Microchemical Journal 220 (2026) 116613

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Analysis and quantification of the propensity of hair dyes to desorb from human hair fibre using an accelerated 48-wash method

Kristina Hetherington [a], Alenka Tidder [a], Bethany J. Tack [a], Meryem Benohoud [a], Dan Nowlan [b], Anwar Zahar [b], Darcy Prater [c], Jeanna C. Zguris [b], Christopher M. Rayner [a,d], Richard S. Blackburn [a,e,*]

[a] Keracol Limited, Nexus, Discovery Way, Leeds LS2 3AA, UK
[b] Hair Innovation & Technology, Aveda, 4000 Pheasant Ridge Drive, Blaine, MN 55449, USA
[c] Hair Color Formulation, Aveda, 4000 Pheasant Ridge Drive, Blaine, MN 55449, USA
[d] School of Chemistry, University of Leeds, Leeds LS2 9JT, UK
[e] Leeds Institute of Textiles and Colour, School of Design, University of Leeds, Leeds LS2 9JT, UK

ARTICLE INFO

Keywords:
Hair dyes
Colour fastness
Quantification of colour loss
Dye extraction
Colour measurement

ABSTRACT

The durability of oxidative hair dyes is a critical factor in consumer satisfaction and hair colour product development. This study introduces an accelerated 48-wash analytical method for quantifying hair dye desorption, combining spectrophotometric colour strength measurements with a back-extraction protocol coupled to HPLC. The approach was first validated using a two-component model dye system comprising *p*-aminophenol and 2-methyl-5-aminophenol, forming a heterodimeric product that could be quantitatively monitored. Hair types studied included Natural White Hair and Bleached Blonde Hair, with dyeing performed using either ammonia or monoethanolamine as the alkalizing agent. Results demonstrate that most colour loss occurs during the first wash, and Bleached Blonde Hair treated with monoethanolamine exhibits the highest dye desorption, reflecting superficial dye deposition and structural vulnerability of chemically processed hair. In contrast, ammonia-based formulations promote deeper dye penetration and improved retention. Colour loss trends quantified by spectrophotometric measurement followed a logarithmic pattern over the accelerated 48 wash test, and strong correlations ($R^2 > 0.95$) were observed between spectrophotometric data and HPLC quantification of dye removal. The robustness of the method was also demonstrated on multi-component dye formulations, and although direct HPLC quantification was challenging for these more complex systems, the spectrophotometric measures serve as a reliable proxy for quantification of actual dye loss, based on the calibration established with the model two-component system. This work provides a quantitative framework for understanding hair dye wash fastness, the influence of hair type and alkaliser, and the mechanisms of dye desorption. The method supports future product innovation by linking molecular-level dye behaviour to long-term colour performance.

1. Introduction

Coloration of human hair is a trend documented throughout history since ancient Egypt [1]. Today, hair dyeing is a commonplace procedure, popular with people of all ages and genders, with coloration processes conducted in both professional salon environments and at home through consumer self-application [2–6]. Forecasts are that by 2029 the global hair colour market will be valued at around $42 billion [7].

Hair dye systems are split into three categories based on the durability of effect produced: temporary dyes which deposit high molecular mass compounds onto the surface of the hair and are easily removed in one or two washes [4]; semi-permanent dyes, where some deposition of the dye within the hair shaft occurs, achieve colour lasting several washes [8]; and permanent dyes, where the dye molecules penetrate the cortex of the hair shaft, resulting in substantially improved colour resistance to multiple shampoo washes [9–11]. Overall, the pursuit of long-lasting permanent hair colour has remained a cornerstone of research and product development based on the high consumer-

* Corresponding author at: Leeds Institute of Textiles and Colour, School of Design, University of Leeds, Leeds LS2 9JT, UK.
E-mail address: r.s.blackburn@leeds.ac.uk (R.S. Blackburn).

https://doi.org/10.1016/j.microc.2025.116613
Received 19 August 2025; Received in revised form 8 December 2025; Accepted 15 December 2025
Available online 17 December 2025

driven expectation for better colour fastness.

Colour formation in permanent oxidative hair dyes relies on complex chemical reactions in an alkaline environment. For many decades, ammonia has been the preferred alkalising agent in hair dye formulations; ammonia creates an alkaline medium that promotes hair cuticle opening and plays a role in generating active peroxide species that lighten existing melanin pigments within the hair [2]. Upon cuticle opening, dye precursors that include at least one primary intermediate (generally *p*-diamines or *p*-aminophenols) and at least one coupler (generally *m*-diamines, *m*-aminophenols, and mono or polyhydric phenols) penetrate and diffuse into the hair cortex wherein colorants are formed *in-situ* in the presence of the alkaliser and hydrogen peroxide [12–14]. Despite ammonia's effectiveness and ease of washing out, ammonia-free dye formulations have been designed to bypass its pungent smell [6,15]. Ammonia-free dye formulations have been developed based on monoethanolamine (MEA); MEA has been studied and deemed safe for use in hair dyes, being capable of creating the alkaline environment needed for dye diffusion into different hair types and textures, but notably devoid of the smell associated with ammonia [15–17]. However, MEA is not without its own drawbacks – the larger molecular size relative to ammonia results in comparatively slower diffusion of MEA within the hair fibre. Accordingly, higher concentrations of MEA need to be incorporated within dye formulations to elicit satisfactory and comparable colour development as observed with ammonia. MEA also causes more damage to hair fibres; scanning electron microscopy studies of hair fibres before and after coloration with MEA-based dyes show an increase in damage [18–20]. The choice of alkalising agent plays a crucial role in colour development within the hair fibre and final colour formed, but it also influences colour durability (colour fastness) and overall condition of the hair. When deciding between ammonia *versus* MEA (or indeed a different alkalising agent) more standardised tools for testing are needed.

Colour fastness testing examines a colorant's ability to resist changes under stress, such as fading or leeching from fibres when exposed to light or washing [2,21]. In the context of hair dyes, colour fastness to washing is the process through which the hair is exposed to typical conditions that consumer hair would undergo in its lifetime, including washing with water and shampoo [22–25]. To assess the crucial aspects of wash fastness and colour fading in dyed hair, the beauty industry employs colour measurement tools, especially utilisation of spectrophotometers. Dyed hair colour is initially measured using the spectrophotometer, subjected to a hand-wash cycle and measured again [23]; the difference in colour before and after washing is expressed as ΔE according to the CIE Lab colour space model. However, wash fastness techniques lack standardisation as handwashing can vary depending upon the operator. In addition, there is no evidence in literature that handwashing has been extended to multiple cycles to determine the point at which dye can no longer freely desorb from the hair shaft, which is due to impracticality of washing a hair tress with consistency by hand over multiple cycles. However, understanding the concentration of dye that remains in hair under even the most extreme washing conditions would be a beneficial benchmark for dye strength, which can direct development of improved dye formulations. Moreover, assessing colour *change* using ΔE values accounts for changes in hue, chroma and lightness, but is not focused on quantification of colour *loss*, making it difficult to ascertain the mechanisms involved in the removal/desorption of dye molecules from the hair. As such, there is a significant need for analytical tools that can answer questions regarding the intricate dynamics of dye-hair protein interactions and quantify dye loss with washing.

Previously, we reported a new rapid method for back-extracting hair dyes that had been applied to morphologically different hair types, designed to provide analytical tools that could provide a deeper understanding of dye behaviour in hair and the propensity of dyes to desorb from hair fibres [26]. The method was adapted from analysis protocols used when studying and conserving dyes present in historical textile artefacts [27–31]. We studied four distinct hair types (bleached hair, natural grey hair, natural white hair, and curly hair), all of which were dyed using a simple two-component system comprising 4-aminophenol (PAP; **1**) and 2-methyl-5-aminophenol (2M5AP, **2**) (Fig. 1). The heterodimeric species that forms under basic conditions (**3**) is a vibrant orange-red colour that notably can be monitored with ease using HPLC and UV–Vis techniques. The heterodimer developed in the hair fibre was back-extracted using an optimised solvent mixture of 2:1 (*v/v*) water:pyridine for 2 h at 40 °C [26]. Visible colour change of the hair was evaluated, and the concentration of dye desorbed from each morphologically different hair type was quantified, which demonstrated differences in dye leeching behaviour for different hair morphologies.

The study herein further develops the novel back-extraction method and its application to understanding colour loss over multiple washes, alongside established spectrophotometric techniques. The research is also designed to ascertain if this method can be applied to more complex hair dye systems that are typical of consumer products. The impetus for this research originates from a lack of comprehensive data (based on a comprehensive literature search using the Web of Science platform for the period 2025 to 1901) on how specific ingredients, such as alkalisers, affect colour retention, as well as how the physical morphology and structure of hair influence dye adsorption and desorption dynamics over multiple washes. Understanding these interactions over multiple wash cycles holds profound implications for consumer satisfaction, product innovation, and the broader practices of hair care. Moreover, this method is envisioned as a potential standardised application in understanding both hair morphology and alkaliser effects on dye behaviour.

