCl, the surface tension was 32.578, whereas, at 1% NaCl, the surface tension decreased to 31.837. The system containing APG did not change much; however, the surface tension did decrease slightly from 29.765 to 29.613 at 0% NaCl and 1% NaCl, respectively.

The 1 wt. % dilutions were made of the formulations mentioned, in order to reduce viscosity, for the foaming study. The first five minutes of foam was recorded and is shown below in Figure 11.

The best way to observe foamability is to analyze the total foam volume as well as how long bubbles last in the liquid phase. After analyzing the foaming for both systems, it can be seen that the salt concentration does not nearly impact the foaming abilities as much as the surfactant. The system containing APG has both a larger total foam volume, and has bubbles that last longer in the liquid phase. The 1% salt for the APG does have a slightly better total foam volume and more dense bubble presence in the liquid phase. The system containing ASL has a smaller total foam and has bubbles that escape to the air–water interface more rapidly. These results are further diminished with the removal of NaCl, showing that the 1% NaCl produces slightly better results. Overall, each system, regardless of the type of surfactant or whether NaCl is present, is homogeneous and quite stable.
4. Discussion

The most efficient way to determine the optimal system of hyaluronic acid combined with a natural surfactant is to focus on the properties of each system. The discussion will further analyze the results found on wet combing force, rheological properties and surface activity of each sample prepared.

4.1. Wet Combing Force

As aforementioned, the wet combing force is measured on a hair tress several times, once before the hair is treated with the conditioning system, once while the system is in the hair, and a final time after the conditioning system has been rinsed out of the hair. The proper equation to calculate the reduction in wet combing force is

\[
\text{Force Required (Pretreated Hair – Treated Hair)} \times 100 = \%\, \text{Reduction}
\]

The higher the reduction value, the better the conditioning and lubricating properties seen in the sample because less friction is present in the treated hair, meaning less force is required to comb through the hair. This illustrates how the reduction in wet combing force has an inverse relationship with the friction coefficient. Given that wet conditioning correlates with the lubrication process, both can be further explained using a Stribeck curve, seen below in Figure 12 [29].

When analyzing the x-axis of the curve, the viscosity is the main contributor because the speed and normal load parameters were held constant during the study. Additionally, the viscosity is observed from right to left on the graphs, so it shows that an initial decrease in viscosity will cause a decrease in the friction coefficient in the hydrodynamic regime. In the mixed regime, as viscosity decreases, the friction coefficient will begin to increase until it reaches the boundary regime, where the friction coefficient will remain constant regardless of viscosity change. Each regime demonstrates a different part of the lubrication process—for example, initial conditioning typically correlates with the hydrodynamic regime, and the mixed regime is the washing of hair and the boundary regime corresponds to dry conditioning. Wet combing has the potential to be found in either the hydrodynamic or mixed regime.
There are two trends exhibited when comparing the reduction in wet combing force between the four different molecular weight hyaluronic acids. Looking specifically at the lower-molecular-weight (8, 90 and 130 kDa) hyaluronic acids, as the molecular weight increases, the viscosity increases, and the reduction in wet combing force decreases, meaning the friction coefficient increases. This could place these Newtonian samples in the hydrodynamic regime. For the highest molecular weight (800 kDa), the reduction in combing force increases slightly from the 130 kDa sample, meaning the friction coefficient decreases while viscosity increases. There are two possible explanations for the increase in reduction found between these two molecular weights. Most likely, because the 800 kDa HA is non-Newtonian, both low shear rate and high shear rate viscosities have to be considered. The first possible explanation occurs at higher shear rates such as $10^5$, where due to shear-thinning behaviors, the viscosity of the 800 kDa would actually be lower than that of the 130 kDa HA. With lower viscosity, yet the same larger reduction in wet combing force and, in turn, smaller friction coefficient, this would put the 800 kDa sample in the hydrodynamic regime as well. For the second possible explanation, at low shear rates, the 800 kDa hyaluronic acid has a larger viscosity, a larger reduction in wet combing force and therefore a smaller friction coefficient. This could show that the sample might be in the mixed regime, and that it was able to form a thicker, lubricating layer on the hair surface which decreased the friction coefficient. This possible change in regime could be confirmed by conducting tribology; however, that was outside of the scope of this study.

