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Application of Electrochemically Reduced Water for New No-Rinse Shampoo: Design and Optimization Using Response Surface Methodology

Sureewan Duangjit [1,*], Phaijit Sritananuwat [2], Sureewan Bumrungthai [2], Tanasait Ngawhirunpat [3] and Kozo Takayama [4]

[1] Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand
[2] Division of Biopharmacy, Faculty Pharmaceutical Sciences, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand
[3] Department of Pharmaceutical Technology, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand
[4] Department of Pharmaceutical Sciences, Faculty of Pharmacy and Pharmaceutical Sciences, Josai University, Sakado 350-0295, Japan
* Correspondence: sureewan.d@ubu.ac.th; Tel.: +66-45-353-630

Abstract: Coronavirus disease (COVID-19) cases are continuing to rise around the world, with more than 607 million confirmed cases and more than 6.51 million deaths as of September 2022. The World Health Organization (WHO) has emphasized the importance of hygiene in the ongoing COVID-19 pandemic, especially in elderly and bedridden patients. The use of no-rinse shampoo represents a simple change in hair-cleansing products. Daily hospital hair washing for the elderly, bedridden, and ICU patients would be more readily adopted. The objective of this study was to design and optimize a new no-rinse shampoo based on electrochemically reduced water (ERW) using response surface methodology. The relationship between coconut-based surfactant mixtures in a no-rinse shampoo and the resulting physicochemical properties, effectiveness (antibacterial and antifungal activity), and stability of the shampoo was investigated. The vesicle size, size distribution, zeta potential, conductivity, pH, foamability, wetting time, turbidity, and stability of the model formulation were optimized. The optimal formulation with the appropriate physicochemical properties and stability was selected. The effectiveness of the optimal formulation was compared to that of commercially available products. The dry shampoo (DS4) containing 3.5% of the mixed detergent with ERW may prevent infection by *Escherichia coli*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Candida albicans*. The no-rinse shampoo based on ERW successfully demonstrated good stability in addition to efficacy in terms of antibacterial and antifungal activity. The treated hair fiber was not significantly different from that of the intact hair fiber. Under the scanning electron microscopy (SEM) and the atomic force microscopy (AFM), the cuticle layer of the treated hair fiber was not damaged. Thus, no-rinse shampoos may reduce the time of hair rinsing and improve the quality of life of caregivers.

Keywords: electrolytic-reduction ion water; electrolytic ionized water; waterless shampoo; dry shampoo; sodium cocoyl glycinate; cocamide diethanolamine

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1. Introduction

The World Health Organization (WHO) has emphasized the importance of hygiene during the current coronavirus (COVID-19) pandemic [1]. As of September 2022, COVID-19 cases continue to rise globally, with more than 607 million confirmed cases and more than 6.51 million deaths. While the race to develop and apply appropriate medicine and vaccinations continues, the WHO has presented key messages and actions for COVID-19 prevention and control, including physical distancing, covering of the mouth and nose,

frequent washing of hands with soap and water, and frequent cleaning of touched surfaces and objects. In routine hair care, a daily wash may be required [2]. The role of opportunistic pathogen (bacteria or fungi) infections in influencing the morbidity and mortality of COVID-19 patients remains less clearly defined [3]. The relationship between pathogen co-infections and the severity of COVID-19 disease in patient outcome is still inadequate [4]. Patients admitted to hospital are exposed to areas of the hospital that are of moderate to high risk. Caregivers have to maintain hygiene for the patients by bathing the bedridden; hair care is a part of that [2]. In addition to being a valuable hygienic activity, the daily hair washing of patients can help to boost their sense of well-being. Hair is an important part of the health indicator [5]. No-rinse, dry, and waterless shampoos or no-rinse shampoo caps are comparatively convenient and may therefore serve as alternatives in supporting hair care, which is essential during these extraordinary times. Moreover, no-rinse shampoos may improve the quality of life of caregivers, which is of growing importance given the increasing trend in the aging population, which has increased by 2.4% over the past 10 years [6]. An ideal shampoo for daily washing should contain only mild detergent, provide a deep clean, and be easily washed off. Nowadays, shampoo is well beyond the stage of pure hair cleanser. The specific shampoo includes herbal extracts and natural essential oils or agents to treat dandruff, dermatitis, and other hair diseases [5]. The use of electrochemically reduced water (ERW) in new clinical applications has been reviewed. ERW is a type of artificially treated water that is popular in Japan and exhibits an alkaline pH of 8 to 10, has negative oxidation–reduction potential, and is rich in hydrogen molecules [7]. Moreover, ERW has an antibacterial effect against many types of bacteria. ERW can be applied for wound healing, advanced tissue care, and dental clinics and has also been used to ensure surface sterilization and food safety for several years [8]. ERW protects several types of neuronal cells by scavenging reactive oxygen species (ROS) due to the presence of dissolved hydrogen and platinum nanoparticles in the ERW [9]. ERW was selected for application as an active compound with several advantages, such as being environmentally friendly and safe for human beings. Surfactants are an essential ingredient in most shampoo formulations and may perform various functions, e.g., removal of dirt (such as sebum, solid particles), foam forming, increasing product viscosity, suspending actives, and solubilizing fragrance components [10]. The background information on the primary surfactants used in shampoo formulation is important. Sulfate-free shampoos are a trend in hair cosmetics [11]. Coconut oil was found to reduce protein loss in both undamaged and damaged hair when used as either a washout or a leave-on product [12]. In this context, we selected three types of coconut-based surfactants that were accompanied by a safety data sheet for use in the no-rinse shampoo formulations developed in this study. Cocamide diethanolamine (DEA) or Comperlan KD® (KD) is a mixture of coconut fatty acid and ethanolamides that is used as a foam booster and viscosity-increasing surfactant in cosmetics. The Cosmetic Ingredient Review (CIR) panel previously assessed the safety of cocamide DEA. The Food and Drug Administration approved cocamide DEA in 745 products in 1994 [13]. Cocamidopropyl betaine (CAPB) or Dehyton KT® (KT) is a zwitterionic surfactant that is synthesized using coconut oil [14] and used primarily as a surfactant in cosmetics. A safety assessment for CAPB was published by the CIR in 1991. CAPB is safe for use in rinse-off products at 30% and should not exceed 3.0% for leave-on products [15]. Sodium cocoylglycinate (SCG) or Eversoft® YCS-30S (ES) is an amino acid derivative that is generally synthesized from coconut fatty acids and amino acid glycine. SCG is one of the few surfactants that naturally produce a creamy foam. Due to its safety and nonirritating properties, SCG is used in several cosmetic products. SCG is practically safe and is used by the CIR [16]. Surfactant mixtures are widely used in numerous practical applications, due to their advantages. Synergistic effects and better performance are important for a wide range of surfactant-based phenomena, including foam, emulsion, cleaning, solubilizing, and detergency [17]. The effect of formulation factors on the physicochemical properties, effectiveness, and stability of a no-rinse shampoo needs to be defined when using a new combination of coconut-based surfactants. The key

to success is the safety of the composition at the used concentration. In the development of a no-rinse shampoo, the properties of the surfactants should be optimized to be as gentle as possible to the hair and eyes. Moreover, the physicochemical properties, effectiveness, and stability of the shampoo formulation must be considered. Response surface methodology (RSM) is a set of statistical and mathematical methods used in experimental design. RSM is a tool for the experimental design and optimization of effect process variables. RSM has been successfully utilized to reduce the number of required experiments [18]. Such an approach is helpful in developing an appropriate formulation. Thus, the complicated relationship between causal (formulation) factors and the response variables, including the physicochemical properties, efficacy, and stability of no-rinse shampoo, can be understood using RSM. The purpose of our present study was to design and optimize a new no-rinse shampoo based on ERW using RSM. A micellar shampoo formulation based on a combination of coconut-based surfactants and ERW was prepared and investigated. The vesicle size, size distribution, zeta potential, conductivity, pH, foamability, wetting time, turbidity, and stability of the model formulation were optimized. The optimal formulation with the appropriate physicochemical properties and stability was selected for a further study in which the effectiveness (antibacterial and antifungal activity) of the optimal formulation was compared to that of commercial products. The findings provide basic information on the effect of the surfactant mixture and ERW on the properties and performance of the optimized no-rinse shampoo. Moreover, a basis for understanding the complicated relationship between the coconut-based surfactant mixtures and the physicochemical properties, effectiveness, and stability of the model formulation was achieved. The properties and effects of shampoo on the hair fiber can be assessed by a variety of methods [19]. Finally, the hair samples treated and untreated with the optimal formulation were analyzed using scanning electron microscopy (SEM) and atomic force microscopy (AFM).

2. Materials and Methods

2.1. Materials

Electrochemically reduced water (ERW), cocamide diethanolamine (cocamide DEA) or Comperlan KD® (KD), cocamidopropyl betaine (CAPB) or Dehyton KT® (KT), and sodium cocoylglycinate (SCG) or Eversoft® YCS-30S (ES) were generously supplied by P. general Ltd. (Bangkok, Thailand). All the other chemicals used were of reagent and cosmetic grade and purchased from Chemical Express Ltd. (Bangkok, Thailand).

2.2. Formulation Preparation Method

Initially, the surfactants and other additives were accurately weighed. The shampoo formulation consisted of a controlled amount of 0.1% lavender oil, 1.0% preservative, and 50% ERW. Distilled water was added to 100 mL. Lavender oil was incorporated in the micelles prior to inclusion in the no-rinse shampoo. The concentrations of surfactants used varied depending on the code −1, 0, and 1, by 1%, 3%, and 5%, respectively, according to the shampoo formulation obtained from the two-level factorial experimental design with a duplicate centroid (F10 and F11; Table 1). A pump bottle was then filled with the optimal shampoo formulation.