2. Experimental section

2.1. Materials

The following chemicals used for the study were supplied from Sigma-Aldrich, UK: *p*-aminophenol (PAP), 2-methyl-5-aminophenol (2M5AP); hydrogen peroxide solution 34.5–36.5 %; acetonitrile for HPLC ≥99.9 %, 1-heptane sulfonic acid sodium salt, potassium phosphate monobasic, (KH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), and ammonia solution (25 % in water). Commercial hair dye and hair care products *Aveda Color Catalyst Conditioner Cream Developer*, *Clairol Professional BW2 powder*, *Clairol 20V Developer* and *Aveda Color Conserve Shampoo* were supplied by Aveda (Blaine, MN, USA); details of the ingredients of these commercial products are provided in Supplementary Information (SI). All other chemicals were purchased from Sigma-Aldrich, UK.

All hair samples were purchased from International Hair Importers and Products, Glendale, NY, USA. Out of 12 different types of hair (in 4 categories: Type 1 = straight, Type 2 = wavy, Type 3 = curly and Type 4 = coily), two different hair types were selected for this study: natural white hair corresponding to Caucasian Type 1 white hair from mixed source (NWH); medium brown hair from mixed source of Caucasian Type 1 and bleached to produce bleached blonde hair (BBH) (see section 2.3).

2.2. Instrumentation

HPLC analysis was conducted using a ThermoFisher Ultimate 3000 with a UV–Vis absorption spectroscopy detector with detection at 280 nm, 465 nm, 475 nm, and 505 nm. UV–Vis spectroscopy analysis was conducted using a Jasco V-530 spectrophotometer and data collected and processed using Jasco Spectra Manager Software version 1.54.03. Colour measurement analysis was conducted using a Datacolor 500 spectrophotometer and collected and processed using Datacolor software version 2.3.3. pH measurement was conducted using a Thermoscientific Orion Star A111 pH meter connected to a Sentek P11 rod glass combination pH electrode.

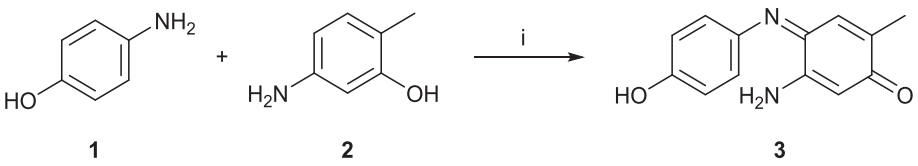

Fig. 1. Two-component hair dye system: PAP (**1**) + 2M5AP (**2**) and the resultant coloured product (**3**) with reaction conditions (i) pH 9 with NH_3 or MEA and H_2O_2.

2.3. Preparation of bleached blonde hair (BBH)

Caucasian type 1 medium brown hair tresses were bleached using a mixture of *Clairol Professional BW2 powder* and *Clairol 20V Developer*, used according to professional instructions. Bleaching formulation was applied to the hair with a brush until thoroughly coated, wrapped in aluminium foil, and kept in an oven for 30 min at 37 °C. After this time, hair tresses were rinsed with warm water and washed using ~0.6 g of 5 % (*w/v*) sodium lauryl sulfate (SLS) aqueous solution by massaging the SLS solution into the hair tresses for 30 s, followed by rinsing with warm tap water for 1 min. This process was carried out to remove all traces of bleaching components from the hair. The tresses were then combed and blow dried with a hair dryer with a diffuser for 5 min on the highest setting. All experiments where BBH was mentioned were done in triplicates unless otherwise stated in the text.

Lightness values (L^*) for the original Caucasian type 1 medium brown hair tresses and the resultant BBH tresses were 23.6 units and 55.9 units, respectively; lightness difference (ΔL^*) between the two samples was 32.2 units and colour difference (ΔE) between the two samples was 41.0 units. Lightness value (L^*) for the NWH tresses was 76.8 units; ΔL^* between the NWH tresses and the BBH tresses was 21.0 units, and ΔE was 23.6 units. See section 2.5 for colour measurement and colour difference calculation details.

2.4. Hair dye application

The model system is composed of two-colour precursors: PAP (**1**) and 2M5AP (**2**). Formulated dye (80 g) was prepared by mixing 1.36 % PAP and 1.54 % 2M5AP into a formulation base (details of ingredients in provided in SI). Formulated dye was then mixed in a 1:1 ratio with *Aveda Color Catalyst Conditioner Cream Developer*. The mixture was applied to the hair with a brush until thoroughly coated, wrapped in aluminium foil, and kept in an oven for 30 min at 37 °C. Following dye application, the hair tresses were washed, rinsed, and dried as described above. All formulated dyeing was done in triplicate unless otherwise stated in the text. Following dye application, the hair tresses (3 replicates) were removed and washed using 1:1 ratio of *Aveda Color Conserve Shampoo* to hair weight (~0.6 g), where the shampoo was massaged into the hair tresses for 30 s, followed by rinsing with warm tap water for 1 min. The tresses were then combed and blow dried with a hair dryer with a diffuser for 5 min on the highest setting.

The **four colour precursors** 4CB dye system contains: PAP (**1**), 2M5AP (**2**), *m*-aminophenol (MAP; **4**), and 2,5-diaminotoluene (TDS; **5**). The **seven colour precursors** 6N dye system contains: PAP (**1**), 2M5AP (**2**), MAP (**4**), TDS (**5**), 1-naphthol (AN; **6**), 2-methylresorcinol (2MR, **7**) and resorcinol (RES; **8**).

2.5. Colour measurement

Hair samples were measured using the spectrophotometer before and after dyeing; dyed hair samples were measured before and after washing, and washed hair samples were measured before and after back-extraction. Four measurements were taken on each side of the hair tress and the mean value calculated. The colour strength (K/S) of dyed samples was calculated from reflection spectra by the Kubelka-Munk function (eq. 1):

$$\frac{K}{S} = \frac{(1 - R_\lambda)^2}{2R_\lambda} \tag{1}$$

where K is the absorption coefficient, S is the scattering coefficient, and R_λ is the reflectance any given wavelength (λ).

For dyed samples, especially for dark and dull shades, it is often preferable to express the total colour strength of the sample through a summation of all K/S values across the visible spectrum. Total colour strength (f_k) is calculated using Eq. 2, which is summation of all K/S values from 400 to 700 nm at 20 nm intervals:

$$f_k = \sum_{400}^{700} \frac{K}{S} \tag{2}$$

The colour on the dyed hair tress was also quantified in terms of L^*, a^* and b^* values within the CIELab system. Colour difference (ΔE) between samples before (L^*_1, a^*_1, b^*_1) and after (L^*_2, a^*_2, b^*_2) back-extraction and after washing was calculated by Eq. 3:

$$\Delta E = \sqrt{\left(L_2^* - L_1^*\right)^2 + \left(a_2^* - a_1^*\right)^2 + \left(b_2^* - b_1^*\right)^2} \tag{3}$$

The human eye can perceive difference in colour when the ΔE value between two samples is greater than 1 unit.

Difference in lightness between hair samples (ΔL^*) was calculated by Eq. 4:

$$\Delta L^* = L_2^* - L_1^* \tag{4}$$

where L^*_1 and L^*_2 are lightness values within the CIELab system for two different samples.

2.6. HPLC analysis

An Agilent Zorbax Eclipse Plus C18 column was used with the mobile phase consisting of: A) 25 mM L^{-1} phosphate buffer pH 6 + 0.1 % heptane sulfonic acid sodium salt; B) acetonitrile; and C) water at 30 °C, using a flow rate of 1.0 mL min^{-1}. Mobile phase A was prepared in a 2 L volumetric flask, 6.12 g KH_2PO_4 (44.8 mmol), 0.76 g Na_2HPO_4 (5.35 mmol) and 2.00 g sodium salt of 1-heptane sulfonic acid (9.87 mmol) dissolved by shaking in water (1 L), then filled up to the mark with water and mixed. The pH of the buffer should be 6.0, otherwise adjusted to pH 6.0 by the addition of 1 M NaOH or 2 M H_3PO_4. Gradient elution of the mobile phase: 0 min 10 % B, 0 % C; 2 min 10 % B, 0 % C; 6 min 40 % B, 0 % C; 10 min 60 % B, 25 % C; 12 min 75 % B, 25 % C; 14 min 95 % B, 5 % C; 18 min 95 % B, 5 % C; 22 min 95 % B, 5 % C; 24 min 10 % B, 90 % C.

Samples for HPLC analysis were prepared as follows: 500 μL of back-extraction solution was added to 500 μL of 1 % (*v/v*) formic acid in methanol to halt any further reaction of starting components to product which would otherwise be encouraged in the basic conditions of the back-extraction material. The solutions were then analysed by HPLC.

