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"Innovative Methodology for Scientific Validation: Assessing Water-Saving Claims of 'Easy to Rinse' Hair Dye Through Initial In Vivo Observations"

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1. Introduction

Cosmetic products manufacturing requires a considerable amount of water consumption across all stages of the product life cycle. In recent years, many companies in the cosmetic industry have increasingly committed to reducing the water footprint (WFP) of their products [1]. The WFP is a comprehensive indicator that evaluates the impact of both producers and consumers on water resources by quantifying water use and pollution throughout the entire supply chain [2].

A study conducted by Cosmetics Europe on the Product Environmental Footprint of Shampoo revealed that only 5% to 20% of the total environmental impact of the life cycle of a shampoo is associated with raw materials, manufacturing, distribution, and packaging. Most of the impact occurs during the consumer-use phase, particularly during product application and disposal, underscoring the importance of strategies aimed at reducing water use in this critical stage [3].

The present study investigates the rinsing phase of a novel, eco-sustainable hair dye formulation. Preliminary *in vivo* assessments, conducted across 13 professional salons and comprising 153 individual test cases, revealed a significant reduction in rinsing time compared to standard alkaline hair dyes. Based on these initial findings, the objective of this work is to develop customized instrumental methodologies to quantitatively evaluate the potential water savings associated with the use of the new formulation.

The selection of analytical techniques was guided by the physicochemical properties of the product [4]. To quantify the release of hair dye from coloured natural hair strands during rinsing, spectrophotometric analysis in the UV-VIS range (200-800nm) was employed. This method enabled the monitoring of dyes concentration in rinse water, providing direct and quantitative data on colour release dynamics [5]. Surface interaction properties between the hair dye and the hair fiber were assessed by measuring the zeta potential (mV) and isoelectric point of coloured natural hair strands. These measurements offered insights into electrostatic interactions influencing hair dye retention and rinsability [6][7]. Furthermore, pH and conductivity (mS/m) are parameters usually employed in cleaning validation processes (FDA 7/93). In this work these parameters in the rinse water were continuously monitored during the rinsing process. These parameters served as an indirect indicator of the presence of the formulation in the rinse water, with higher values correlating with increased release of product from the hair fibers [4]. Together, these analytical approaches provided a comprehensive understanding of the rinsing behavior of the system.

The methodologies presented herein support the "easy to rinse" product claim, establish a robust framework for the evaluation of rinsability in cosmetic formulations, and contribute to the development of future products with improved environmental sustainability, enabling objective comparison with benchmark or alternative formulations.

2. Materials and Methods

An innovative eco-designed oxidative hair dye formulation (*Coloration A*) was evaluated in combination with its designated hydrogen peroxide-based developer. For comparison, four additional oxidative hair dyes – *Coloration 1*, *2*, *3* and *4* – were tested with their respective oxidizing emulsions. All experiments were performed on natural level 5 human hair strands (20 x 4.5 cm), supplied by Imhair S.r.l. (Italy). Prior to dye application, the strands were pretreated using bleaching powder mixed with the appropriate hydrogen peroxide developer, following the manufacturer's recommended mixing ratio. The dye mixtures (*Coloration* + developer) were prepared and applied on hair strands according to standard manufacturer guidelines, using an analytical balance, appropriate application tools, and a digital timer to ensure consistent processing times. Following the colour development phase, the dyed hair strands were rinsed under agitation in 200 mL of distilled water for 2 minutes. This rinsing step was repeated in another 200 mL volume of water until the rinse water appeared visually clear. Aliquots of the rinse water were collected after each wash cycle for subsequent analysis.
UV-Vis spectrophotometric analysis was performed across the 200-800 nm wavelength range (UV-Vis Shimadzu 1900, Italy). For each formulation analyzed, a stock solution was prepared to determine the maximum absorbance wavelength (λ max), which was then used to monitor dye release in the rinse water over successive washes. The percentage of product washed was calculated based on the decrease in absorbance at λ max. Additionally, the pH and conductivity (mS/m) of the rinse solutions were measured using benchtop pH and conductivity meters (XS Instruments, Italy), immediately after collection.
To assess changes in the hair fiber surface charge, the dyed hair strands were categorized into wet and dry groups and analyzed using an electrokinetic analyzer (SurPASS 3, Anton Paar GmbH, Austria). For the characterization of surface charge, a portion of treated hair strand (600–700 mg) was mounted in the Cylindrical Cell of the SurPASS 3 electrokinetic analyzer (Anton Paar GmbH, Austria). Measurements were conducted using:
0.003 mol/L KCl solution for the evaluation of the native hair-water interfacial charge and to evaluate the interaction with the cosmetic formulations.
The zeta potential (ζ) was calculated based on the Helmholtz–Smoluchowski equation (Eq. 1):