Table 1. Experimental design for two factors and model formulation of shampoo.

Form.	Code		RSM-1		RSM-2		RSM-3	
	*X*1	*X*2	KT (%) [+/−]	KD (%) [0]	KT (%) [+/−]	ES (%) [−]	KD (%) [0]	ES (%) [−]
F1	−1	−1	1	1	1	1	1	1
F2	−1	0	1	3	1	3	1	3
F3	−1	1	1	5	1	5	1	5
F4	0	−1	3	1	3	1	3	1
F5	0	0	3	3	3	3	3	3

Table 1. *Cont.*

Form.	Code		RSM-1		RSM-2		RSM-3	
	*X*1	*X*2	KT (%) [+/−]	KD (%) [0]	KT (%) [+/−]	ES (%) [−]	KD (%) [0]	ES (%) [−]
F6	0	1	3	5	3	5	3	5
F7	1	−1	5	1	5	1	5	1
F8	1	0	5	3	5	3	5	3
F9	1	1	5	5	5	5	5	5
F10	0	0	3	3	3	3	3	3
F11	0	0	3	3	3	3	3	3

Abbreviations: Formulation (Form.); X_1 and X_2 were causal factors; [−], [+/−], [0] were anionic, zwitterionic, and nonionic surfactant; Comperlan KD® (KD); Dehyton KT® (KT); Eversoft® YCS-30S (ES); Response surface methodology (RSM).

2.3. Measurement of Vesicle Size, Size Distribution, Zeta Potential, and Conductivity

The mean vesicular size, size distribution, zeta potentials, and conductivity of the model shampoo formulations were measured by photon correlation spectroscopy (PCS) (Zetasizer Nano Series, Malvern Instruments, Worcestershire, UK). All samples were collected at an ambient temperature of 25 °C. Measurements were collected using 1 mL of the shampoo formulations, where at least three independent samples were used for each shampoo.

2.4. pH Measurement

The pH of the shampoo formulations was measured using a pH meter (FiveEasy Plus pH meter FP20, Mettler Toledo, Bangkok, Thailand) on the undiluted shampoo, with at least three independent samples for each shampoo.

2.5. Foamability Measurement

The procedure utilized nitrogen to produce foam and measure foam formation using a slightly modified version of the procedure in [20]. The shampoo formulation (10 mL) was placed in a 500 mL beaker; 10 mL/min nitrogen gas flowed under the shampoo solution for 1 min until the maximal amount of foam had been generated, which was measured as volume. The foamability value was calculated using the following equation:

$$\text{Foamability value} = [\text{foam volume} - \text{shampoo volume}]/\text{foam volume}$$

The resulting foam was measured immediately after 3 and 6 min and until no further bubble formation was observed.

2.6. Wetting Time Measurement

The method according to a previous study [20] was used to measure wetting time. The canvas was cut into 1 × 1 inch squares to achieve the same average weight. The canvas was floated over the surface of the shampoo formulation, and the start-up time was recorded. The required time for the canvas to begin to flow was accurately recorded and marked as wetting time.

2.7. Dirt Dispersion Measurement

The method of dirt dispersion was measured using a slightly modified [16]. In brief, 10 mL of distilled water (DW) was added to a large test tube. One hundred microliters of shampoo was added, and then, fifty microliters of ink was filled into the test tube. The mixture was blended and stirred for one minute with the vertex® mixture. The amount of ink in the foam was measured by the scale [21].

2.8. Turbidity Measurement

All shampoo formulations were pre-classified from the formation of different micelles (mixed micelles) in turbidity testing [22]. Measurements of 1 mL of shampoo at 400 nm were obtained using a UV–vis spectrophotometer (Mettler Toledo, Bangkok, Thailand).

2.9. Stability Testing

Heating–cooling cycles were used to measure the accelerated stability of the shampoo formulation. All samples were stored at 4 °C for 48 h and then at 45 °C for 48 h for a total of 6 cycles. The initial shampoo was classified as Day 0, and the incubated shampoo was categorized as Cycle 6. All samples were stored under 30 ± 2 °C/75 ± 5% RH for 3 months, following the "long term for products in primary containers semipermeable to water vapor" ASEAN Guidelines for the study of drug-product stability.

2.10. Antibacterial and Antifungal Test

The following methodology, according to a previous study [23], was used to measure the sterilization effect, with a slight modification: 1.0 mL of different pathogens (*Escherichia coli* [*E. coli*] ATCC 25922, *Staphylococcus epidermidis* [*S. epidermidis*] DMST 15505, and *Staphylococcus aureus* [*S. aureus*] ATCC 25923 representing bacteria, and *Candida albicans* [*C. albicans*] ATCC 10231 representing fungi) was added into 4.0 mL of the shampoo formulation. The mixture was incubated at 37 °C and 30 °C for the bacteria and fungi, respectively. To determine the antibacterial and antifungal effects, 100 µL of the solution was collected at 0 h, 2 h, 6 h, and 24 h and cultured on an agar plate. The colonies were counted and converted into the number of microbes per 1 mL. Normal saline was used for a control group. Ampicillin and tetracycline were used in the positive control for antibacterial activity, and amphotericin B was used in the positive control for antifungal activity.

2.11. Determination of Optimal Formulation

Full details of the simultaneous optimization by RSM can be found in previous publications [24–26]. The relationships between the causal factors and the response variables are readily understandable by RSM. RSM can be used to estimate a stable and reproducible simultaneous optimal formulation. It has been applied to pragmatic cases to optimize pharmaceutical formulations and does not require any complicated procedures, such as an artificial neural network. Optimization of the shampoo formulation based on RSM was conducted with the dataset obtained for the model formulations. The optimal formulation of the shampoo was defined as an adequate physicochemical property. Small vesicular size (less than 1000 nm), small size distribution (0.2–0.4), and high zeta potential (±30 mV) were the latent variables that influenced formulation stability. Moreover, nanosized vesicles may encapsulate and maintain the lavender oil throughout the shelf life of the no-rinse shampoo. An optimal formulation should have a minimal vesicular size value, minimal size distribution, maximal zeta potential, and minimal instability. The experimental shampoo formulation was prepared according to the optimal shampoo formulation determined by RSM to confirm the reliability of the optimization.

2.12. Scanning Electron Microscopy (SEM) Analysis

The SEM procedure was analyzed using a slightly modified method [27]. Hair was provided by a local hair salon. This human hair study was performed at Ubon Ratchathani University and met the requirement of the Ethics of Human-related Research Committee of Ubon Ratchathani University (ID no. UBU-REC-84/2565). The area of the hair sample near the nape was collected. A total 3 long hair strands (approximately 50 cm long) were cleaned, cut (1–3 cm long), and used; they received the DS1 optimal formulation for 24 h and underwent distilled water treatment. Each hair sample observed under SEM was the same strand. The hair sample was prepared for conventional SEM analysis. Briefly, the dried hair sample was placed on pieces of aluminum and coated with gold. Images from

the hair samples were captured through Schottky field emission SEM (JEOL JSM-7610F Plus, Toyko, Japan) at 15 kV and at magnifications of 250×, 500×, and 1000×.

2.13. Atomic Force Microscopy (AFM)

An atomic force microscope (AFM model XE-100, Park Systems, Suwon, Korea) was used to estimate the topography using an NSC36C cantilever (MikroMasch, Wetzlar, Germany) in contact mode. A standard spring constant of 0.6 $N \cdot m^{-1}$ was used to determine the compliance of each tip with a typical resonance frequency of 65 kHz. The damaged portions of the cuticle and the images of cuticle peel and cuticle density were also observed by the AFM [28]. An AFM scan was completed prior to the SEM analysis.

2.14. Ethics in the Animal Study

This animal study was performed at Ubon Ratchathani University and met the requirement of the Ethics Committee for the Care and Use of Laboratory Animals (ID no. 51/2563/IACUC). Dirt was swabbed from the back of the dogs (n = 3) using a cotton bud which was then immersed in 10 mL of sterile normal saline per each swab. Then, 0.5 mL of the dirt solution was added into the 4.5 mL shampoo formulation. The mixture was mixed and converted into the number of microbes per 1 mL at 0 h, 2 h, 6 h, and 24 h, and maintained at 37 °C and 30 °C for bacteria and fungi, respectively

2.15. Computer Programs

Design Expert® version 11 release 11 (Stat-Ease, Inc., Minneapolis, MN, USA) was used to draw the response surface for each response variable.

3. Results

3.1. Preliminary Study

The preliminary study was conducted to prepare the basic formulation of the shampoo using a single surfactant (KD, KT, and ES). The mean vesicle size, size distribution, zeta potential, and stability (Day 0 and Cycle 6) of the shampoo formulations were assessed (Figure S1). The vesicle of most of the initial (Day 0) shampoo formulations was less than 1000 nm. The size distribution, zeta potential, and conductivity of the shampoo formulations was dependent on their primary surfactants. The low percentage difference of physicochemical properties between Day 0 and Cycle 6 was classified as a stable formulation. The stability study indicated that 1% KD and 3–5% of ES in the formulation led to a stable shampoo. Please see Appendix A for more information.