2.7. Back-extraction method

Details for the optimization of the back-extraction method have been reported [26]. Dyed and washed hair samples were submerged in 20 mL of the back-extraction solution consisting of 2:1 (*v/v*) water:pyridine and stirred for 2 h at 40 °C. **Caution: pyridine liquid and vapour is highly flammable and has acute toxicity being harmful if swallowed, inhaled, or in contact with skin. Pyridine waste material must be**

disposed of in accordance with national and local regulations (please refer to suppliers Safety Data Sheets for more information). In our previous work [26], we considered whether more environmentally friendly solvents could be used, but water:pyridine mixtures were particularly effective for this back-extraction method and could be used safely in a controlled laboratory environment.

The hair samples were then removed from the back-extraction solution and rinsed using tap water for 30 s. The tresses were then combed and blow dried with a hair dryer with a diffuser for 5 min on the highest setting.

The colour of dyed hair tresses was measured before and after washing using spectrophotometric methods described above, and percent colour loss calculated using Eq. 5:

$$\%\text{colour loss} = \frac{f_k(i) - f_k(w)}{f_k(i)} \times 100 \quad (5)$$

where $f_k(i)$ and $f_k(w)$ are f_k values of the initial hair tress before washing (i) and after washing (w), respectively.

2.8. Washing method

A critical aspect of this research was the development of a standardised method for accelerated wash fastness testing. Washing individual hair tresses for 48 separate washes by hand is prohibitively time-consuming for experimentalists, so the development of a technique with much higher throughput is extremely desirable. The accelerated wash fastness testing method was developed using a Roaches Washtec P A2 wash fastness testing machine, which was designed for repeatable, reliable washes in colour fading from fabrics compliant with ISO standards.

The accelerated wash fastness testing method was conducted using a Roaches Washtec P A2 wash fastness testing machine. Each dyed hair tress (~0.6 g) was enclosed in a 300 mL capacity stainless-steel wash pot with 8 mL of shampoo (corresponding to hair:shampoo ratio of ~1:13.3 by weight) and 80 mL water, at 40 °C and washed for a specific duration.

In order to establish a comparable ratio of number of hand washes to time equivalent in the accelerated wash fastness testing method, we conducted a series of hair dyeings using commercial hair dye samples and washed them for up to 24 washes and then compared the % colour loss data (f_k values) over different time intervals of testing. These data were averaged and a relationship established with good correlation (R^2 = 0.918; see SI). From the equation of the trendline associated with these data it was possible to relate number of hand washes to washing time in the accelerated wash fastness testing method, as show in Table 1.

The total number of hair tresses for each hair type was ten, comprising nine for each wash cycle and one that was not subjected to washing (referred to as hair tress at 0 washes). Following each test, irrespective of duration, hair tresses were rinsed with warm tap water for 1 min. The tresses were then combed and blow dried with a hair dryer with a diffuser for 5 min on the highest setting. Colour of the tresses was then measured as per the method in Section 2.5.

Aveda Color Conserve Shampoo was chosen for this washing research as it is a highly representative example of a shampoo used by consumers to wash dyed hair. The purpose of this research was to demonstrate that the back-extraction and colour loss quantification method described was applicable over multiple washes. The performance with this specific shampoo and its comparison to other shampoos was not the purpose of the research, rather to establish that the washing performance of one highly commercially relevant example shampoo could be determined using this novel methodology.

Table 1
Calculated time in accelerated wash fastness testing method equivalent to number of hand washes.

Number of hand washes	Time in accelerated wash fastness testing method (min)
1	3
3	6
6	9
9	12
12	15
15	22
20	35
24	71
48	109

2.9. Software

Software for all data processing was Microsoft 365 Excel. Software for producing all individual chemical structures and Fig. 1 was ChemDraw version 23.0.1. Software for producing Figs. 2 and 5 was Microsoft 365 PowerPoint. Software for producing Figs. 3, 4, 6, 7, 8, 9, and 10 was Microsoft 365 Excel.

3. Results and discussion

3.1. Hair, dye, and alkaliser selection

The method is anchored in the model system utilizing the two-component dye system, comprising the primary intermediate PAP (**1**) and the coupler 2M5AP (**2**). This combination affords a vibrant orange-red colour upon application on hair and gives the heterodimeric dye product (**3**) (Fig. 2A). The method investigates the wash fastness of hair dyed using the simple PAP + 2M5AP dye system over multiple washes (Fig. 2B) and colour change of the dyed hair over an accelerated 48-wash cycle assessed by spectrophotometry (Fig. 2C); this simulates colour change that occurs over a longer and more practical lifespan of the dye on hair. The analysis is coupled with back-extraction and analysis by HPLC (Fig. 2D) over the same accelerated 48-wash cycle, using the method established in our previous work [26]; quantification of each dye constituent was possible after establishing calibration curves for the individual components (**1**, **2** and **3**) detected at 280 nm. HPLC is

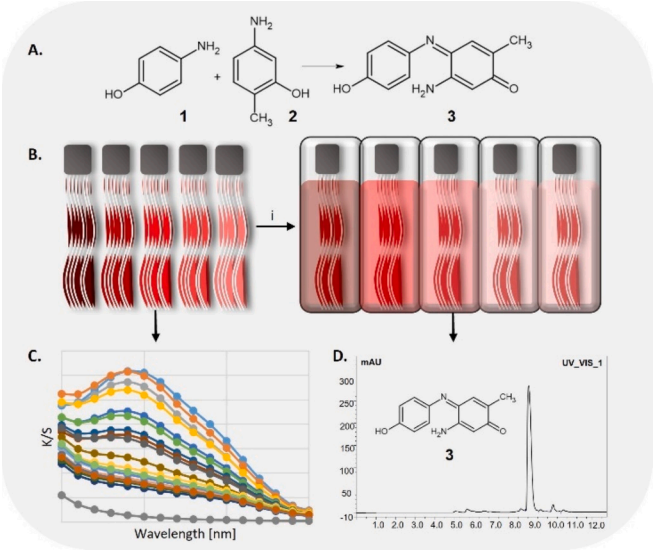

Fig. 2. Accelerated 48-wash method combined with back-extraction method: **A.** Two-component dye system (PAP (**1**) + 2M5AP (**2**)) and the resultant-coloured heterodimer product (**3**) with reaction conditions of pH 9 with NH_3. H_2O and H_2O_2; **B.** Hair tress journey after dyeing and subsequent colour loss over multiple washing cycles: (i) dyed and washed hair samples are submerged in the back-extraction solvent for 2 h at 40 °C; **C.** Analysis of hair tress before and after washing and after back-extraction using spectrophotometry. **D.** Analysis of back-extraction solution using HPLC and UV–Vis by detection of compound **3**.

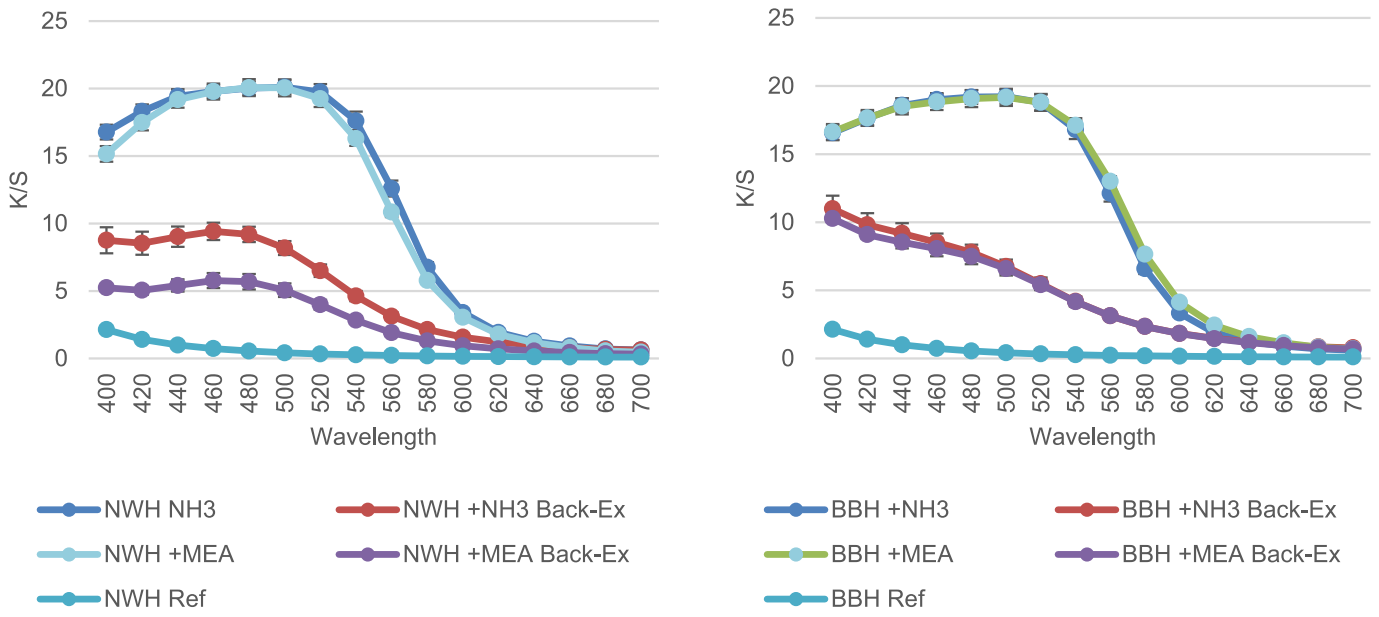

Fig. 3. Colour strength (*K/S*) of NWH (left) and BBH (right) dyed with both the PAP + 2M5AP formula that contains ammonia or the PAP + 2M5AP formula that contains MEA both before and after back-extraction.