$$\zeta = \frac{\mathrm{d}U_{str}}{\mathrm{d}\Delta p} \times \frac{\eta}{\varepsilon_{rel} \times \varepsilon_0} \times \kappa_B$$

$dU_{str}/d\Delta p$ is the streaming potential coupling coefficient calculated as the slope of the linear dependence of the streaming potential U_{str} on the pressure gradient Δp applied between both ends of the permeable hair plug, η and ε_{rel} are the dynamic viscosity and the dielectric coefficient of water, ε_0 is the permittivity of free space, and κ_B is the electric conductivity of the aqueous solution [6][7].

Zeta potential (mV) measurements were recorded at baseline (t0), after 5 minutes (t5min) and after 10 minutes (t10min) of immersion in electrolyte solution. Bleached but undyed hair strands served as negative control. Each sample was measured in triplicate, and the mean values were reported. Zeta potential (mV) was also determined as a function of pH (ranging from 6.0 to 2.0) in order to identify the isoelectric point of each treated hair sample.

3. Results

3.1 SurPASS 3 Analysis

The strands of each group, group A (dry strands) and group B (wet strands), are analyzed to obtain the following parameters: pH, conductivity (mS/m) and zeta potential (mV) at initial time (t0), after 5 minutes (t5min) and after 10 minutes (t10min). The results obtained from the analysis of the dyed strands are compared with those obtained from the analysis of the bleached strand used as a baseline.

3.1.1 Zeta-potential (mV)

The results of Zeta potential (mV) obtained with SurPASS 3 of Group A and B are shown in tables 1 and 2, respectively.

Table 1. The table shows the averages of the 3 replications performed for each sample of Group A.

TIMING	BASELINE	COLORATION A	COLORATION 1	COLORATION 2	COLORATION 3	COLORATION 4
t0	0,40	-1,33	-1,09	-1,15	-2,47	-1,82
t5min	0,86	7,40	7,32	6,11	4,19	3,22
t10min	0,79	6,58	6,12	5,17	3,57	2,67

Table 2. The table shows the averages of the 3 replications performed for each sample of Group B.

TIMING	BASELINE	COLORATION A	COLORATION 1	COLORATION 2	COLORATION 3	COLORATION 4
t0	0,40	-1,65	-0,50	-1,50	-1,73	-1,45
t5min	0,86	3,95	3,34	3,69	4,07	6,00
t10min	0,79	3,82	2,79	3,73	3,26	5,15

3.1.2 pH

The results of pH obtained with SurPASS 3 of Group A and B are shown in tables 3 and 4, respectively.

Table 3. The table shows the averages of the 3 replications performed for each sample of Group A.

TIMING	BASELINE	COLORATION A	COLORATION 1	COLORATION 2	COLORATION 3	COLORATION 4
t0	6,44	6,11	6,23	6,09	6,05	5,83
t5min	6,10	6,21	6,35	6,19	6,10	5,97
t10min	6,74	6,26	6,39	6,24	6,16	6,01

Table 4. The table shows the averages of the 3 replications performed for each sample of Group B.

TIMING	BASELINE	COLORATION A	COLORATION 1	COLORATION 2	COLORATION 3	COLORATION 4
t0	6,44	5,90	5,79	6,09	6,06	6,08
t5min	6,10	5,58	6,05	6,16	6,17	6,14
t10min	6,74	6,05	6,09	6,20	6,21	6,19

3.1.3 Conductivity (mS/m)

The results of conductivity (mS/m) obtained with SurPASS 3 of Group A and B are shown in tables 5 and 6, respectively.

Table 5. The table shows the averages of the 3 replications performed for each sample of Group A.

TIMING	BASELINE	COLORATION A	COLORATION 1	COLORATION 2	COLORATION 3	COLORATION 4
t0	15,35	12,72	15,18	14,68	15,12	15,42
t5min	11,49	9,38	9,91	10,96	11,18	13,17
t10min	12,69	9,31	9,67	9,60	10,17	11,38

Table 6. The table shows the averages of the 3 replications performed for each sample of Group B.