The effects of hyaluronic acid concentration on the reduction in wet combing force were also explored. Pure 800 kDa hyaluronic acid at 0.25, 0.5, 0.75 and 1 wt. %, as seen in the flow curves, are non-Newtonian, and the overall viscosity increases as concentration increases, so viscosity at different shear rates does not need to be considered when utilizing the Stribeck curve. Another observed trend is that as concentration increases, the reduction in combing force increases, so the friction coefficient decreases. It can be assumed these samples reside in the mixed regime, which can be understandable given that the mixed regime correlates with wet combing after conditioning.

Next, the effects of HA concentration, combined with either ASL or APG, on the reduction in wet combing force were studied. Regardless of the surfactant, the samples containing 0.5 or 1 wt. % HA were non-Newtonian and the samples without HA were Newtonian. This does not affect the comparison of viscosity because even at higher shear rates, the viscosities of the samples containing HA will not drop below the sample without HA. That being said, as the concentration of hyaluronic acid increases, the overall viscosity increases and the reduction in wet combing force increases. In terms of the curve, as viscosity increases, the friction coefficient decreases, meaning these samples may also be

![Figure 12. Stribeck curve.](image-url)
in the mixed regime. When observing the difference between the two surfactants present, ASL has a slightly higher viscosity than APG at each HA concentration, and consequently, ASL has a better reduction in wet combing force. This is most likely due to its ability to form a thicker layer on the hair’s surface, reducing the friction force, if the samples are in fact in the mixed regime.

Lastly, the Stribeck curve can also be used to explain the correlation between salt addition on two systems—1 wt. % HA + 10 wt. % ASL and 1 wt. % HA + 10 wt. % APG. When 1 wt. % NaCl is added to the system with ASL, the viscosity decreases, but the reduction in wet combing force increases meaning the friction coefficient decreases. This result can infer that this system is probably in the hydrodynamic regime. On the other hand, when salt is added to the system containing APG, viscosity increases, wet combing force reduction decreases and the friction coefficient increases. This could also potentially place the samples in the hydrodynamic regime. As previously stated, both the hydrodynamic and the mixed regime may be used to describe wet combing—it ultimately depends on the thickness of the sample to dictate which regime. Since viscosity is so crucial, the rheological properties of the systems are explored next.

4.2. Rheological Properties

While viscosity has already been discussed slightly in the wet combing force section, it is important to further analyze the profiles of each system. Starting with the viscosity profiles of the different molecular weight hyaluronic acids, it was observed that the three lower-molecular-weight hyaluronic acids, at 8, 90 and 130 kDa, were Newtonian and had relatively low viscosities. While there are no previous studies to our knowledge of hyaluronic acid solution at such low molecular weights, it can be assumed that since they are so low, entanglements were most likely unable to be formed, or if they were formed, the entanglement density was very low. The 800 kDa HA was non-Newtonian, with shear-thinning tendencies, and has relatively large viscosity at lower shear rates. Overall, the trend seen is that as molecular weight increases, viscosity increases, possibly because as molecular weight increases, more entanglements occur, especially at 800 kDa. This similar trend had been observed in several studies, showing hyaluronic acid has the ability to become more entangled at higher molecular weights [15,16,30].

Second, the effects of HA concentration were studied using rheology. It can be seen that all four concentrations produce non-Newtonian, shear-thinning behavior and as concentration increases, viscosity increases. This could potentially be due to an increase in either density of entanglements present as concentration increases, an increase in the length of the entanglements, or a slight variation in shape of the entanglements. In a study by Ambrosio et al., it was shown that both molecular weight and concentration have a proportional relationship with viscosity but that molecular weight had a larger impact on rheology [30]. This is demonstrated in the two graphs showing both sets of flow curves, given that changing molecular weight changes the entire behavior of a solution and concentration only slightly changes the overall viscosity.