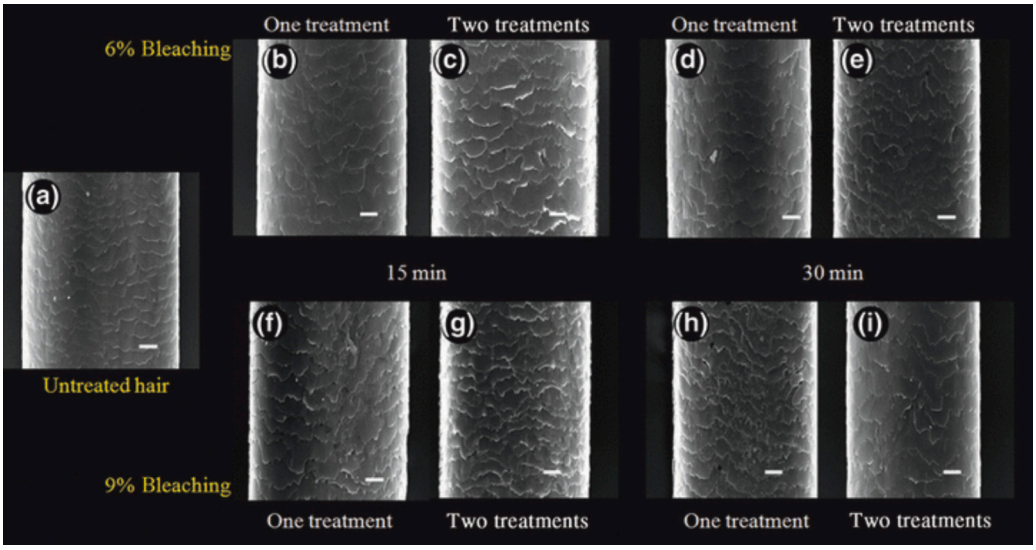

Fig. 4. SEM images of the untreated hair (a) and bleached hairs with 6 % (b–e) or 9 % (f–i) bleaching agent with one (b,d,f,h) or two treatments (c,e,g,i) for 15 (b,c,f,g) or 30 min (d,e,h,i) [35].

the preferred analytical tool for the quantitative analysis of this dye combination, which combines well with hair colour strength measurement, to provide evidence for and enhances confidence in understanding of the molecular structures involved in observed and quantified colour changes.

This study focuses on two distinct hair types: Natural White Hair (NWH) and Bleached Blond Hair (BBH), both characterized by their absence of melanin pigmentation, which ensures minimal background colour when measuring hair colour appearance with a spectrophotometer. This is critical for precise measurement of colour change and recording of accurate and reproducible data, essential for supporting mechanistic research study. Furthermore, the two types of hair selected facilitate the investigation into the effects of damage induced by chemical processing (notably bleaching) on dye adsorption and desorption, in comparison with untreated, less damaged natural hair. BBH typically exhibits modifications in chemical and physical structure to unbleached hair, as well as alterations in hair morphology, including the pattern and spacing of hair cuticles [32–34].

Adsorption of dye components onto hair is influenced by a confluence of factors including the physical morphology and structure of the hair, but also by the specific chemical structural features of the heterodimer(s) that facilitate the binding to the hair surface and cortex. Chemical features of these heterodimers that may influence such interactions include: a) hydrophobic moieties that can interact with hydrophobic regions of hair proteins; b) functional groups capable of forming hydrogen bonds with the keratin structure; c) presence of ionic groups in the dye molecule enabling electrostatic interactions with oppositely charged sites in the hair; d) van der Waals forces that can contribute to adsorption of small molecules onto the hair surface, especially when multiple VDW interactions occur simultaneously; and e) the molecular size and shape of the monomers and heterodimers that can influence their ability to penetrate the hair cuticle and interact

effectively with the hair cortex. Therefore, the monitoring and quantification of the heterodimers throughout dyeing and washing processes are pivotal to the methodological strategy adopted in this study.

Lastly, two dye formulations were developed, one using ammonia and the other employing monoethanolamine (MEA), in order to evaluate the effect of different alkalisers on overall wash fastness of the dyed hair. This was undertaken with two reasons in mind: firstly, to understand the impact of alkaliser on colour development on the two distinct types of hair; secondly, to understand impact of alkaliser on dye desorption from hair fibre.

3.2. Model system and back-extraction quantification

Two dye formulations, one with ammonia and one with MEA, were applied to both NWH and BBH tresses in triplicate, and these were back-extracted using the 2:1 (*v/v*) water:pyridine solvent for 2 h at 40 °C, as per previous work [26]. This was done to understand the effect of the two different alkalisers on both colour development and desorption of dye during back-extraction based on two different hair types. Visual inspection revealed no discernible difference in the dyed hair tresses (Figs. S1 and S2). Measurement of the colour strength (*K/S*) by spectrophotometry supported the visual observations (Fig. 3), where it was observed that a very similar amount of colour (from the dye product **3**) was deposited in the hair, regardless of hair type or alkaliser used. The data for the average values of the dyed hair tresses nearly overlapped, reinforcing the validity of the chosen model system for this study and its suitability for the detailed analysis of the becoming of hair dye molecules over time and potential for application when investigating multiple washes.

The specific chemical structure of the heterodimer (**3**) plays a crucial role in binding to the hair surface and cortex, thereby enhancing its resistance to desorb. Although colour uptake was similar between BBH and NWH, the back-extraction process yielded insightful observations. BBH exhibited stronger colour retention compared to NWH, suggesting more robust dye-hair interactions or that molecular size and shape, in combination with open cuticles, provided easier access to the hair cortex; and, regardless of the alkalising agent used, the damaged structure of BBH facilitated uniform dye removal, underscoring the significance of hair condition in the dyeing process. This is evidenced through exemplar Scanning Electron Microscopy (SEM) images of the morphological changes of the hair surface that occur after bleaching (Fig. 4 [35]). Untreated hair (Fig. 4a) has a relatively complete and uniform appearance of the cuticle scales, and the edges of the cuticle scales are clearly separated; in contrast, it is observed that the cuticle scales of bleached hair are irregular and lifted with some pitting and breaking. The degree of morphological damage to the hair surface is dependent upon the bleaching conditions; Fig. 4d is representative of BBH used in this study (one treatment with 6 % hydrogen peroxide for 30 min), and although the damage observed here is not as severe as observed for those using 9 % hydrogen peroxide or repeat bleaching applications, the changes observed compared to undamaged hair (*i.e.* NWH) are still significant.

In addition, NWH dyed with MEA experienced greater colour loss upon back-extraction and retained less colour compared to NWH dyed with ammonia, indicating that dye molecules in MEA-based formulations treated penetrate the NWH structure less effectively than with ammonia, with dye molecules located at the surface being more easily washed away due to weaker interactions between dye and hair.

To elucidate the molecular composition of the colour, the 2:1 (*v/v*) water:pyridine solvent system was employed for the back-extraction, chosen for its efficacy in removing dye without degrading the dye molecules or causing side-reactions, and also preserving the structural integrity of the hair for subsequent spectrophotometric analysis [26]. The components extracted, dye precursors and the heterodimeric dye product, were then analysed and quantified using HPLC. Notably, no dye precursors (PAP nor 2M5AP) were back-extracted, which was expected as previous studies showed these two components do not stick to hair and that they wash off in post-dyeing rinsing [26]. However, successful desorption of the heterodimer **3** was obtained after stirring the hair tress in the back-extraction solvent for 30 min (and at the 2-h end point) as monitored and quantified by HPLC (Fig. 5).

As expected, the greatest amount of dye back-extracted resulted from dyeings using formulations that contain MEA when applied to BBH (101 % ± 0.4 %), which suggests that MEA plays a potential role in further damaging the hair shaft (reducing dye retention), with the combination of these parameters pushing the coloration system to extreme in terms of damage and desorption. It should be noted that although the amount of dye extracted for this data point is very slightly over 100 % it is within an acceptable margin of error for the calibration curve; it is not possible to extract more than 100 % of dye in practice.

Significantly, desorption of dye from BBH dyed with MEA is higher after 30 min of back-extraction (76 %) when compared to all other combinations of hair type and alkaliser, which suggests that most of the dye does not penetrate deeply enough within the hair. Conversely, desorption of dye from BBH dyed with ammonia was significantly lower after 30 min (30 %) and comparatively lower compared to the MEA dyeing after 2 h (86 %).