TIMING	BASELINE	COLORATION A	COLORATION 1	COLORATION 2	COLORATION 3	COLORATION 4
t0	15,35	14,18	15,34	15,48	15,05	13,99
t5min	11,49	8,76	10,71	9,60	13,70	9,61
t10min	12,69	8,41	9,15	9,48	11,92	8,60

3.1.4 Isoelectric point

From a pH- Zeta potential (mV) graph, the isoelectric point of each treated strand was calculated and compared with the value of the baseline strand. The analysis was performed in a pH range between 6 and 2 and the results are reported in table 7.

Table 7. The table shows the isoelectric point of each strand.

	BASELINE	COLORATION A	COLORATION 1	COLORATION 2	COLORATION 3	COLORATION 4
pH	4,87	3,05	3,74	3,32	-	3,01

3.2 Spectrophotometric analysis

A stock solution is prepared for each sample, and it is analyzed in a spectrophotometer to obtain the maximum absorption wavelength that will be used to analyze subsequent rinse solutions. Spectrophotometric analysis of the rinse water of dyed strands allows the % release to be calculated by monitoring the decrease in concentration of the sample within the water. The results are summarized in figure 1 below, reporting the ratio of the quantity of color eliminated from *Coloration A* with rinsing after 200 and 600 ml, compared to other Colorations. Based on the data obtained, for the same rinse volume (200mL or 600mL), the effectiveness of the *Coloration A*, in terms of ease of rinsing, is higher than the other samples.

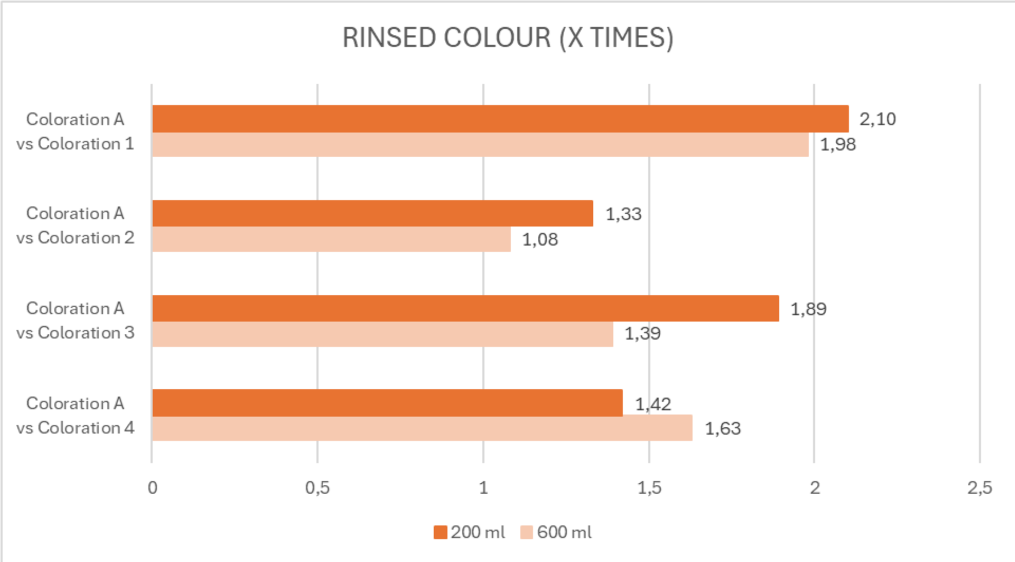

Figure 1. Confrontation between *Coloration A* and other Colorations by evaluating the amount of dye washed related to the volume of rinse water.

3.3 pH and conductivity (mS/m) analysis

An aliquot of each volume of rinse water is collected to perform pH and conductivity (mS/m) analysis and the variation in these parameters, due to the presence of the dye released by the strand, is evaluated. The results relating to the volume of water necessary for each sample to bring the pH and conductivity values back to the initial conditions are reported in table 8.

Table 8. The table shows the volumes of rinse water necessary to bring pH and conductivity values back to initial conditions, for each sample analyzed.

