

# Evaluation of efficacy of permanent hair dyes – intense red

## Abstract

**Introduction:** The aim of this work was to evaluate persistence of red-colored hair dyes and possible damage caused due to protein losses and due to hair thermal resistance.

**Methodology:** All analyses were carried out by means of diffuse reflectance spectrometry, thermogravimetry and derivative thermography (TG/DTG); color measures were assessed using the CIELCH (L\*C\*h\*) scale.

**Results and discussion:** Results demonstrated that dye B on virgin and discolored hair caused greater protein losses (202.49% and 51.86%, respectively) than dye A on virgin and discolored hair (93.67% and 38.11%, respectively). Thermogravimetry analysis demonstrated that dye A had a higher thermal resistance to protein denaturation than dye B on both virgin (difference of 4.63°C) and discolored (difference of 2.42°C) hair. Results also demonstrated that dye A faded faster than dye B, as spectrophotometric analysis showed dye B persisted throughout 15 washes and also had lower values for luminosity and saturation than dye A.

**Conclusion:** As dye A had higher values of saturation and luminosity, color fading became perceptible after 10 washes.

**Keywords:** hair dye, color persistence, hair color fading, protein loss

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Camila Skaf Maia,<sup>1</sup> Robson Miranda da Gama,<sup>2</sup> Carla Aparecida Pedriali Moraes<sup>1</sup>

<sup>1</sup>Laboratório de Pesquisa, Tecnologia em Cosméticos, FATEC Diadema-Luigi Papaiz, Brasil

<sup>2</sup>Laboratório de Pesquisa do Curso de Farmácia, Faculdade de Medicina do ABC, Brasil e LESIFAR Laboratório Escola Semi Industrial de Farmácia, Universidade Santo Amaro, Brasil

**Correspondence:** Carla Aparecida Pedriali Moraes, FATEC Diadema – Luigi Papaiz, Laboratório de Cosméticos, 483 Luiz Merenda Avenue, Diadema, 09931-390 São Paulo – SP, Brazil, Tel +5501 14 09 22328, Email [capedriali@hotmail.com](mailto:capedriali@hotmail.com)

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## Introduction

Oxidative dyes are complex formulations, as they must provide intense and persistent color to hair; at the same time, their compounds must ensure hair fibers suffer the least amount of damage.

The human hair is constituted by four main structures: cuticle, cell membrane complex, cortex and medulla with its subunits.<sup>1,2</sup> The cuticle is the most external structure, acting as a barrier protecting cortex and medulla from chemical substances.<sup>1-3</sup> The cell membrane complex is responsible for cell cohesion, while the cortex confers mechanical properties to the hair fiber.<sup>1,2</sup>

Permanent oxidative hair dyes are constituted of three main components: an oxidizing substance in an alkaline environment (hydrogen peroxide and ammonia), precursor compounds and coupling agents. Precursor compounds are oxidized by the oxidizing substance leading to release of oxygen, and then are combined with coupling agents producing the desired color. The molecular size of this complex must be high enough for it not to be easily released by the cortex.<sup>4</sup> Permanent hair dye is the only type of dye capable of lightening and darkening the natural color of hair up to four tones; this happens due to opening of cuticles, which can also, on the other hand, lead to reduction of hair softness, resistance and brightness, causing the hair to look “unhealthy”.<sup>5,6</sup>

The aim of this work was to evaluate persistence of red hair dyes and possible damage caused to hair fibers due to protein loss and due to thermal resistance. Analyses were carried out using diffuse reflectance spectrophotometry, thermogravimetry and derivative thermogravimetry (TG/DTG); color measures were determined

using the CIELCH (L\*C\*h\*) scale. Permanent dyes which confer intense red color were preferred for studying, as their fading speed in comparison to other dyes are faster and there is still a lack of studies in scientific literature dedicated to investigation of such types of dyes.<sup>7</sup>

## Materials and methods

### Materials

Due to commercial reasons, brand names were not divulged and thus were labeled simply A and B. The kits for dye A and B consisted of a flask containing oxidizing substance of 20 volumes, a tube for cream color, a sachet containing a concentrate made of fruit and flower oils (dye A) and a sachet containing mask for conditioning treatments (dye A and B). All other reagents were of analytical grade and were purchased from either Sigma<sup>®</sup> or Merck<sup>®</sup>.