3.3. Wash fastness analysis

Wash fastness testing emulates routine shampooing to evaluate the resistance of hair dye to fading over successive washes. This is assessed by measuring the colour loss (from f_k) and colour change (ΔE) after a set number of wash cycles, providing insight into the ability of the colour molecules to withstand washing with warm water and shampoo. In this study, we assessed the colour strength (*K/S*) of dyed hair (relative to reference hair) by spectrophotometry coupled with the back-extraction and subsequent quantification of the dye molecule **3**. In duplicates, dyed hair tresses underwent a series of washes, with hair colour strength measured after 0, 1, 3, 6, 9, 12, 15, 20, 24 and 48 equivalent accelerated wash cycles. After washing, the tresses were back-extracted with the 2:1 (*v/v*) water:pyridine solvent system. HPLC quantified the residual colour on the hair post-wash; an overview of the developed method is presented in Fig. 6.

Both NWH and BBH dyed with the PAP + 2M5AP system using ammonia or MEA as alkaliser exhibit a consistent trend of logarithmic colour loss over an accelerated 48 wash cycles (Fig. 7 A); most significant colour loss is observed in the first wash, with BBH treated with MEA having the greatest initial colour loss (17.1 %), followed by BBH treated with ammonia (12.8 %), followed by NWH treated with ammonia (11.4 %), with NWH treated with MEA showing lowest initial colour loss (9.5 %). Fig. 7B shows a plot of colour loss relative to the natural logarithm of the number of washes from 3 washes onwards, where it is observed that there is a significant linear correlation ($R^2 = 0.88–0.96$); from the slope (m) of the equations of these trendlines, it is clear that after the first wash, colour loss, relative to the number of washes, is a similar magnitude of change for both hair types in both conditions (m = 0.12–0.15). This demonstrates the importance of the first wash in determining subsequent colour fastness of the hair dyeings but also suggests that in the rudimentary PAP + 2M5AP system that colour loss increases proportionate to the number of washes equally, independent of hair type and alkaliser, despite the total magnitude of the loss differing across these variables. After 48 accelerated washes, BBH dyed with MEA demonstrates the greatest total colour loss, whereas NWH tresses dyed with ammonia have the lowest.

Upon examining the colour data collected from each experiment, it becomes evident that the back-extraction post-washing removes a portion of the residual colour on the hair. Dyeing with ammonia results in more colour remaining 'locked' into the hair following back-extraction, compared to formulations that contain MEA. The underlying hypothesis is that MEA does not facilitate the opening of the hair cuticle as effectively as ammonia, preventing dye precursors from penetrating the hair cortex, as observed in results from the initial back-

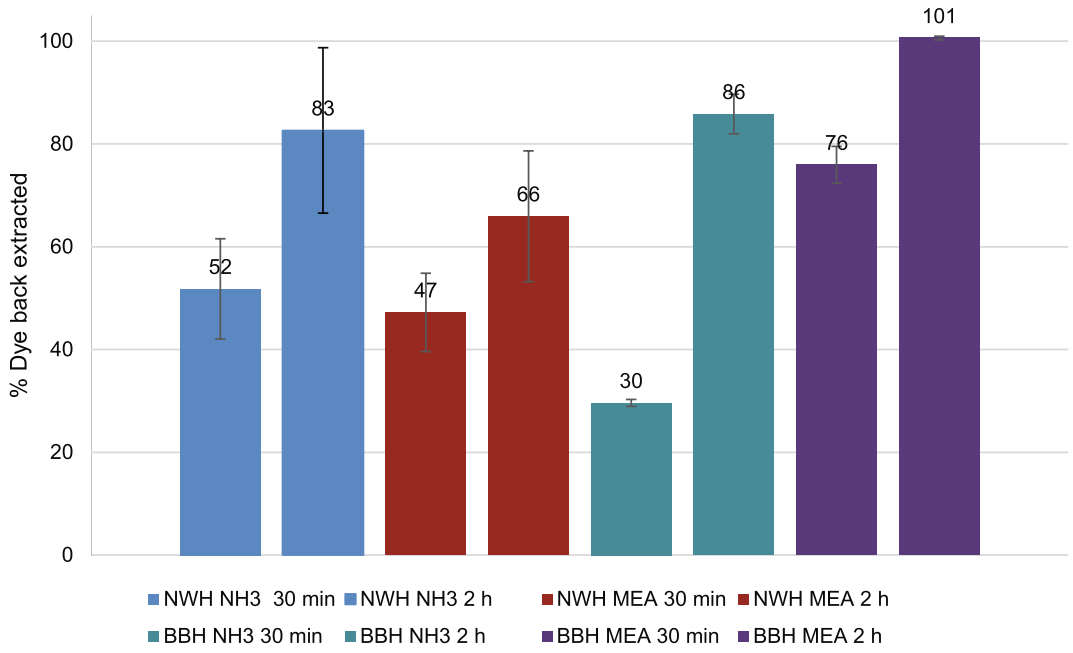

Fig. 5. HPLC quantified dye desorption measured at 30 min and 2 h of back-extracting the hair tresses.

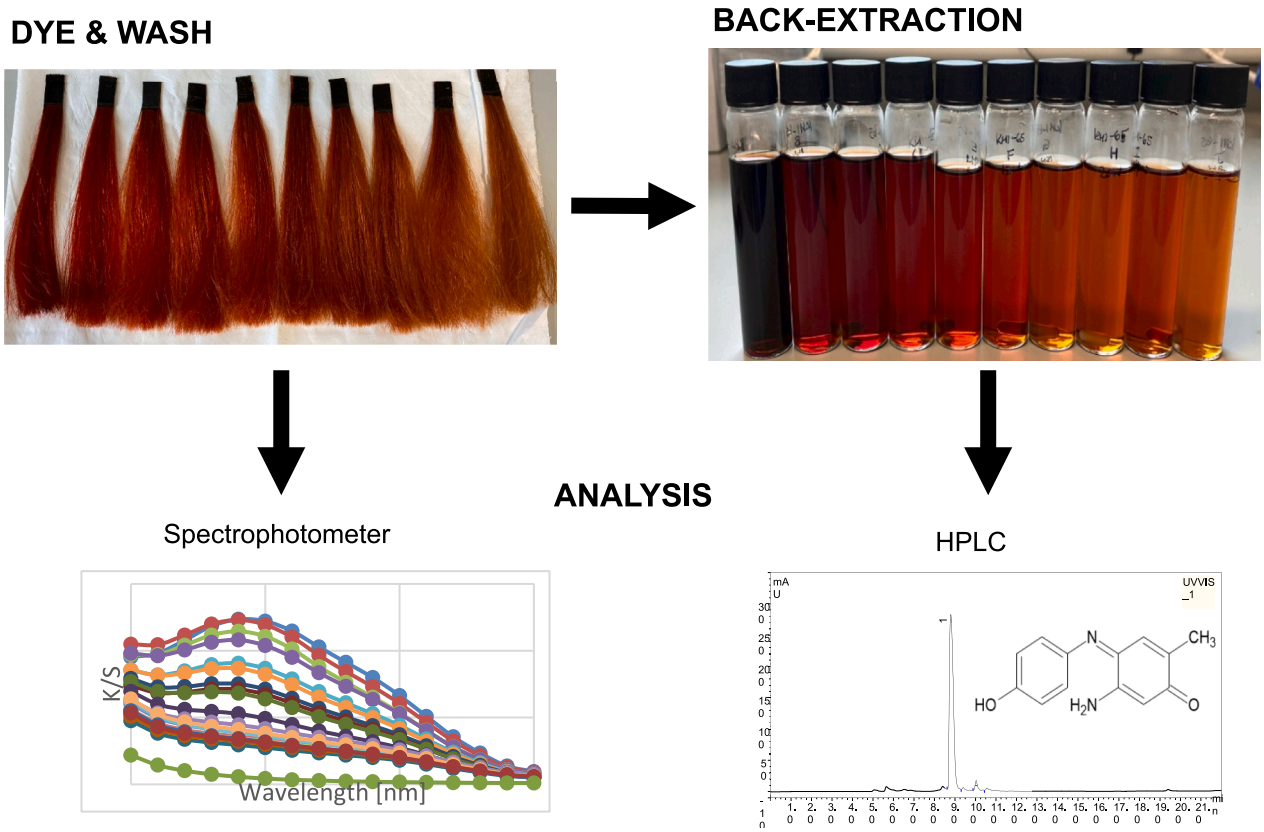

Fig. 6. Wash fastness method: Hair tresses undergo dyeing & washing. The dyed hair samples are then back-extracted. Hair tresses are analysed before and after extraction using spectrophotometry and back-extraction solution also analysed using HPLC.

extraction of the hair (in triplicate). Consequently, the heterodimeric product forms predominantly on the surface of the hair, making it more susceptible to being readily washed away during routine shampooing and by the back-extraction solvent washing with water and shampoo but also by the back-extraction solvent.