3.2. Identification of the Response Surface by RSM

Eleven formulations of shampoo were established. The amounts of lavender oil, preservative, and ERW were fixed at 0.1%, 1%, and 50%, respectively. The concentration of surfactants (KD, KT, and ES) affected the foam boosting, sebum solubilizing, and cleansing functions of the shampoo and varied from 1% to 5% w/w according to the formulation obtained from the two-level factorial experimental design with the duplicate centroid (F10 and F11). The physical appearance and visual turbidity of the model shampoo formulations are shown in Figure 1.

The combination of coconut-based surfactants suggested that the combined formulations were more stable than the single-surfactant formulations under the accelerated condition (Day 0 and Cycle 6), as shown in Figure 2. The relationship between the two surfactants is complicated by the formation of the mixed micelles. Although numerous studies have been published on the interactions between surfactants and polymers, the complex behavior of mixed additives in solutions is far from fully understood [29]. Thus, the complicated relationship between the formulation factors, i.e., KD, KT, and ES, and the response variables, namely shampoo vesicle size, size distribution, zeta potential, conductivity, pH, and stability, could be understood using RSM.

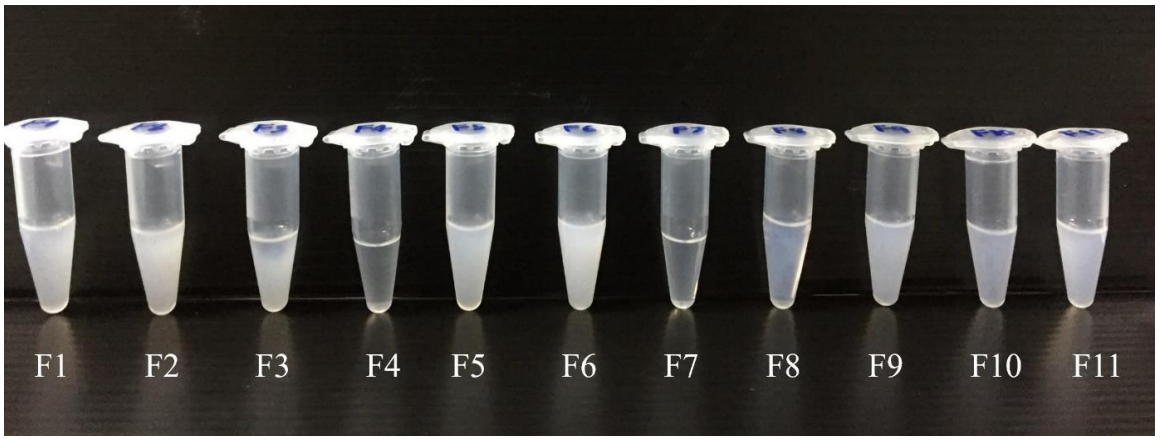

Figure 1. The physical appearance and visual turbidity of model formulations of shampoo of RSM-1. Abbreviations: Response surface methodology (RSM).

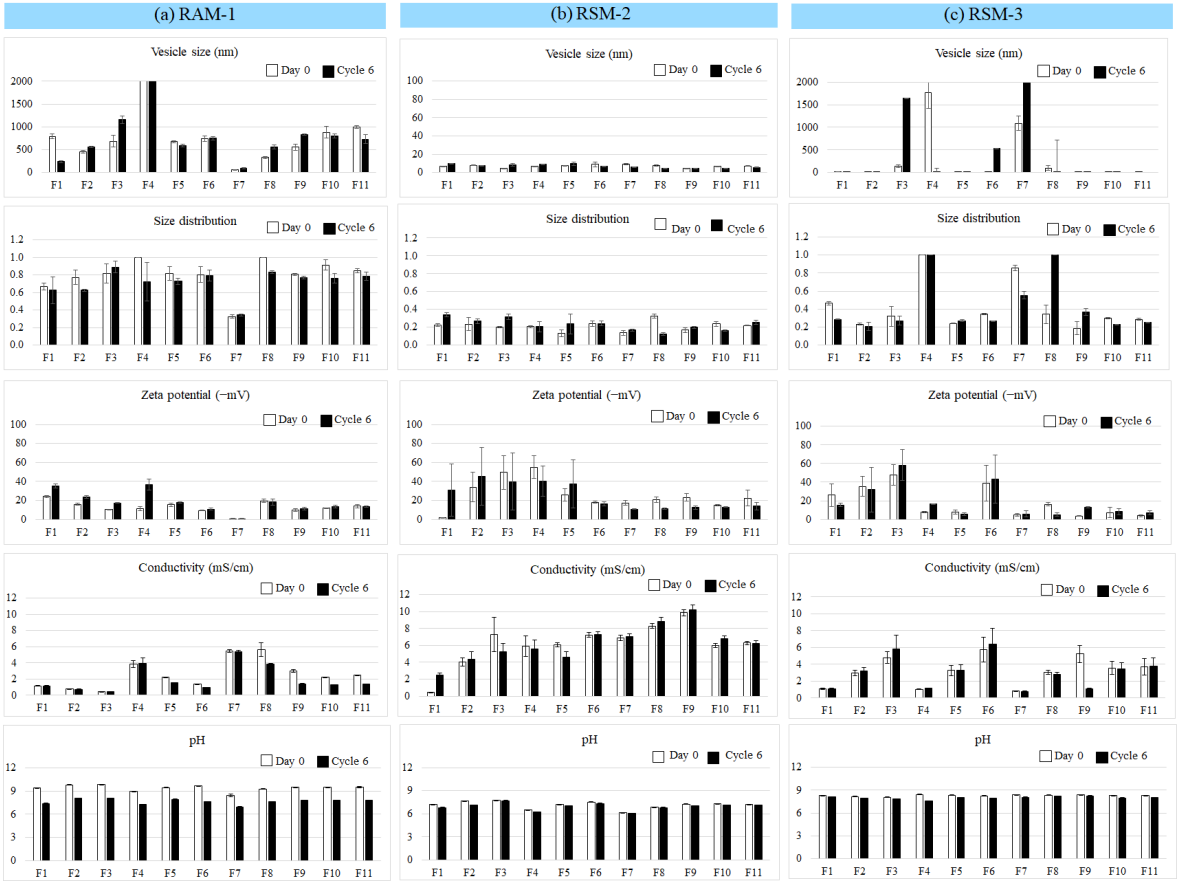

Figure 2. The physicochemical properties and the stability of different shampoo formulations: (**a**) RSM-1, (**b**) RSM-2, and (**c**) RSM-3.

The types and quantity of surfactants were selected as causal factors (X_n). The physicochemical properties, including vesicle size, size distribution, zeta potential, conductivity, pH, foamability, and wetting time were selected as the basic characteristics (latent variables). Antimicrobial (antibacterial and antifungal) activity was selected as the response variable. The response surface estimated by RSM shows the relationship between the causal factors and the latent variables (e.g., vesicle size, size distribution, zeta potential, conductivity, and pH) and the relationship between the causal factors and the response variables (e.g.,

physicochemical stability). The effect of the coconut-based KD, KT, and ES surfactants on the physicochemical properties of the shampoo formulation is shown in Figure 3.