After, the rheological properties of systems containing HA combined with ASL or APG were analyzed. It was shown that for both surfactant systems, the samples that did not contain any HA were low viscosity, Newtonian systems. This is understandable given that HA has the ability to help build viscosity and the surfactants alone typically have lower viscosities at a concentration of 10%, confirmed by Pingali, Benhur and Amin, as well as Moore et al. [17,18]. As the concentration of HA increased in both systems, they become non-Newtonian, shear-thinning and high viscosity samples. This can elude that as HA is introduced, entanglements may be easily formed, or micelles form given that there is now a surfactant present in these systems.

The last rheological properties that need to be examined are the flow curves when salt is added to the systems containing 1% HA and 10% of surfactant, either ASL or APG. When 1 wt. % NaCl is added to the system with ASL, the viscosity decreases very slightly at low shear rates but then remains constant with the system containing 0 wt. % NaCl
as the shear rate increases. A similar trend is observed with the APG system, where at higher shear rates, the viscosities are the same; however, the viscosity increases slightly as sodium chloride is added at the lower shear rates. These, very minor changes could even be considered insignificant and ultimately shows that the potential for entanglements seems to rely more on the concentration of hyaluronic acid more than whether or not sodium chloride is present in the system.

4.3. Surface Activity: Surface Tension at the Air–Water Interface and Foaming

Now that wet combing force and rheology have been analyzed, exploring surface tension will help further optimize the materials used to create an efficient conditioning shampoo. As formerly determined, the surface tension was only observe for systems containing the 800 kDa HA, due to its desired rheological profile when compared against the other molecular weight hyaluronic acids. Therefore, the effects of hyaluronic acid concentration were studied and it could be seen that as concentration increases, surface tension value increase as well. This could potentially be due to the fact that at higher concentrations, HA begins to block the air–water interface enforcing a hydrophobic effect. A similar trend was exhibited in the study conducted by Krause et al. because at very small concentrations, HA was able to decrease surface tension; however, past a concentration of 0.35 wt. %, surface tension values increases due to a steric hinderance of molecules on the surface [19].

Continuing on, the concentration effects of HA paired with either ASL or APG on surface tension were measured. Regardless of surfactant, a very similar trend was observed where HA concentration increased, the surface tension values increased; however, these values had a much lower starting point, given that a surfactant was now present in the system. Surfactants naturally lower the surface tension because they adsorb quickly at the surface. When comparing the individual surfactants, APG has a slightly lower surface tension recorded at each HA concentration than ASL. In an experiment conducted by Sulek, it was discovered that APG significantly lowers the surface tension from that of water because APG easily forms a surface phase that differs significantly from the bulk phase [23]. Ultimately, as the hyaluronic acid concentration increases, there is more potential for competition against the surfactants at the air–water interface; however, the surfactants still form most of the surface phase.

The last surface tension analysis was focused on the addition of sodium chloride to a system containing 1 wt. % HA and either 10 wt. % ASL or 10 wt. % APG. When studying the system containing ASL, as salt concentration increased, the surface tension drastically decreased. This trend is confirmed in an experiment conducted by Li et al. who studied the effects of salt on sophorolipid at the air–water interface [31]. It was observed that salt addition not only lowered the critical micellar concentration (CMC) and surface tension values for ASL but also increased the maximum adsorption capacity, showing NaCl shields the charge of the hydrophilic headgroups, favoring the surface activity of ASL. This can ultimately show that salt increases surface activity, therefore decreasing the surface tension values. Observing the system with APG, as salt concentration increased, surface tension did not change significantly, only slightly decreased. An experiment conducted by Staszak et al. explains the effects of NaCl on different anionic, zwitterionic and nonionic surfactants [32]. It was discovered that when sodium chloride was added to nonionic surfactants, such as APG, there typically a very small or insignificant effect on the CMC or surface tension values, most likely due to a salting in or out of the hydrophobic groups. This was seen in our experiment given the insignificant change in surface tension value for the APG system.