Colour data aligns with the HPLC quantification outcomes (as seen in Table 2 and Figs. S6-S9), showing that % dye loss (calculated from the dye extracted and quantified by HPLC) correlates with % colour loss (calculated from f_k values measured by spectrophotometry). This is further exemplified through remarkably close correlation (R^2 =

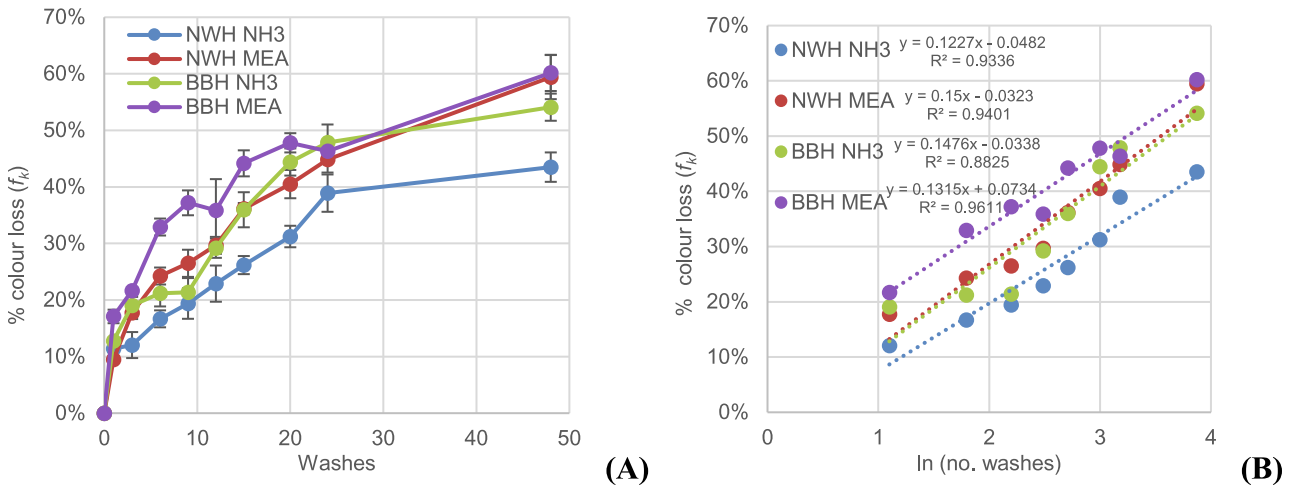

Fig. 7. A. % colour loss (based on f_k) during accelerated washes (0–48) for NWH and BBH dyed with the PAP + 2M5AP formulation that contains either ammonia or MEA as alkaliser. **B.** same % colour loss data in **A** plotted against natural logarithm of the number of washes (equations of linear trendlines shown with accompanying R^2 value). Error bars show standard deviation.

Table 2
Comparison of colour change analysis using spectrophotometric methods (% colour loss from f_k and ΔE) with HPLC back-extraction methods (% dye loss) for NWH and BBH dyed with the PAP + 2M5AP formulation that contains either ammonia or MEA as alkaliser. Observations after 1 wash and 48 accelerated washes ± standard deviation.

Hair type and alkaliser	Number of washes (accelerated)	Spectrophotometry		HPLC
		% colour loss (from f_k)	ΔE	% dye loss
NWH NH_3	1	11.4 ± 0.1	2.7 ± 0.3	21.1 ± 1.5
NWH MEA	1	9.5 ± 0.5	4.3 ± 0.4	19.6 ± 0.5
BBH NH_3	1	12.8 ± 0.1	2.3 ± 0.1	15.9 ± 1.0
BBH MEA	1	17.1 ± 1.2	7.2 ± 0.2	36.0 ± 2.5
NWH NH_3	48	43.5 ± 2.6	10.0 ± 1.0	45.1 ± 2.8
NWH MEA	48	59.4 ± 3.9	16.2 ± 1.5	82.5 ± 1.2
BBH NH_3	48	54.1 ± 2.4	15.8 ± 1.2	80.4 ± 2.1
BBH MEA	48	60.2 ± 3.2	17.2 ± 1.3	88.3 ± 1.7

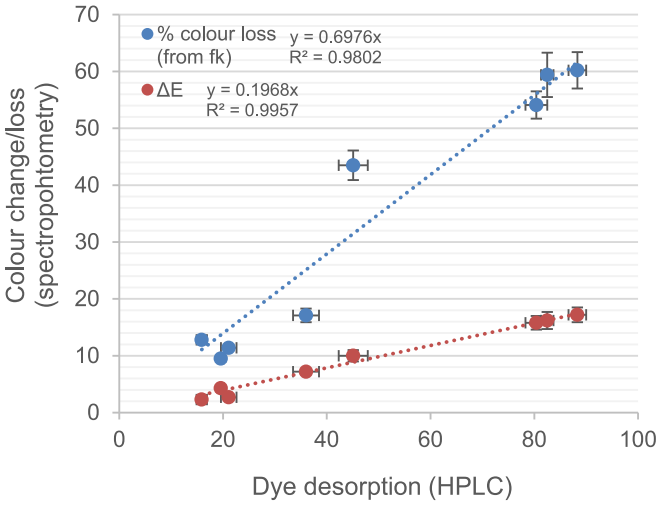

Fig. 8. Correlation between quantification of colour loss/change *via* spectrophotometric analysis compared with quantification by back-extraction and HPLC for hair dyed with the PAP + 2M5AP system, independent of hair type, formulation alkaliser and the number of washes (equations of linear trendlines shown with accompanying R^2 value). Error bars show standard deviation.

0.98–0.99) between quantification of colour loss/change *via* spectrophotometric analysis compared with quantification by back-extraction and HPLC for hair dyed with the PAP + 2M5AP system, independent of hair type, formulation alkaliser and the number of washes (Fig. 8). Hair treatments such as bleaching inflict damage on hair fibres, leading to protein degradation from the cuticle to the cortex, and it is understandable that a pronounced impact of the alkalisers was evident with the BBH and MEA, resulting in higher percentage of colour loss compared to those with ammonia. After 48 accelerated washes, the trends observed in the first wash persist, underscoring the considerable influence of hair's structural characteristics and the choice of alkaliser on the dye retention and colour stability.

This preliminary study involving NWH and BBH used the simple model system comprising a straightforward two-colour precursor dye system of PAP + 2M5AP that produced a single-coloured heterodimer, which could be easily monitored by HPLC, and thus facilitated the establishment and validation of the analytical method. This method provided quantifiable insights into dye loss and colour change throughout the washing cycles of dyed hair. The method's robustness was evaluated against two more intricate dye formulations: a four-component brown (4CB) and a market available seven-component brown (6N). The expansion to more complex dyes meant that quantification of % dye loss *via* extraction and HPLC analysis was problematic due to the formation of multiple coupling product structures, especially to darker shades such as browns; it is known that during oxidative coupling between some precursors formation of oligomeric species occurs *via* formation of dye dimers that are unstable and can be further oxidised to oligomers [9,36]. However, it is envisioned herein that colour analysis and quantification by both % colour loss (from f_k) and colour change (ΔE) from the simple PAP + 2M5AP dye system could be extended to these more complex dyes.

3.4. Four-component brown (4CB) dye system

The 4CB dye system represents a more sophisticated dye formulation, incorporating four colour precursors which afford a brown colour: PAP (**1**), 2M5AP (**2**), MAP (**4**) and TDS (**5**), with the potential to form six

distinct heterodimers. The complexity of the reaction products of this four-component system meant that it was not possible (at least in the work reported herein) to monitor % dye loss using the HPLC method described for the model PAP + 2M5AP system.

In terms of assessment of colour loss/change *via* spectrophotometric methods, both NWH and BBH dyed with the 4CB system using ammonia or MEA as alkaliser exhibit a consistent trend of logarithmic colour loss over 48 accelerated wash cycles (Fig. 9 A); colour loss observed in the first wash varies significantly (Table 3), with BBH treated with MEA having the greatest initial colour loss (21.4 %) > > NWH treated with ammonia (9.8 %) = NWH treated with MEA (9.8 %) > BBH treated with ammonia (7.5 %). Fig. 9B shows a plot of colour loss relative to the natural logarithm of the number of washes from 3 washes onwards, where it is observed that there is significant linear correlation (R^2 = 0.86–0.96); from the slope (m) of the equations of these trendlines, it is clear that after the first wash, colour loss, relative to the number of washes, is a similar magnitude of change for both hair types in both conditions (m = 0.09–0.17).

(A) (B)

This again demonstrates the importance of the first wash in determining subsequent colour fastness of the hair dyeings and suggests that in the four-component 4CB system that colour loss increases proportionate to the number of washes equally, independent of hair type and alkaliser, despite the total magnitude of the loss differing across these variables. After 48 accelerated washes (Table 3), BBH dyed with MEA demonstrates the greatest total colour loss > NWH dyed with MEA > BBH dyed with ammonia > NWH dyed with ammonia, which were the same trends as observed for the PAP + 2M5AP system.