PARAMETER	COLORATION A	COLORATION 1	COLORATION 2	COLORATION 3	COLORATION 4
pH	7,69	7,70	7,70	7,78	7,69
CONDUCTIVITY (mS/m)	29,88	28,78	28,24	26,89	28,44
VOLUME (ml)	600	1600	800	1200	1000

These results can be expressed as the percentage of water saved using *Coloration A* compared to the other tested Colorations and it was calculated according to the following formula (Eq. 2):

$$\% \text{ Water Saved} = [(VolCi-VolCA)/VolCi]x100$$

Where VolCi represents the volume of water required to completely rinse *Coloration 1*, *2*, *3* or *4* from the hair strands, and VolCA represents the volume of water required to completely rinse *Coloration A* from the hair strands.

The average percentage of water saved was determined by calculating the arithmetic mean of the individual percentage obtained for each comparison. Overall, the use of *Coloration A* resulted in an average water saving of 41.25% compared to the other Colorations.

3.4 Panel tests results

Prior to the main study *in vivo* assessments were conducted across 13 professional salons globally. More than 150 evaluations were performed by expert hair professionals, demonstrating positive outcomes regarding the key performance indicator “easy to rinse” and “fast to rinse”. Following the instrumental analysis, additional *in vivo* assessments were carried out, once again yielding favorable results. In total, the study encompassed over 180 *in vivo* evaluations.

These findings reinforce the robustness of the full study and provide strong support for the final product claim: *in vivo* test (consumer test) allow to understand the perceived performance of the product in a consumer-relevant context.

4. Discussion

The aim of this study was to provide analytical support to *in vivo* evidence concerning the rinsing ease of a new eco-sustainable hair dye formulation. To this end, targeted analytical methodologies were employed, selected according to the specific physicochemical properties of the tested product, with the goal of objectively validating the “easy to rinse” claim.

Among the techniques used, spectrophotometric measurements were used to quantify the concentration of pigments released into the rinse water. The observed decrease in pigments concentration with each wash allowed for a precise assessment of colour washed and, consequently, the rinsability of the product from the hair. This approach offered objective insight into the release kinetics and rinsing efficiency of the product. Further support was provided by data on pH and conductivity (mS/m) of the rinse water. Changes in these parameters, associated with the presence of residual product, delivered indirect yet consistent confirmation of the spectrophotometric findings. In particular, the correlation between elevated pH and conductivity (mS/m) values and higher hair dye concentrations in the rinse water suggests a more efficient rinsability of the hair dye final product from the hair. Moreover, the combined analysis of pH and conductivity (mS/m) values of the rinse water enabled the estimation of an average water saving of 41.25% during rinsing, compared to the amounts typically required by conventional alkaline hair dyes.

An additional level of analysis was provided by zeta potential (mV) analysis permitting to determine the isoelectric point of the hair fiber and the electrostatic interaction between the hair dye and the hair surface. Monitoring these parameters after repeated washes revealed the integrity of hair fibers before and after treatment and the degree of the interaction between the hair dye and the hair fiber, indicating the performance of the product over time. The choice of analytical methods investigated in the study was closely linked to the characteristics of the tested formulation. For example, the presence of colored pigments justified the use of spectrophotometry, while the highly basic nature of the hair dye made pH monitoring particularly relevant.

While these techniques proved effective in this context, their application to other categories of cosmetic products, especially those with different physicochemical profiles, may require significant adaptation.

Overall, the study offers an innovative contribution to the evaluation of cosmetic products from both functional and sustainability perspectives. The described methodologies provide a reproducible framework for the assessment of eco-friendly formulation and the validation of functional claims such as “easy to rinse” claim. This analytical integrated approach establishes a robust model for future screening of cosmetic products in support of sustainable innovation.

5. Conclusion

The result of this study, obtained through a focused and integrated analytical approach, confirmed the rinse efficiency highlighted during panel testing of a new environmentally sustainable hair dye formulation. Key methodologies – including zeta potential (mV) analysis, hair fiber isoelectric point determination, spectrophotometry and the monitoring of pH and conductivity (mS/m) – demonstrated a quantifiable reduction in water consumption, with an average decrease of 41.25% compared to the market average for alkaline hair dye systems.
These findings substantiate the “easy to rinse” product claim and underscore the value of the proposed analytical framework as a robust and reproducible tool for evaluating both functional performance and environmental impact in cosmetic formulations.

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