### Sample preparation and discoloration

A total of 30 hair samples obtained from Asian individuals were used in the study; they weighted about 1g each and had approximately 20 cm of extension each. Half of the hair samples were subjected to a discoloration procedure. For such, a paste containing a mixture of 100 mL hydrogen peroxide (volume 30) and discoloring powder (50g) at a 2:1 ratio was used.<sup>8</sup>

### Dye application and washing

Dye application was carried out according to manufacturer instructions: the contents of the tubes containing hair dye were mixed with the contents of the flask containing oxidizing agent (20 volumes); the sachet containing oils was mixed was well when the kit

A was used. A total of 5.0g of the mixture was applied to hair samples, which were allowed to rest for 30 minutes. Next, the hair samples were washed with water at  $37.0 \pm 5.0^\circ\text{C}$  for some seconds. A solution of 15% sodium lauryl sulfate (2mL) was then applied to hair samples by gentle massaging locks of hair with fingertips for 30 seconds. Hair samples were again washed with water flowing at a rate of 1.8L/min until all detergent was removed. Hair conditioning substances present in the kits were used to condition locks of hair, which were then again washed and allowed to dry at room temperature under a relative humidity of  $60\% \pm 5\%$  for 48 hours.<sup>1</sup>

### Diffuse reflectance spectrophotometry and statistical analysis

Color of locks of hair was assessed with the aid of a reflectance colorimeter Konica Minolta CM 3700-A, model CIELCH ( $L^*C^*H^*$ ), capable of numerically representing the most recent color assessed, developed by X-Rite.

A total of five readings along the hair fiber were performed. Hair samples colored with hair dye A or B were washed 1, 5, 10 or 15 times. Results were statistically analyzed with the aid of software OriginPro® 8.0 SR0 (OriginLab, Northampton, USA). Results obtained for luminosity ( $L^*$ ), red-green ( $a^*$ ) and yellow-green ( $b^*$ ) coordinates, chroma ( $c^*$ ) and hue ( $h^*$ ) were subjected to parametric analysis, the means corresponding to each parameter assessed being compared by univariate analysis (one-way ANOVA). Results for each sample were compared regarding variance between samples, standard-deviations and variations due to washes.<sup>9</sup>

### Evaluation of protein loss

A total of 50mg of hair samples, weighted on an analytical balance (Marte-AD500-500g/1mg), were placed into a 250mL Erlenmeyer flask containing 15.0 mL of distilled water, which was then subjected to sonication using a TransSonic T460 (Elma – Brazil) for 40 minutes. Next, the solution was filtered with filter-paper and a 2.0mL aliquot was used for quantification of protein loss. The Lowry method modified by Peterson used for quantification of proteins involves the transfer of 2.0mL of a stock-solution of albumin to test tubes, which is then mixed with solution a for 15 minutes using a minishaker at 1000rpm, followed by a 10 minute resting time. Next, 1.0mL of the previously prepared Folin-Ciocalteu reagent is added to the solution, which is then agitated for 15 seconds followed by a 30 minutes resting time, protected from light.<sup>10</sup> The same procedures were carried out with previously processed hair samples. After reactions have ended, solutions were subjected to readings by UV-vis spectrophotometry (Biospectro SP) at 750nm.<sup>10,11</sup> Readings for each dilution were carried out in quadruplicates; distilled water was used in order to obtain blank readings.

### Thermogravimetry (TG) and derivative thermogravimetry (DTG) analysis

An amount between 0.5 and 1.0g of sample was placed in the thermal balance of the equipment (Shimadzu DTG-60); samples remained under dynamic air atmosphere ( $50\text{mL}\cdot\text{min}^{-1}$ ) and were heated at a  $10^\circ\text{C}/\text{min}^{-1}$  ratio starting at a temperature of  $25^\circ\text{C}$  until an endpoint temperature of  $800^\circ\text{C}$  was reached. The methodology here employed is an adaptation of the method described by Gama (2010).<sup>10</sup>

## Results

### Evaluation of color persistence

The hue is synonymous of color, nuance or tone. This term refers to wavelengths of both directly emitted and reflected light, as seen by the human eye: main colors red and blue and other colors as results of the mixture of this main colors<sup>(12)</sup>. Figure 1(a) and 1(b) show results regarding analysis of color persistence in hue graphs. These images refer to wavelengths of both directly emitted and reflected light, as seen by the human eye: main colors red and blue and other colors as results of the mixture of this main colors.<sup>12</sup>

Statistically significant differences were observed for samples A1 and B1 when assessing color fading at the 1<sup>st</sup>, 10<sup>th</sup> and 15<sup>th</sup> washings. These samples suffered darkening at the 5<sup>th</sup> washing.

Hair samples colored with dye A suffered hair color tone variations as washings were carried out. No statistical differences were observed between samples at the 10<sup>th</sup> washing when compared to natural hair samples (N), indicating that the dye was being continuously removed throughout the washings.