3.5. Seven-component brown (6N) dye system

The 6N dye system is an intricate dye formulation that incorporates seven colour precursors: PAP (**1**), 2M5AP (**2**), MAP (**4**), TDS (**5**), AN (**6**), 2MR (**7**) and RES (**8**), with the potential to form twenty distinct heterodimers. The complexity of the reaction products of this seven-component system meant that it was not possible (at least in the work reported herein) to monitor % dye loss using the HPLC method described for the model PAP + 2M5AP system.

In terms of assessment colour loss/change *via* spectrophotometric methods, both NWH and BBH dyed with the 6N system using ammonia or MEA as alkaliser exhibit a consistent trend of logarithmic colour loss over 48 accelerated wash cycles (Fig. 10 A); colour loss observed in the first wash varies significantly (Table 4), with BBH treated with ammonia having the greatest initial colour loss (15.5 %), followed by BBH treated with MEA (13.1 %), followed by NWH treated with MEA (8.2 %), with NWH treated with ammonia showing lowest initial colour loss (5.9 %). Fig. 10B shows a plot of colour loss relative to the natural logarithm of the number of washes from 3 washes onwards, where it is observed that there is linear correlation (R^2 = 0.70–0.93); from the slope (m) of the equations of these trendlines, it is clear that colour loss, relative to the number of washes, varies significantly relative to the alkaliser used with values dyeings using MEA comparable for both hair types (m = 0.12–0.14), but clearly different to values for both hair types dyed using ammonia (m = 0.03–0.04).

This suggests that colour loss from these complex multi-component systems is significantly lower when dyeing with ammonia compared

Table 3
Comparison of colour change analysis using spectrophotometric methods (% colour loss from f_k and ΔE) with HPLC back-extraction methods (% dye loss) for NWH and BBH dyed with the 4CB formulation that contains either ammonia or MEA as alkaliser. Observations after 1 wash and 48 accelerated washes ± standard deviation.

Hair type and alkaliser	Number of washes (accelerated)	Spectrophotometry	
		% colour loss (from f_k)	**ΔE**
NWH NH_3	1	9.8 ± 2.5	1.5 ± 0.1
NWH MEA	1	9.8 ± 0.1	2.2 ± 0.2
BBH NH_3	1	7.5 ± 0.4	1.3 ± 0.1
BBH MEA	1	21.4 ± 1.2	4.6 ± 0.3
NWH NH_3	48	43.3 ± 3.6	10.0 ± 0.8
NWH MEA	48	53.4 ± 3.2	13.7 ± 0.2
BBH NH_3	48	49.6 ± 4.4	12.6 ± 0.5
BBH MEA	48	59.8 ± 3.2	16.8 ± 1.1

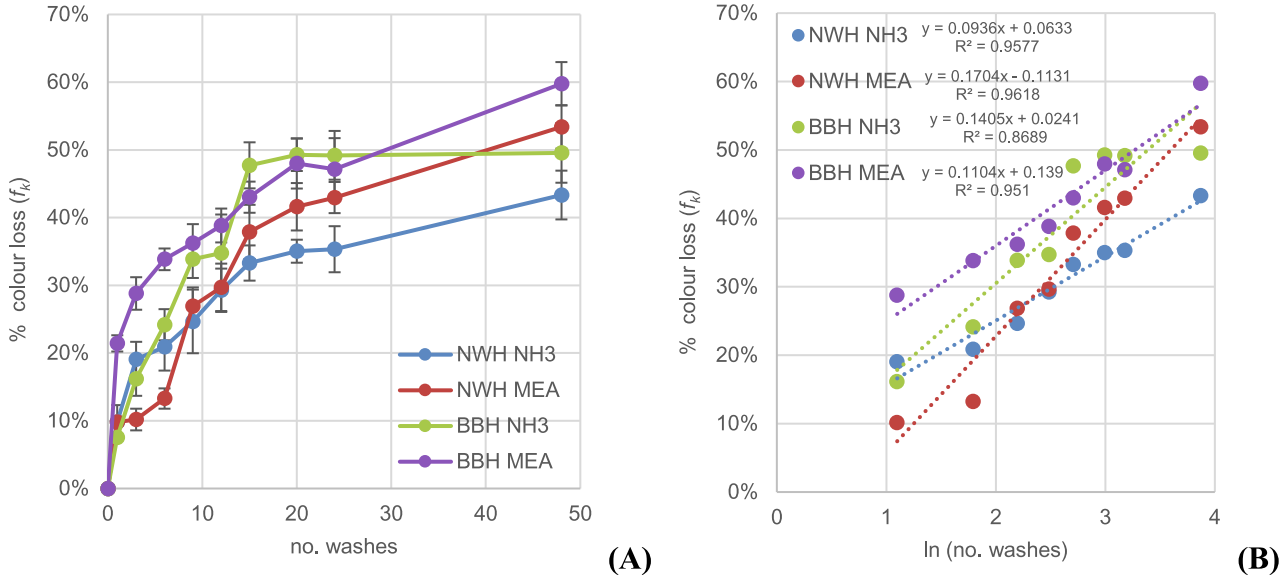

Fig. 9. A. % colour loss (based on f_k) during accelerated washes (0–48) for NWH and BBH dyed with the 4CB formulation that contains either ammonia or MEA as alkaliser. **B.** same % colour loss data in **A** plotted against natural logarithm of the number of washes (equations of linear trendlines shown with accompanying R^2 value). Error bars show standard deviation.

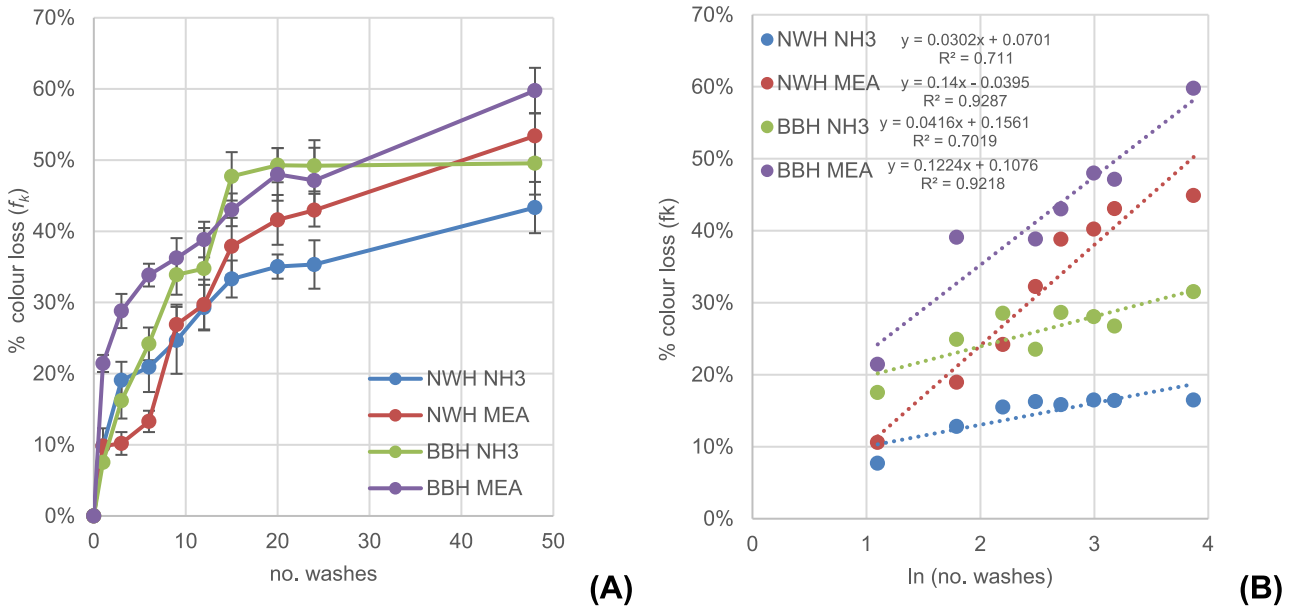

Fig. 10. A. % colour loss (based on f_k) during accelerated washes (0–48) for NWH and BBH dyed with the 6N formulation that contains either ammonia or MEA as alkaliser. **B.** same % colour loss data in **A** plotted against natural logarithm of the number of washes (equations of linear trendlines shown with accompanying R^2 value). Error bars show standard deviation.

Table 4
Comparison of colour change analysis using spectrophotometric methods (% colour loss from f_k and ΔE) with HPLC back-extraction methods (% dye loss) for NWH and BBH dyed with the 6N formulation that contains either ammonia or MEA as alkaliser. Observations after 1 wash and 48 accelerated washes ± standard deviation.