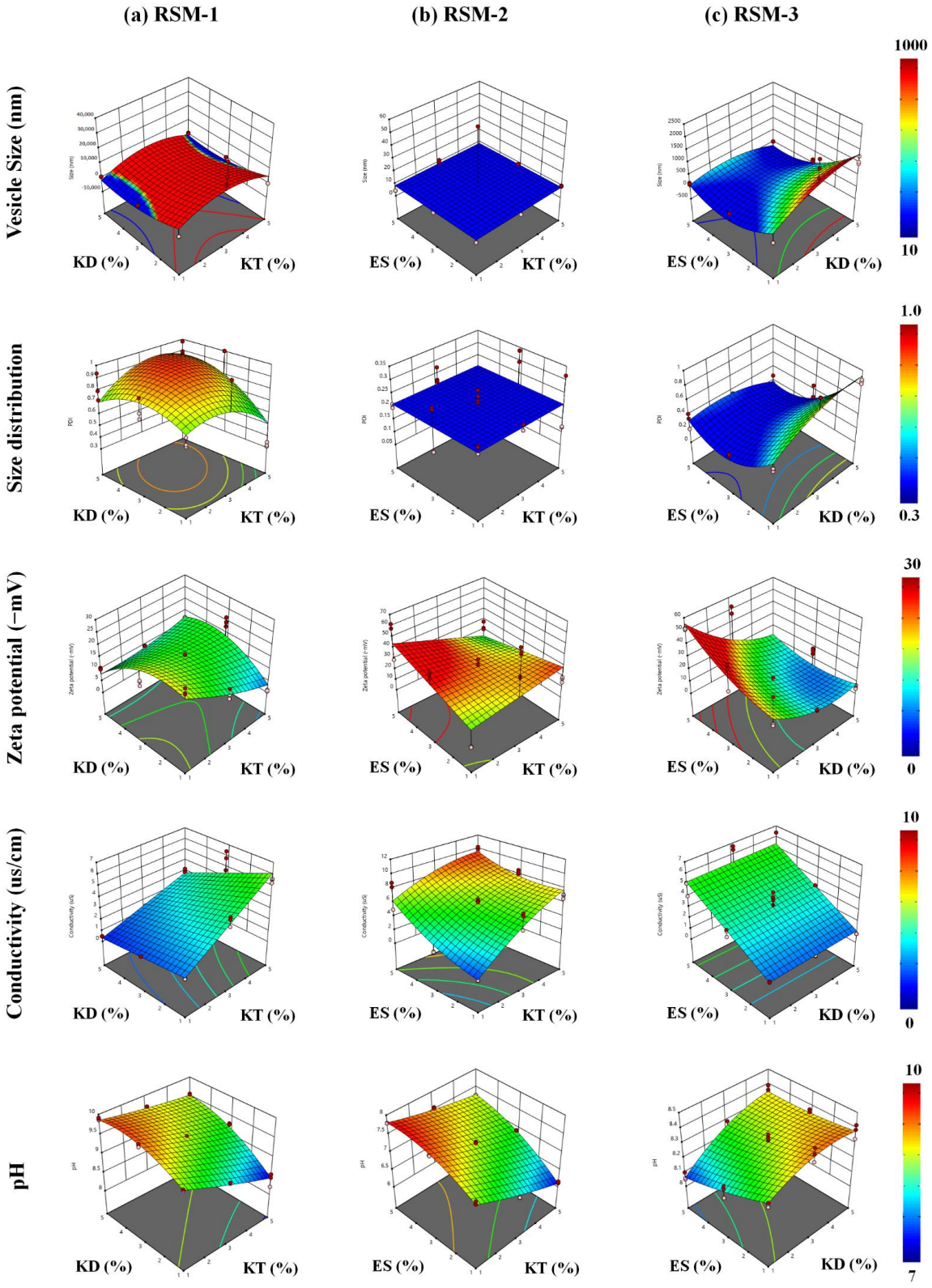

Figure 3. The response surface of the results for the model formulation of shampoo of (**a**) RSM-1, (**b**) RSM-2, and (**c**) RSM-3. Abbreviations: Comperlan KD® (KD); Dehyton KT® (KT); Eversoft® YCS-30S (ES).

The response surface estimated by RSM displayed the relationship of causal factors with the latent and/or response variables. However, the diagnostic plot based on the design of the experiments of each response had to coincide well with the response surface. The plot of the normal residuals, the predicted versus actual values, and the residual versus the runs of RSM-1–3 were plotted (Figure S2). The normal probability plot of the externally studentized residuals was the suggested metric to use for this plot. The results indicated whether the residuals followed a normal distribution, in which case the points were followed with a straight line. Some moderate scatter is expected even with the normal datasets. The close fit between the plots of the predicted and actual values demonstrated that the model made fairly accurate predictions. These results suggested that a higher-order model would be required to improve the fit since some of the data points are split evenly over the 45-degree line. The plot of the residual versus the run order provided a latent (lurking) variable that influenced the response during the experiment. Most of the random scatter tended to fall within the externally studentized residuals range, indicating reliability. Thus, some latent variables (foamability, wetting time, and turbidity) were not illustrated in this study. Please see Appendix B for more information.

Figure 3a shows the response surfaces of the latent variables between KD and KT, including the vesicle size, size distribution, zeta potential, conductivity, and pH estimated by RSM. The response surfaces indicate that an increase in KT results in a significant increase in size and conductivity and a slight decrease in size distribution, zeta potential, and pH in the formulation. An increase in KD results in a slight decrease in size and an increase in pH in the formulation. The accuracy and reliability of the response surfaces were determined using one-way analysis of variance (ANOVA). There were some scatter points (F4) outside the externally studentized residual range (Figure S2(a3)). Thus, the reliability of the original dataset is important for estimation by RSM. The RSM of the foamability, wetting time, and turbidity is not shown due to the values not being significantly different between each formula. The foamability of all the formulations was over 0.98 and stable for more than 30 min. Bubbles were still found in the samples after 60 min of incubation time. The wetting times of all the formulations varied in the range of 1–50 min depending on the surfactant concentration in the shampoo formulation. High wetting times were found for shampoo formulations with low surfactant concentrations, while those with high surfactant concentrations showed minimal wetting times [30], whereby wetting time represents the cleaning ability of the shampoo. Thus, low wetting times are preferred and indicate the good ability of a shampoo to diffuse and wet the hair shaft.

Figure 3b displays the response surfaces of latent variables between KT and ES. The response surfaces revealed that an increase in KT results in a slight increase in conductivity and a slight decrease in pH in the formulation. An increase in ES results in a significant decrease in size, size distribution, and pH; a significant increase in zeta potential; and a slight increase in conductivity in the formulation. The random scattering of the size distribution, zeta potential, and conductivity indicates the achievement of being within the externally studentized residuals range, as well as the reliability of the optimization (Figure S2(b3)).

Figure 3c shows the response surfaces of the latent variables between KD and ES. The response surfaces suggested that an increase in KD results in a slight increase in size, size distribution, and pH and a slight decrease in zeta potential in the formulation. An increase in ES results in a significant decrease in size and size distribution, a slight decrease in pH, a significant increase in zeta potential, and a slight increase in conductivity in the formulation. The reliability of the size distribution, zeta potential, conductivity, and pH was adequate, as shown in Figure S2(c3).

The interaction between surfactants has a strong effect on the consistency index of the physicochemical properties of the shampoo formulation [29]. The estimated response surface was interpreted in terms of the interactions between the two surfactants. The same surfactant was in different environments of formulations, resulting in different effects. Surfactants are a fundamental ingredient used in pharmaceuticals for a variety of purposes.

The interactions between surfactants and the complex behavior of mixing solutions still need to be studied case by case.

Figure 4 illustrates the response surfaces of the different percentages of each variable. The percentage of difference was calculated by considering the difference between the physicochemical characteristic values at Day 0 and Cycle 6. Minus and plus values represent a decrease and an increase in one value, respectively. The minimal instability or high-stability formulation is presented by the zero percentage of difference (green area of the response surface). The relationship between the causal factors and the response variables (e.g., physicochemical stability) was determined. RSM-2 was mostly demonstrated to be found in the green area of the response surface (Figure 4b). This technique was utilized in our previous study, a computerized design study that estimated the stability of the model formulation by RSM [31]. The fraction in the quantity of the surfactant mixtures of shampoo formulation demonstrated the feasibility of the formulations to acquire favorable physicochemical stability. The results revealed the ability of computer software (Design Expert®) to estimate the formulation stability of the no-rinse shampoo. Using the experimental design, the number of measurements required to simultaneously determine the effects of combining the surfactants on the stability of the shampoo formulations were reduced. Almost all the shampoo formulations were found to be stable under 30 ± 2 °C and 75 ± 5% RH for at least 3 months in a long-term stability study. Thus, the accelerated condition was also utilized in our study to observe the formulation in critical condition.

3.3. Formulation Optimization Using RSM

The shampoo formulation was optimized on the basis of the original dataset using RSM. The search directions for the response variables were set to produce high stability of the formulation and to use minimal surfactant. The optimal formulations of the waterless shampoo predicted by RSM-1, RSM-2, and RSM-3 are shown in Table 2. The experimental shampoo formulation was prepared to confirm the reliability of the optimization, as shown in Table 3. The three experimental optimal formulations of the waterless shampoo were prepared and used to fill a pump bottle. The foamability of the optimal formulations in the pump bottles was established and is displayed in Figure 5. According to the good stability of RSM-2, the experimental RSM-2 optimal formulation was selected as an appropriate formulation in this study. The antibacterial and antifungal activity of the experimental RSM-2 was further investigated.

3.4. Comparative Study

The experimental RSM-2 optimal shampoo formulation was prepared and defined as dry shampoo (DS) 1. The DS1 was compared to a commercially available product. Moreover, the alternative optimal formulation with lower surfactant concentration and lower desirability than those of DS1 was prepared. The combination of KT and ES at 6%, 4.8%, and 3.5% was classified into high, medium, and low surfactant concentrations and defined as DS1, DS2, and DS3, respectively. The alternative optimal formulation with low surfactant concentration and ERW was established as DS4. DS5 was the commercial dry shampoo in the market. The composition of the comparative formulation is shown in Table 4. The formulation of the shampoo that inhibited bacteria and/or fungi was selected as the appropriate formulation in this study. The formulation of the shampoo that inhibited common human bacteria and/or animal pathogen bacterial strains, Gram-negative (*E. coli*) and -positive (*S. aureus* and *S. epidermidis*) and fungi (*C. albicans*), was measured at 0, 2, 6, and 24 h of incubation.