The systems comparing the salt effects were further used for a foaming study. When comparing the samples of 1% HA with 10% APG to the samples with 1 % HA and 10% ASL, the samples had a larger total foam volume as well as more stable bubbles lasting in the liquid phase. This is understandable because foaming and surface tension often correlate and APG’s surface tension is lower than ASL’s. This is also confirmed by Hirata et al., who mention how sophorolipids have overall lower foamability than most surfactants and
Zhou et al. who discuss APG’s very stable foaming abilities [27,28]. When 1 % of salt is added to both samples, there is a very small difference between the samples without salt, but it can be seen that the bubbles are slightly more stable in the liquid phase. This again is most likely due to the correlation of surface tension since the surface tension does decrease when salt is added, which should, in turn, enhance foaming properties.

5. Conclusions

The wet combing force reduction, rheological properties and surface activity of a sustainable conditioning system containing hyaluronic acid partially in combination with ASL or APG were investigated for the first time. Effects of hyaluronic acid molecular weights and concentration were explored, in addition to combining 800 kDa HA with either ASL or APG. Lastly, the effect of adding sodium chloride to a system containing 1% HA and 10% surfactant was analyzed.

The wet combing force highlighted the significant impact of HA molecular weight, concentration and the type of surfactant present in the system on the ability to increase the reduction in force required and decrease the friction coefficient of the hair tress. The lowest molecular weight of hyaluronic acid (8 kDa) produced the largest reduction between molecular weights. As the concentration of the 800 kDa hyaluronic acid increased, the reduction in wet combing force also increased. Hyaluronic acid with ASL produced a larger wet combing force reduction when compared against APG. Salt had a smaller effect on the reduction, but sodium chloride increased the reduction for ASL and decreased the reduction for APG.

The rheological properties directly impacted wet combing force reduction so molecular weight and concentration of HA had the largest impact on rheology. As molecular weight increased, systems turned from Newtonian to non-Newtonian with shear-thinning behaviors. Overall, the viscosity, specifically at low shear rates, increased as molecular weight increased. Similarly, as the concentration of hyaluronic acid increased, viscosity increased, with all systems exhibiting non-Newtonian, shear-thinning behavior, the desired profile for a conditioning shampoo. The surfactant and salt addition had a very small effect on the overall viscosity.

The surface activity was tested to optimize the conditioning system into an effective cleansing system as well. As hyaluronic acid concentration increased, whether alone or in combination with a natural surfactant, surface tension increased. However, when the HA is used with a surfactant, the overall surface tension is still significantly lower. The lowest surface tension is found with pure APG at 29.189 mNm. The lowest surface tension for a system containing hyaluronic acid is 0.5% HA with 10% APG at 29.45. Effect of salt concentration was also analyzed and it was found that as salt concentration increased, surface tension either decreased slightly or had an insignificant change. Foaming ability seemed to correlate with surface tension because the samples containing salt had slightly better foaming abilities than those without and the systems containing APG had better foamability than the systems with ASL.

Overall, the system containing 1 wt. % hyaluronic acid, 10 wt. % acidic sophorolipid and 1 wt. % NaCl exhibited an excellent reduction in wet combing force, a desired rheology profile through the formation of entanglements, and a decent decrease in surface tension with suitable foam generation. This system is highly biocompatible, natural and sustainable without compromising these essential performance parameters. Future studies should focus more on tribological properties to confirm lubrication regimes.

6. Patents

An international patent application has been filed with the number PCT/US2021/033398 utilizing work reported in this manuscript.
Author Contributions: Each author had a specific role in this study, outlined here. Conceptualization, S.A.; methodology, K.Y.; software, K.Y.; validation, K.Y.; formal analysis, K.Y.; investigation, K.Y.; resources, K.Y.; data curation, K.Y.; writing—original draft preparation, K.Y.; writing—review and editing, S.A.; visualization, K.Y.; supervision, S.A.; project administration, S.A. All authors have read and agreed to the published version of the manuscript.

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