The colors of samples A2 and B2 (b) were statistically different; the color of samples A2 and B2 after the 10<sup>th</sup> washing when compared to the color of the same samples prior to previous washings was also statistically different (1<sup>st</sup> and 5<sup>th</sup> washings). The hue of sample A2 increased 22.0%, while this increase was of 25.73% for sample B2, but the average hue values for dye A were still 32.01% higher than the values for dye B.

The resulting color when virgin hair (N) was treated with dyes A and B was dark, as a result of the ability of the human eye to detect light and dark combined with color saturation. However, previously discolored locks of hair when treated with dye A were brighter. The final resulting red colors when locks of hair are treated with dyes A2 and B2 are very distinct from each other, the red color being more intense for dye A2 than dye B2 due to color saturation, as it influences the intensity by which the color is perceived by the human eye.

### Evaluation of protein loss

Results obtained regarding protein loss demonstrated that dye A caused a lesser protein loss from the cuticle when applied to virgin (93.67%) or discolored (38.11%) hair than dye B when applied to virgin (202.49%) or discolored (51.86%) hair, when compared to standard samples N (natural hair-100%) and D (discolored hair-100%). Samples D, A2 and B2 had a protein loss of 202.55%, 279.74% and 307.60%, respectively, when compared to a virgin hair sample (N). When compared to reference standards of discolored hair (D), the same samples had a smaller loss of protein, as described in Table 1; this happened due to previous discoloring damaging the hair fiber, which in turn allowed better penetration of the dyes as the structure of the cuticle had been weakened. Results regarding assessment of protein loss are described in Table 1.

### Thermal analysis

Results regarding thermal analysis are described in Table 2. The first event refers to loss of water, which occurred at temperatures ranging from  $55.47$  to  $65.45^\circ\text{C}$ ; loss of mass (loss of locks of hair) varied from 9.70 to 12.32%. The second event was observed at temperatures ranging from  $232.07$  to  $236.52^\circ\text{C}$ , and respective loss of

mass varied from 19.06 to 23.67%, as demonstrated in Table 2.

Thermogravimetry results evidenced dye A had a higher resistance to temperature (A1 9.57% at 66.37°C and A2 12.32% at 59.16°C) than dye B (B1 9.87% at 56.70°C and B2 11.35% at 55.47°C). It was also verified that dye A1 was more resistant to temperature than virgin hair by itself (N) (9.78% at 65.45°C). The greatest difference of loss of mass in percentage between samples was of 22.32%, and the greatest temperature difference was of 10.9°C (55.47 to 66.37°C).

The second event refers to keratin denaturation at the beginning of thermal degradation; at this point, the temperature reached was close to 235°C. Natural hair and discolored hair had losses of mass of 21.83% and 22.47%, respectively. A1 and A2 samples had losses of mass of 23.67% and 19.06%, respectively. Samples B1 and B2, on the other hand, reached lower temperatures, 232.07°C (23.54%) and 234.10°C (22.57%), respectively. The loss of mass was more evident at the second event due to keratin degradation, which is the most abundant protein found in hair fibers.<sup>13,14</sup>

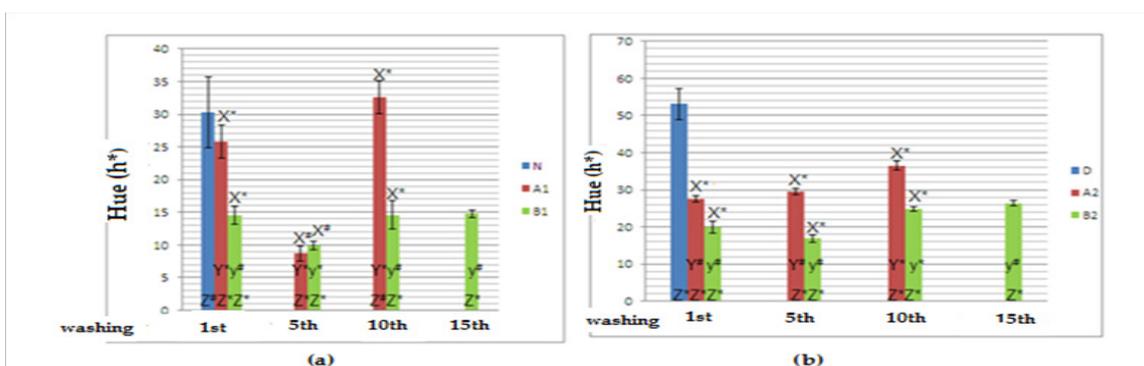


Figure 1 Hue graphs obtained from dyed virgin (a) and discolored (b) locks of hair.