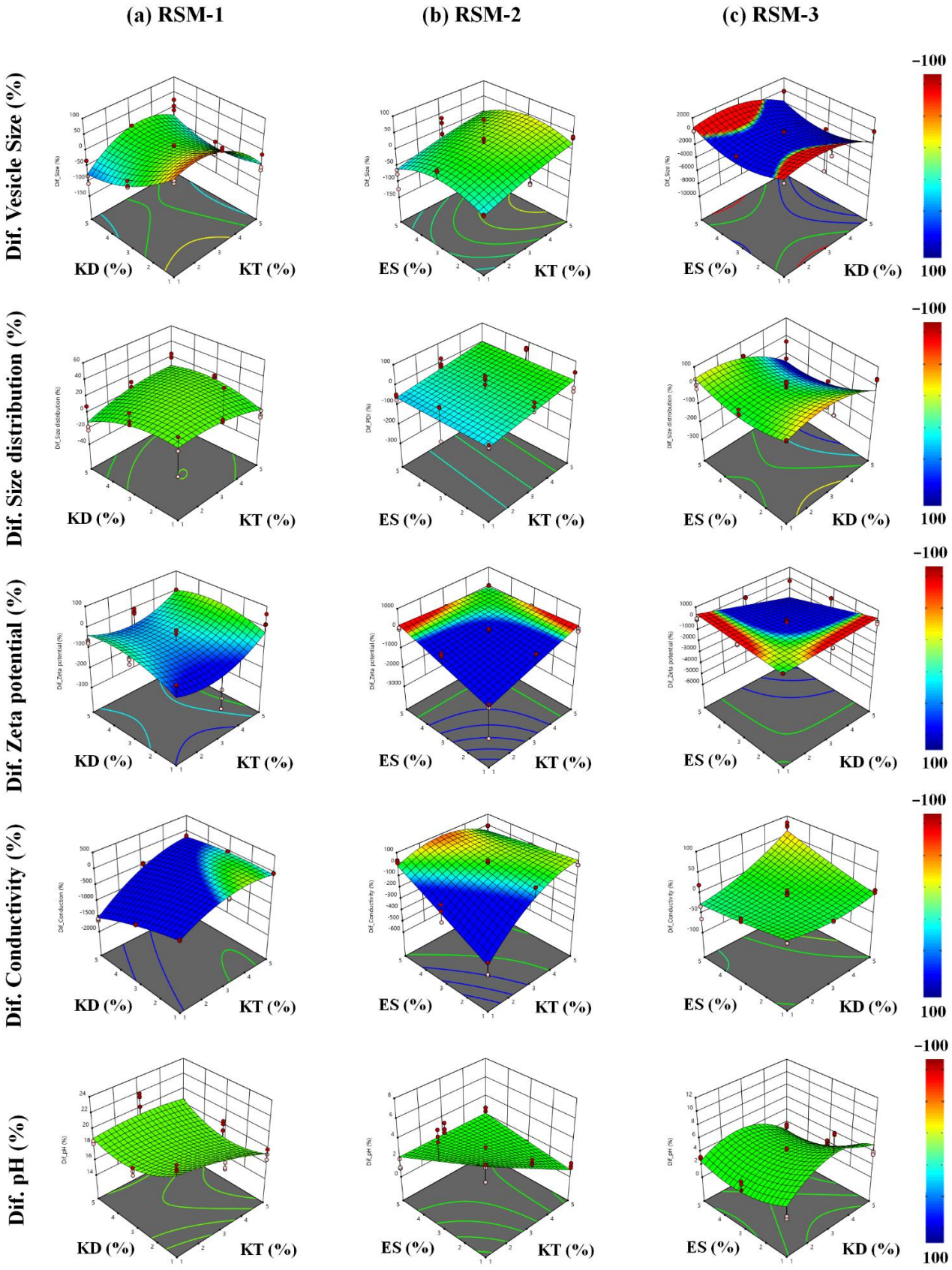

Figure 4. The response surface of the stability results for the model formulation of shampoo of (**a**) RSM-1, (**b**) RSM-2, and (**c**) RSM-3.

Table 2. The predicted optimal formulations of waterless shampoo of RSM-1, RSM-2, and RSM-3.

Optimal Formulation	KD (%) [0]	KT (%) [+/−]	ES (%) [−]	Size (nm)	PDI	Zeta Potential (−mV)	Conductivity (μS/cm)	pH	Foamability	Wetting Time (min)	Desirability
RSM-1	2.2	1.0	-	61.7	0.8	22.5	0.9	9.7	0.98	16.4	0.69
RSM-2	-	2.3	3.7	8.6	0.2	30.2	5.7	7.5	0.98	13.3	0.66
RSM-3	1.7	-	4.1	3.6	0.2	32.6	4.3	8.2	0.98	18.9	0.79

Abbreviations: [−], [+/−], [0] were anionic, zwitterionic, and nonionic surfactant; polydispersity index (PDI).

Table 3. The experimental optimal formulations of waterless shampoo of RSM-1, RSM-2, and RSM-3.

Optimal Formulation	Size (nm)	PDI	Zeta Potential (−mV)	Conductivity (μS/cm)	pH	Foamability	Wetting Time (min)	Turbidity	Dirt Dispersion (cm)
RSM-1	407.17 ± 11.55 #	0.65 ± 0.06	20.73 ± 0.93 *	1.01 ± 0.03 *	9.6 ± 0.01 *	0.98 *	0.11 ± 0.02	0.788 ± 0.00	0.45 ± 0.05
RSM-2	4.38 ± 0.05	0.13 ± 0.02	32.90 ± 0.45 *	6.01 ± 0.22 *	7.5 ± 0.02 *	0.98 *	5.75 ± 1.12	0.012 ± 0.00	0.60 ± 0.10
RSM-3	9.06 ± 0.25	0.38 ± 0.01	34.87 ± 14.81 *	4.64 ± 0.98 *	8.4 ± 0.00 *	0.98 *	3.14 ± 1.49	0.004 ± 0.00	0.77 ± 0.06

%Bias = (predicted value − experimental value)/experimental value × 100%, *—≤10% bias, #—≥100% bias.

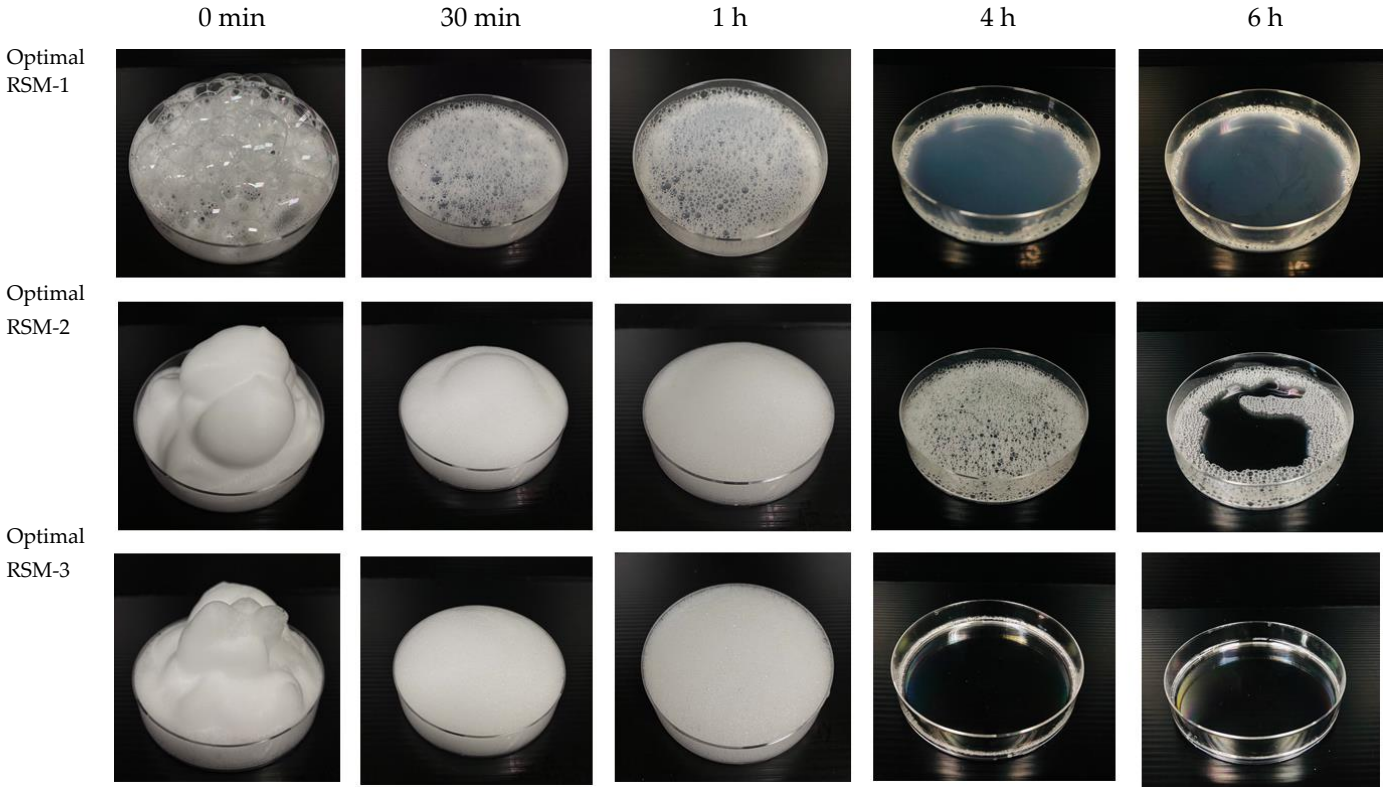

Figure 5. The foamability of optimal formulations with electrochemically reduced water (ERW).

Table 4. The composition of dry shampoo formulations.

Optimal Formulation	KT (%)	ES (%)	Lavender Oil (%)	Preservative (%)	ERW (%)	Aqua qs to (%)
DS1	2.3	3.7	0.1	1	-	100
DS2	3.8	1.0	0.1	1	-	100
DS3	0.5	3.0	0.1	1	-	100
DS4	0.5	3.0	0.1	1	50	100
DS5	n/a	n/a	n/a	n/a	-	100

n/a = unknown data.

Regarding antibacterial activity, all the formulations, including the commercial dry shampoo, had the highest antibacterial effect at 24 h of incubation. The combined for-

mulation with ERW eradicated *E. coli* and *S. epidermidis* at the initiation time, while the formulation with 3.5% (DS4) had a longer period. For *S. aureus*, all formulations except DS1 had little inhibitory activity during a short incubation period (0–2 h) but exhibited gradually increased activity in a longer period (6–24 h) (Table 5).

Table 5. Antibacterial and antifungal activities.

E. coli	**(CFU/mL)**			
Groups	**0 h**	**2 h**	**6 h**	**24 h**
DS1	0	0	0	0
DS2	0	0	0	0
DS3	$2.98 \pm 0.5 \times 10^6$	$1.20 \pm 0.33 \times 10^6$	$9.3 \pm 3.2 \times 10^4$	0
DS4	0	0	0	0
DS5	$3.10 \pm 0.14 \times 10^6$	$8.50 \pm 0.70 \times 10^2$	0	0
125 ug/mL Ampicillin	$>3.0 \times 10^6$	$1.31 \pm 0.42 \times 10^4$	$1.95 \pm 0.95 \times 10^4$	$1.00 \pm 0.02 \times 10^3$
250 ug/mL Ampicillin	$>3.0 \times 10^6$	$4.3 \pm 0.28 \times 10^5$	$2.55 \pm 0.84 \times 10^5$	$3.20 \pm 0.85 \times 10^3$
Negative	$>3.0 \times 10^6$	$>3.0 \times 10^6$	$>3.0 \times 10^6$	$>3.0 \times 10^6$
S. epidermidis				
DS1	0	0	0	0
DS2	0	0	0	0
DS3	$>3.0 \times 10^6$	$2.30 \pm 0.04 \times 10^6$	$2.93 \pm 1.51 \times 10^5$	$1.19 \pm 1.03 \times 10^3$
DS4	0	0	0	0
DS5	3.65 ± 0.06 x10^5	0	0	0
125 ug/mL Ampicillin	$>3.0 \times 10^6$	$6.60 \pm 1.98 \times 10^5$	$6.35 \pm 1.20 \times 10^4$	$2.01 \pm 1.83 \times 10^3$
250 ug/mL Ampicillin	$>3.0 \times 10^6$	$1.08 \pm 0.34 \times 10^5$	$3.82 \pm 0.21 \times 10^4$	$1.05 \pm 0.07 \times 10^3$
Negative	$>3.0 \times 10^6$	$>3.0 \times 10^6$	$>3.0 \times 10^6$	$>3.0 \times 10^6$
S. aureus				
DS1	$3.86 \pm 0.40 \times 10^5$	1.00×10^3	0	0
DS2	$1.13 \pm 0.71 \times 10^4$	$2.00 \pm 1.41 \times 10^3$	0	0
DS3	$2.52 \pm 0.67 \times 10^6$	$2.26 \pm 0.91 \times 10^6$	$8.70 \pm 2.88 \times 10^5$	$2.79 \pm 2.12 \times 10^5$
DS4	$>3.0 \times 10^6$	$3.72 \pm 0.62 \times 10^5$	$2.21 \pm 0.70 \times 10^5$	$1.25 \pm 1.89 \times 10^1$
DS5	$1.07 \pm 0.09 \times 10^6$	$1.45 \pm 0.78 \times 10^3$	$1.25 \pm 0.21 \times 10^2$	0
125 ug/mL Ampicillin	$6.91 \pm 0.57 \times 10^6$	$5.11 \pm 0.85 \times 10^6$	$3.78 \pm 0.49 \times 10^6$	4.00×10^4
250 ug/mL Ampicillin	$>3.0 \times 10^6$	$4.86 \pm 0.76 \times 10^6$	$>3.0 \times 10^6$	$2.17 \pm 0.85 \times 10^5$
250 ug/mL Tetracycline	$>3.0 \times 10^6$	$2.30 \pm 0.21 \times 10^5$	0	0
Negative	$>3.0 \times 10^6$	$>3.0 \times 10^6$	$>3.0 \times 10^6$	$>3.0 \times 10^6$
C. albicans				
DS1	$7.65 \pm 1.91 \times 10^4$	0	0	0
DS2	$1.21 \pm 0.64 \times 10^4$	0	0	$1.31 \pm 1.53 \times 10^3$
DS3	1.84×10^5	4.30×10^5	7.82×10^4	7.75×10^4
DS4	$1.70 \pm 0.55 \times 10^5$	$2.04 \pm 0.70 \times 10^4$	$1.15 \pm 1.32 \times 10^4$	$3.28 \pm 3.27 \times 10^3$
DS5	$1.52 \pm 0.28 \times 10^4$	3.26 ± 0.17 x10^4	0	0
2 ug/mL amphotericin B	$1.49 \pm 0.23 \times 10^5$	$2.01 \pm 0.31 \times 10^3$	$3.05 \pm 1.07 \times 10^2$	$1.73 \pm 0.16 \times 10^4$
Negative	$1.57 \pm 0.29 \times 10^5$	$2.00 \pm 0.08 \times 10^5$	$>3.0 \times 10^6$	$>3.0 \times 10^6$

The result for antifungal activity was established using a *C. albicans* model. The formulations with medium and high surfactant concentrations of the mixture (4.8% and 6%) had greater antifungal activity than that containing 3.5% of the mixed detergent. Adding ERW in the low surfactant concentration of the mixture increased its efficacy. The results indicated that the experimental RSM-2 optimal formulation with ERW (DS4) had antibacterial and antifungal effects against *E. coli*, *S. epidermidis*, *S. aureus*, and *C. albicans*.

Formulation composition was a factor affecting the antibacterial and antifungal activity of the shampoo formulation. A previous study reported the antibacterial effect of ERW [32]. An inflammatory skin condition found in both humans and animals (dogs) is caused by *S. epidermidis*, *S. aureus*, and *C. albicans* [33], while intestinal pathologies and extraintestinal infections of humans and pets are also caused by *E. coli*. Thus, the application of this no-rinse shampoo formulation may prevent infection by bacteria (*E. coli*, *S. epidermidis*, *S. aureus*) and fungi (*C. albicans*).

The cuticle peel and cuticle density can be identified through morphology monitoring using SEM and AFM. The comparison of untreated and treated hair revealed no specific peeling of the cuticles, and no cuticle density reduction was observed, as shown in Figure 6. These results revealed that the cuticle peeling and cuticle density of the treated hair fiber were not significantly different from those of the intact hair fiber.

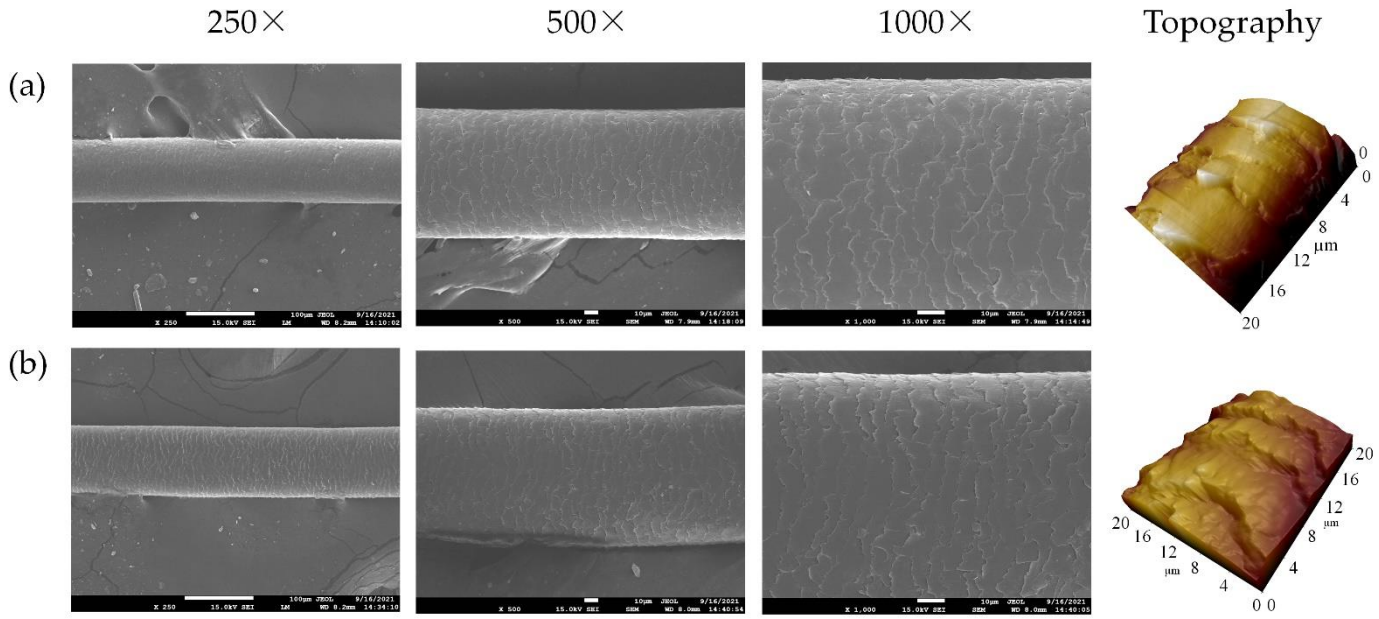

Figure 6. Comparison of scanning electron microscopy (SEM) and atomic force microscopy (AFM) images of no-rinse hairs: (**a**) untreated and (**b**) DS1 optimal formulation treated hair via the immersion method.

4. Discussion

Considering the physicochemical properties, effectiveness, and stability of the 33 model formulations obtained from RSM-1, RSM-2, and RSM-3, the no-rinse shampoo that had good stability and could inhibit bacteria and/or fungi was selected as the appropriate formulation. ERW and a gentle coconut-based surfactant were selected as components in the primary composition of the no-rinse shampoo due to their several benefits and safety. Moreover, lavender oil was also selected as a promising candidate for the efficient enhancement of conventional antiseptics, as demonstrated in a previous study [34].