N, natural hair; D, discolored hair; A1, virgin hair treated with dye A; A2, discolored hair treated with dye A; B1, virgin hair treated with dye B; B2, discolored hair treated with dye B; X, comparison between hair samples treated with dyes A and B; Y, comparison between hair samples treated with dye A throughout several washings; y, comparison between hair samples treated with dye B throughout several washings; Z, comparison between treated hair samples and the standard reference sample; # no statistical significance, considering  $\alpha=5\%$ ;  $p \leq 0.05$ ,  $n=5$  for all samples; \*statistical significance, considering  $\alpha=5\%$ ;  $p \leq 0.05$ ,  $n=5$  for all samples.

Table 1 Protein loss by hair samples as assessed by the Lowry method

Samples	mg/g of hair	%
N	0.03762	100
A1	0.07286	93.67
B1	0.1138	202.49
D	0.0762	100
A2	0.10524	38.11
B2	0.11572	51.86

Abbreviations: N, natural hair; D, discolored hair; A1, virgin hair treated with dye A; A2, discolored hair treated with dye A; B1, virgin hair treated with dye B; B2, discolored hair treated with dye B

Table 2 Results obtained from thermal analysis at 1<sup>st</sup> and 2<sup>nd</sup> events

Samples	mg of hair	1 <sup>st</sup> event loss of mass (%)	1 <sup>st</sup> event temperature peak (°C)	2 <sup>nd</sup> event loss of mass (%)	2 <sup>nd</sup> event temperature peak (°C)
N	6.95	-9.70	65.45	-21.83	235.83
A1	8.86	-11.23	63.23	-22.47	235.75
B1	10.87	-9.57	66.37	-23.67	235.09
D	8.15	-12.32	59.16	-19.06	236.52
A2	6.08	-9.87	56.70	-23.54	232.07
B2	5.67	-11.35	55.47	-22.57	234.10

Abbreviations: N, natural hair; D, discolored hair; A1, virgin hair treated with dye A; A2, discolored hair treated with dye A; B1, virgin hair treated with dye B; B2, discolored hair treated with dye B

## Discussion

The results here shown demonstrated that hair samples treated with dye A had their color fade sooner than samples treated with hair dye B. This might have happened because the kit for dye A contained 3.2mL of oils to be added during the mixing process at a proportion of 1:1 (50mL of hydrogen peroxide to 50g of dye). These oils are film making hydrophobic emollient substances which interfere with the diffusion of dyeing substances through hair fibers, compromising the coloring process.<sup>15</sup>

The kit for dye B, on the other hand, has a third more oxidizing agents than the kit for dye A, which probably caused formulation B to thoroughly react with melanin and keratin, allowing the colored polymer to be formed inside the cortex of hair fibers more efficiently. As shown in Figure 1, this caused virgin hair samples treated with dye B to remain colored even after the 15<sup>th</sup> wash.

Discolored locks of hair had higher color saturation, as these samples lacked natural color pigmentation. It was also possible to observe that even though the dyes used are standardized, as both are labelled 6.6 – intense red, the resulting color on hair samples was different possibly due to the different composition of precursor and coupling agents found in each formulation. Routine procedures, such as washing, can damage hair fibers, be them virgin or colored, due to lipid removal. Use of hair dryers and straighteners, due to functioning at excessive temperatures (nearly 200°C), compromises the integrity of proteins which compose the structure of hair fibers.<sup>5</sup> Analysis of protein loss evidenced that the coloring process damages hair fibers; the greater the damage caused to the hair cuticle, the greater the loss of proteins.<sup>15</sup> Dye B caused a greater protein loss than dye A due to the higher amount of hydrogen peroxide in its formulation.

The analyses of protein loss and thermal resistance were carried out in order to evaluate the actions of conditioning agents present in each hair dye kit assessed in this study. The use of conditioning agents in oxidative dyes aims to reduce the damage caused to hair fibers.<sup>14,15</sup> The study performed by Gama (2010)<sup>10</sup> demonstrated that active compounds such as silanetriol, panthenol, PEG-12 and dimethicone have thermoprotective properties. Conditioning agents, such as cationic and ammonia quaternary surfactants, cationic polymers and emollients found in both formulations assessed were probably the responsible for conferring resistance to temperature after the coloring processes had been carried out.

## Conclusion

It can be concluded that hair colored with dye A suffered less losses of protein and had better thermal resistance due to the higher amount of conditioning agents found in the formulation, and also due to color fading faster. As for actual coloring, dye B was more efficient than dye A, as the color provided by it lasted longer. Even though it was tried to use hair dyes of same standardization (both are labelled 6.6 – intense red), the final color seen on locks of hair was different when comparing both dyes. Coloring applied to discolored hair allowed for better visualization of the red color when compared to coloring applied to natural hair.

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## Conflict of interest

Authors declare that there is no conflict of interest.

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