4.1. Preliminary Study

The results of the preliminary study indicated a significant difference between the physicochemical stability of the single-surfactant and the combined-surfactant formulations. The percentage of the difference between Day 0 and Cycle 6 of almost all the single-surfactant formulations was over 100% (Figure S1). A few single-surfactant formulations composed of 1% KD and 3–5% ES were stable. Comparing the stability of the single-surfactant and the combined-surfactant formulations, the results suggest that compared to a single surfactant, the interaction between the two surfactants may stabilize the micellar formulation to a larger extent. The physicochemical stability of a surfactant formulation of KT-combined ES (RSM-2) at Day 0 was slightly different compared to that at Cycle 6 (Figure 2). In the classification of the instability of a formulation, all the parameters affecting the physicochemical properties (vesicle size, size distribution, zeta potential, conductivity, and pH) were emphasized. Physicochemical properties such as vesicle size and size distribution may be affected by the preparation method [35], while intrinsic physicochemical properties, e.g., zeta potential, conductivity, and pH, are directly influenced by the formulation factor. Considering the physicochemical stability parameter,

the instability of the surfactant formulations may be established by the zeta potential [36], conductivity [37,38], and pH [36] of the surfactant formulation. The interaction among the formulation factors or compositions may affect the total zeta potential, conductivity, and pH value. The zeta potential, conductivity, and pH were utilized as common parameters to establish the instability level of the surfactant formulation in this study [39]. However, temperature and concentration are also intensive factors that affect these parameters in individual surfactants [40] Hence, heating–cooling cycles were used to measure the stability of the model formulation in this study because, under long-term stability, as tested at 30 ± 2 °C and 75 ± 5% RH for 3 months, all the formulations were stable, possibly indicating that the incubation conditions used here were not critical and adequate for testing stability.

4.2. Identification of the Response Surface by RSM

Response surface methodology (RSM) is a tool for estimating effective factors and is used to design models by predicting the interactions among causal factors (surfactant concentration), latent variables (vesicle size, size distribution, zeta potential, conductivity, and pH), and response variables (stability parameters and antimicrobial activity). Moreover, RSM can be used to identify the optimal conditions of variables for desirable responses [41]. The interactions between surfactants and the complex behavior of surfactant mixtures still need to be investigated on a case-by-case basis because the physicochemical characteristics of the micellar formulation affect their effectiveness [42] and stability [43,44]. Proper causal factors were chosen in the study, which confirmed their significance via the preliminary experiment [45].

Almost all human organisms use a lipid barrier as a protection against the outer environment. In the case of hair, lipid barriers such as fatty acids, cholesterol, glycolipids, and ceramides play an active role in protection (e.g., preventing foreign material from entering and internal moisture loss). With routine washing with shampoo, these barriers may lose their function [28]. The surfactants in this study are practically safe and used by the CIR. Thus, we needed to characterize and understand the optimization techniques for simultaneously estimating the concentration of various surfactants. A proper no-rinse shampoo should be optimized to be as gentle to hair and eyes as possible with a minimal concentration of surfactants. The effectiveness and stability of the no-rinse shampoo was determined according to latent variables (vesicle size, size distribution, zeta potential, conductivity, and pH). Thus, the minimal concentration of surfactants to maximize the desirable responses of the latent variables was established.

The ideal vesicle size of nanocarriers for drug targeting is in the generally accepted range of 10–200 nm. A larger vesicle size may be beneficial in terms of drug reservoir accumulation at the target organ. Vesicles of a smaller size may have a better chance of penetrating the targeted organ, with those at the 40 and 50 nm nanoscale demonstrating the greatest effect [43]. Nanotoxicity should be examined [43] in further applications of these micellar shampoo formulations. In this context, i.e., the development of no-rinse shampoos, this nanoscale requirement for optimized penetration into hair follicles does not seem to be relevant due to the topical mode of application. However, a nanoscale vesicle size is beneficial with regard to the physicochemical stability of a formulation. The no-rinse shampoo obtained from RSM-3 had the lowest stability in vesicle size compared to that of the no-rinse shampoo obtained from RSM-1 and RSM-2 (Figure 4C).

Narrow size distribution results in the ideal dispersion of nanocarriers. The size distribution or polydispersity index (PDI) has been utilized as an index for the uniformity of nanocarrier formation. Formulations with monodispersed nanocarriers are highly stable, while those with a wider range of dispersion have lower stability. The formulation with a size distribution of 0.15 demonstrated high stability and retained homogeneous dispersion [46]. The size distribution of almost all the formulations in RSM-2 and RSM-3 showed narrow size distribution under 0.3 (Figure 3B,C). PDI values over 0.7 indicated that the vesicles had very broad size distribution. In lipid-based drug-delivery systems, a PDI of

0.3 and below is acceptable and indicates homogenous formulation [47]. However, the no-rinse shampoos obtained from RSM-1, RSM-2, and RSM-3 were stable in size distribution, as shown in the green area of the response surface in Figure 4. Size distribution was affected by the formulation factor (surfactant concentration) and the method of preparation (e.g., ultrasonication or agitation). In this study, the surfactant concentration and agitation technique may have been factors affecting size distribution. The possible relationship was reported in two ways; first, at low surfactant concentration, the size distribution was lower in the case of ultrasound techniques. Second, at high surfactant concentration, the size distribution was low in both techniques [48]. With low surfactant concentration and the agitation technique, the size distribution of some formulations was high.

The zeta-potential range of –10 to 10 mV was approximately neutral. A zeta potential of less than –30 or greater than 30 mV was considered to be strongly anionic and strongly cationic, respectively [39]. In this study, the formulations in RSM-1, RSM-2, and RSM-3 showed a negative charge between 0 and –30 mV, depending on their compositions (Figure 3). Although the primary composition of the shampoo formulation was aqua, the zeta potential was a total net charge of all compositions. KD, KT, and ES were nonionic, zwitterionic, and anionic surfactants, respectively. In this context, an essential oil, such as lavender oil, may affect the total net charge of the micellar formulation [49]. The surface charge or zeta potential may influence the dust particles repelled from hair. The relationship between electrostatic charge and dust particles is influenced by the intensity of the electric charge. Increased electric-charge intensity improved the amount of dust particles removed from the solid surface [50]. Stability was also highly dependent on the zeta potential of the nanocarriers [36,51]. A high zeta potential can prevent the aggregation of nanocarriers because of their strong repellent forces among carriers [52]. In the case of nonionic surfactants, steric stability can prevent the agglobulation of nanocarriers (e.g., nanoemulsions [53] and nanocapsules [54]), as in the present study. The zeta potential of the no-rinse shampoo obtained from RSM-1, RSM-2, and RSM-3 was not strongly anionic, but the stable formulation, as displayed in the green area of the response surface in Figure 4, may have been affected by steric stability. The zeta potential ranges of –5 to +5, –20 to +20, –30 to +30, and –60 to +60 mV had fast aggregation, short-term stability, good stability, and excellent stability, respectively. However, these ranges were valid for low-molecular-weight surfactants with a pure electric stabilizer. Neither of these is the case for high-molecular-weight stabilizers, which act mainly by steric stabilization. The zeta potential of $\pm$20 mV was the maximum desirable for a combination of electrostatic and steric stabilization [51]. Extreme zeta potentials, both negative and positive charge, can cause great repulsion, whereas repulsive forces between carriers with similar electric charge can prevent aggregation. As human hair and cellular membranes are negatively charged, positive nanocarriers can permeate membranes more than negative nanocarriers can. However, positive nanocarriers generally cause more toxicity related to the disruption of cell walls [39]. In this context, the negative charge of the no-rinse shampoo was appropriate for removing dust particles from the hair and scalp.

Several methods for critical micelle concentration (CMC) endpoint determination were introduced in a previous study [55]. Conductivity was used to identify the endpoint for the same type of surfactant as in micellization. The formation of micelles may be simulated by a conductivity measurement [39]. In this context, the CMC cannot be directly measured due to its mixed micelle behavior. However, the CMC was estimated by using a proper parameter [33]. Conductivity was an intrinsic factor that directly represented the physicochemical properties of the micellar formulation. This factor is also dependent on zeta potential and pH; thus, it was important to report the environmental conditions for accurate determination [39]. In this study, the conductivity of a mixed surfactant system was investigated. Conductivity was directly influenced by the surfactant (type and concentration), as shown in Figure 3. The difference in conductivity between Day 0 and Cycle 6 of the no-rinse shampoo obtained from RSM-1 fell in the green area less than that of RSM-2 and RSM-3 (Figure 4A–C). Conductivity could distinguish the stability of the

combined-surfactant formulations [38]. The results revealed that the mixed surfactant of RSM-2 and RSM-3 resulted in the formulation of a proper no-rinse shampoo.

The pH was reported to affect the stability of the nanocarriers. A shift in pH and/or surfactant concentration may alter the zeta potential, which promotes the repulsive forces between nanocarriers [36]. The pH was directly influenced by the surfactant (type and concentration), as shown in Figure 3. The alteration in surfactant type and concentration resulted in the precise transformation of the pH value. The difference in pH stability between Day 0 and Cycle 6 was within the acceptable green area of the response surface (Figure 4). The micelle in the no-rinse shampoo formulation was stable under the pH of its formulation. However, the physiological pH of hair fibers and the scalp is acidic, and the hair surface carries a negative charge [56]. A low-pH shampoo can minimize hair damage and promote scale tightening [30]. A high pH can induce hair swelling and cuticle-scale opening; thus, foreign or residual matter may remain in the deep hair cortex. However, in "no-tears" shampoos, the pH should be neutral (pH ~7.0) [57]. The standard pH of shampoos may depend on their application. The no-rinse shampoo obtained from RSM-2 had an appropriate pH for the "no-tears" concept, as shown in Figure 3.

Considering the physicochemical properties via the response surface of the vesicle size, size distribution, zeta potential, conductivity, and pH of the obtained model formulation from RSM-1, RSM-2, and RSM-3, the micellar formulation from RSM-2 obviously had good stability and the appropriate properties of a no-rinse shampoo. RSM is again helpful in predicting the physicochemical properties and stability of the no-rinse shampoo [31]. However, all the model formulations from RSM-1, RSM-2, and RSM-3 were optimized in a further study.

4.3. Formulation Optimization Using RSM

Desirability was applied for parameter optimization [58]. Accordingly, the determined quality characteristics of an optimal formulation were transformed into a dimensionless desirability index. The index of desirability was between 0 and 1. Index 0 corresponded to a completely undesirable response, while Index 1 was attributed to an absolutely desired response of the optimization [59]. The predicted optimal formulations of the no-rinse shampoo of RSM-1, RSM-2, and RSM-3 are shown in Table 2. The desirability index of the optimal formulations of no-rinse shampoos RSM-1, RSM-2, and RSM-3 were 0.69, 0.66, and 0.80, respectively (Figure S3). In most cases, a desirability index over 0.7 is accepted [60]. Please see Appendix C for more information. However, the application of desirability approaches may be combined with a traditional experimental design when the behavior of the complicated relationship is studied [61,62]. Thus, the experimental shampoo formulation was prepared to confirm the reliability of the optimal values, as shown in Table 3. The percentage bias was calculated to ensure the accuracy and reliability of the optimal values. The results indicated that the zeta potential, conductivity, pH, and foamability of the experimental values of RSM-1, RSM-2, and RSM-3 were lower than the 10% bias, while the percentage biases of the vesicle size and size distribution were more than 10%. Considering the exhibited foamability in Figure 5, the optimal formulation of RSM-2 was selected for further study as an appropriate formulation due to its stable foam.

4.4. Comparative Study

The experimental RSM-2 optimal formulation of no-rinse shampoos was compared to a commercial dry shampoo. The antibacterial and antifungal effects of the dry shampoo (DS) are shown in Table 4. The results indicated that DS4 with ERW had antibacterial (*E. coli*, *S. epidermidis*, and *S. aureus*) and antifungal (*C. albicans*) effects, although the mixture was characterized by a low surfactant concentration. Surfactants are used in cleaning products to dissolve dirt particles, as foaming enhancers, and for their antimicrobial properties. The dry shampoo formulations used KT and ES as zwitterionic and anionic surfactants, respectively. To determine microbial activity, general canine and feline pathogens, namely

Gram-negative (*E. coli*) and -positive (*S. aureus* and *S. epidermidis*) bacteria and a fungus (*C. albicans*) were used as the model organisms [63,64].

Compared with no treatment (normal saline solution), all the formulas had antibacterial and antifungal effects. In *E. coli* and *S. epidermidis*, all the formulations (DS1–DS5) reduced the number of pathogen cells to 0 and had an effect for 24 h of treatments, while the antibiotic activity gradually reduced cell viability along with incubation time. The presented mechanism means that the surfactant kills pathogens in an initiation period of explosion related to cell-structure degradation. However, some pathogens, such as *S. aureus* and *C. albicans*, might need a longer contact time or a higher concentration to die. A previous study showed that both surfactants have antimicrobial activity with a variation in pathogen cell type. The minimal inhibitory concentration (MIC) of KT is more than 90 mg/mL for *Pseudomonas aeruginosa*, *E. coli*, and *Enterococcus hirae* and 0.06–1.5 mg/mL for *S. aureus* [65], while it showed the strongest antifungal activity with a 0.075–1.5 mg/mL MIC for *Malassezia restricta* [66]. ES is a member of an amino acid base surfactant group that also exhibits antimicrobial activity with an MIC of 1.68, 46.0, and 1.68 mg/mL for *S. aureus*, *E. coli*, and *C. albicans*, respectively [67]. Enhanced activity was found in gradually increased concentrations of KT and ES. The DS3 formula had a higher number of viable cells than that of DS1 in each incubation time and in all pathogens. The concentration of the surfactant mixture could be the factor of microbial activity because DS1 comprised 2.3% KT and 3.7% ES, which was greater than the 0.5% KT and 3.0% ES in DS3. DS1 and DS4 were found to be the most appropriate shampoo formulations in this study. Please see Appendix D for more information.

Although KT and ES are gentle surfactants, increases in concentration were limited by irritation and toxicity effects. The structure of surfactants used is shown in Figure S4. A previous study reported that ERW itself is also antibacterial [32]. The bactericidal action of ERW is suggested by its hydroxide ion (OH–) level and low oxidation reduction potential [68]. A combination with ERW increased antimicrobial activity in a low surfactant concentration mixture (DS4) and might be an additive or synergistic effect. The pH of ERW is 8 to 10, which makes it an alkaline solution that can facilitate the interface affinity between microbial and anionic surfactant ES [69]. Thus, the application of this no-rinse shampoo's formulation may prevent pathogen infection by both bacteria and fungi. Adding ERW enhanced the antimicrobial activity of the formulation.

This study investigates the surface and structure of human hair using SEM and AFM. The morphology and topography image of the cuticle layer on the hair surface of the untreated and treated hairs are presented. The results indicate that the cuticle layer of the treated hair fiber was not damaged and modified. However, the composition of DS1 may deposit on the cuticle surface as a no-rinse procedure.

5. Conclusions

The findings provided basic information on the effect of a coconut-based surfactant mixture and ERW on a no-rinse shampoo using the response surface method. The results obtained from the experimental formulations confirmed the ability of RSM to estimate the responses in terms of physicochemical properties and the stability of the formulation, which could reduce the number of measurements required to simultaneously determine the effects of combining surfactants. RSM-2 was a successful optimal shampoo formulation in our study, composed of a coconut-based surfactant at a low surfactant concentration mixture of 3.5% with ERW and a high surfactant concentration mixture of 6%, and may prevent infection by *E. coli*, *S. epidermidis*, *S. aureus*, and *C. albicans*. The used concentration was also not exceeded for the leave-on products. With the design and optimization of a new no-rinse shampoo, some limitations were resolved. Hygiene is important during the current COVID-19 pandemic. Daily hair washing for the elderly, bedridden, and ICU patients would be more readily adopted. Thus, no-rinse shampoos may improve the quality of life of the caregivers. The use of ERW may be expanded to no-rinse shampoos. Safety via

the skin irritation test and the efficacy of the no-rinse shampoo should be further studied with elderly, bedridden, and ICU patients.

6. Patents

There are petty patents resulting from the work reported in this manuscript.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cosmetics9050104/s1, Figure S1: the physicochemical properties and the stability of different single-surfactant shampoo formulations: (**a**) KD, (**b**) KT, and (**c**) ES.% Different = (Day 0 − Cycle 6)/Day 0 × 100%, ≠ — ≥ 100% different, Figure S2: the diagnostic plot of (1) the normal plot of residuals and (2) the predicted vs. actual and (3) residual vs. run of (**a**) RSM-1, (**b**) RSM-2, and (**c**) RSM-3, Figure S3: the desirability index of the optimal formulations of no-rinse shampoos RSM-1, RSM-2, and RSM-3, Figure S4: structure of (**a**) cocamide diethanolamine, (**b**) cocamidopropyl betaine, and (**c**) sodium cocoyl glycinate.

Author Contributions: Conceptualization, S.D. and P.S.; methodology, S.D., P.S., S.B. and T.N.; software, S.D. and K.T.; validation, S.D., P.S. and S.B.; formal analysis, S.D.; investigation, S.D., P.S. and S.B.; resources, S.D.; data curation, S.D.; writing—original draft preparation, S.D.; writing—review and editing, S.D., P.S., S.B., T.N. and K.T.; visualization, S.D. and T.N.; supervision, S.D. and T.N; project administration, S.D.; funding acquisition, S.D. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Human hair study was performed at Ubon Ratchathani University and met the requirement of the Ethics of Human-related Research Committee of Ubon Ratchathani university (ID no. UBU-REC-84/2565), and the study conformed to the following recognized standards: Declaration of Helsinki, the Belmont Report, the Council for International Organizations of Medical Sciences (CIOMS) guidelines, and the International Conference on Harmonization in Good Clinical Practice (ICH-GCP). The animal study was performed at Ubon Ratchathani University and met the requirement of the Ethics Committee for the Care and Use of Laboratory Animals (ID no. 51/2563/IACUC).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

The physicochemical properties and the stability of different single-surfactant shampoo formulations: (a) KD, (b) KT, and (c) ES are shown in Figure S1. The results indicated that the percentage of the difference between Day 0 and Cycle 6 of almost all the single-surfactant formulations was over 100%. The single-surfactant formulations were less stable than the combined-surfactant formulation under the accelerated condition (Day 0 and Cycle 6)

Appendix B

The diagnostic plots of (1) the normal plot of residuals and (2) the predicted vs. actual and (3) the residual vs. run of (a) RSM-1, (b) RSM-2, and (c) RSM-3 are shown in Figure S2.

Appendix C

The desirability index of the optimal formulations of the no-rinse shampoos RSM-1, RSM-2, and RSM-3 is shown in Figure S3.

Appendix D

The structure of (a) cocamide diethanolamine, (b) cocamidopropyl betaine and (c) sodium cocoyl glycinate is shown in Figure S4.

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