Hair type and alkaliser	Number of washes (accelerated)	Spectrophotometry	
		% colour loss (from f_k)	**ΔE**
NWH NH_3	1	5.9 ± 1.5	1.4 ± 0.1
NWH MEA	1	8.2 ± 0.1	1.7 ± 0.2
BBH NH_3	1	15.5 ± 1.4	3.0 ± 0.2
BBH MEA	1	13.1 ± 1.2	3.1 ± 0.1
NWH NH_3	48	16.5 ± 1.6	8.4 ± 0.5
NWH MEA	48	44.9 ± 3.4	11.3 ± 0.7
BBH NH_3	48	31.5 ± 2.4	9.7 ± 0.2
BBH MEA	48	59.8 ± 3.8	16.0 ± 0.8

to MEA and may be related to the ability of ammonia to open the hair cuticle and enable penetration of the more complex oligomeric systems into the hair cortex. Once these oligomers form within the hair cortex, their larger molecular size will increase their retention within the hair relative to smaller dimeric reaction products that are much more easily removed; conversely, if these oligomers form on the periphery of the hair (as may be the case with MEA-based formulations), their propensity to be removed during washing increases. After 48 accelerated washes (Table 4), BBH dyed with MEA demonstrates the greatest total colour loss > NWH dyed with MEA > BBH dyed with ammonia > NWH dyed with ammonia, which were the same trends as observed for the PAP + 2M5AP system and the 4CB system.

3.6. Comparison of data between the PAP + 2M5AP system and the multi-component systems

The presence and location of its blocking methyl group means that PAP can couple only once with 2M5AP, preventing formation of larger molecular species [9,36] and this justifies is use as a predictable model system. However, the formation of trimer or other dark-coloured oligomeric species is possible for unblocked systems [26], and this is likely for the seven-component brown (6N) dye system, which has the potential to form twenty distinct heterodimers, but also oligomeric species that may be responsible for the brown colour of the dye. As molecular size of the dye species increases its water solubility decreases, and where these dyes are mechanically entrapped within hair through this *in-situ* reaction, their reduced water solubility and larger molecular size is likely to increase their wash fastness. This is evident in the spectrophotometric data in discussed previously, where the colour change observed for the 6N system is generally lower than the 4CB and PAP + 2M5AP systems.

It has already been discussed that the complexity of the reaction products of the multi-component hair dye systems (4CB and 6N) meant that it was not possible in the work reported herein to monitor % dye loss using the HPLC method in the same way as described for the model PAP + 2M5AP system. However, the learnings from the simpler dimeric system can be applied to multi-component systems that incorporate these compounds within the wider mixture. To exemplify this, if we examine data in Fig. 8 for the PAP + 2M5AP system (where both spectrophotometric data are related to % dye loss), we can calculate the relationship between different spectrophotometric data relative to % dye loss (from HPLC data), thus ΔE = % colour loss × 0.28. If we then compare data within Tables 2 to 4 in terms of the relationship between % colour loss (calculated from f_k values) and colour change (ΔE), as shown in Fig. 11, we see that for the PAP + 2M5AP system ΔE = % colour loss × 0.28, for the 4CB system ΔE = % colour loss × 0.26, and for the 6N system ΔE = % colour loss × 0.27. If we then extrapolate these data, we can relate the magnitude on dye loss (actual removal of dye molecules) directly to colour loss/change data where these same trends are observed and all based on the PAP + 2M5AP system. Thus, this analytical method serves as a basis for not only understanding the visual colour loss/change observed, but also a quantification of the amount of

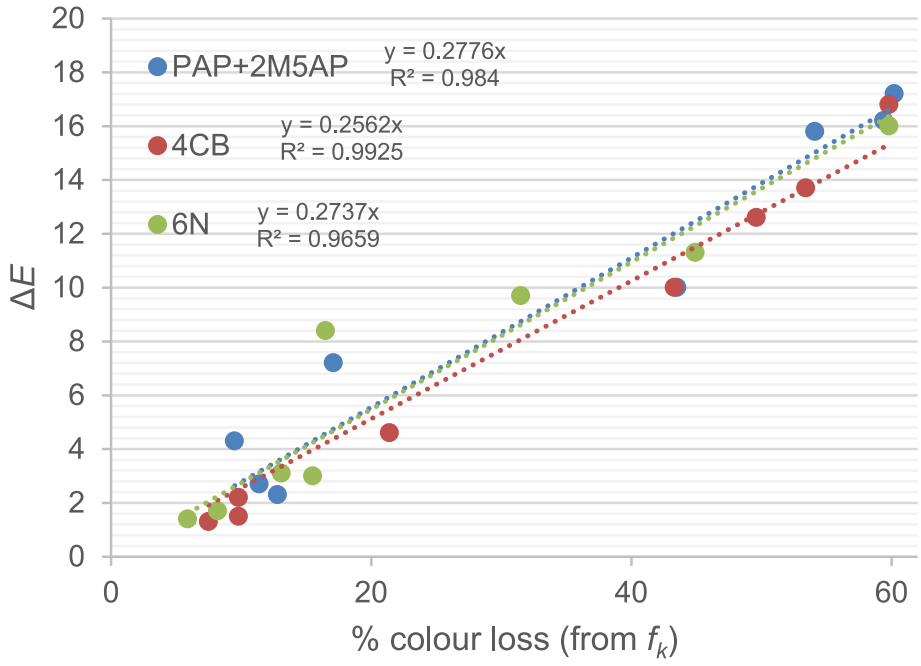

Fig. 11. Relationship between % colour loss (based on f_k) and ΔE for the PAP + 2M5AP, 4CB, and 6N hair dye systems.

applied dye product removed during washing.

4. Conclusions

This study introduces a novel method for analysing the wash fastness of dyed hair across 48 accelerated wash cycles, employing two complementary analytical techniques: spectrophotometry to measure colour strength (K/S) and back-extraction coupled with quantitative HPLC analysis to determine the concentration of residual dye molecules. The method is validated using a simple two-component PAP + 2M5AP system where the correlation between spectrophotometric % colour loss and HPLC % dye loss is strong. The robustness of the method is also demonstrated when applied on multi-component dye formulations, and although direct HPLC quantification is challenging for these more complex systems, the spectrophotometric measures (% colour loss, ΔE) serve as a reliable proxy for quantification of actual dye loss, based on the calibration established with the model system.

The methodological approach was designed to provide insights into the interactions between hair dye and hair, enhancing our comprehension of hair dye chemistry. The findings contribute significantly to our understanding of the extent to which dye is likely to desorb from the hair throughout the dye's lifespan. These observations made also suggest that while damaged hair tends to lose colour more rapidly when washed to untreated hair, it retains higher levels of residual colour, a paradoxical observation that underscores the complex interplay between hair condition, dye performance and role of ingredients in dye formula. This study not only advances our understanding of hair dye chemistry but also aims to understand how much dye is likely to desorb from the hair over the lifetime of the dyeing and aims to potentially transform consumer experiences with innovation in hair colour products. This new analytical method and its application in hair research and development aims to foster innovation in hair colour products, offering a scientific basis for the development of more durable and consumer-friendly hair dye solutions.

CRediT authorship contribution statement

Kristina Hetherington: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Alenka Tidder:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis. **Bethany J. Tack:** Methodology, Investigation, Formal analysis. **Meryem Benohoud:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Dan Nowlan:** Writing – review & editing, Validation, Resources, Project administration, Methodology, Formal analysis, Conceptualization. **Anwar Zahar:** Writing – review & editing, Validation, Methodology, Formal analysis. **Darcy Prater:** Writing – review & editing, Validation, Methodology, Formal analysis. **Jeanna C. Zguris:** Writing – review & editing, Validation, Resources, Project administration, Methodology, Formal analysis, Conceptualization. **Christopher M. Rayner:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Formal analysis, Conceptualization. **Richard S. Blackburn:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Ethics declaration

Review and/or approval by an ethics committee was not needed for this study because the human hair used in this study was ethically sourced and purchased from International Hair Importers & Products, Inc. from willing participants who are paid for their tress.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2025.116613.

Data availability

Data will be made available on request.

References

[1] V. Sherrow, Encyclopedia of Hair: A Cultural History, Greenwood Press, Westport, 2006.
[2] C.R. Robbins, Chemical and Physical Behavior of Human Hair, 4th ed, Springer, New York, 2002.
[3] S. Harrison, R. Sinclair, Hair coloring, permanent styling and hair structure, J. Cosmet. Dermatol. 2 (2003) 180–185.
[4] S.A. da França, M.F. Dario, V.B. Esteves, A.R. Baby, M.V.R. Velasco, Types of hair dye and their mechanisms of action, Cosmetics 2 (2015) 110–126.
[5] S. Kwon, S. Lee, J. Jang, J.B. Lee, K.S. Kim, Quantifying the effects of repeated dyeing: morphological, mechanical, and chemical changes in human hair fibers, Heliyon 10 (2024) e37871.
[6] M.-L. Lind, S. Johnsson, C. Liden, B. Meding, A. Boman, Hairdressers' exposure to hair dyes during different hair dyeing tasks, Contact Dermatitis 77 (2017) 303–310.
[7] D. Petruzzi, Global hair coloring market size 2022 & 2029, Statista (2023). Available at: https://www.statista.com/statistics/972997/global-hair-color-market-value/.
[8] J.H. Franco, B.F. Da Silva, M.V.B. Zanoni, Assessment of semi-permanent hair dyes in wash water from beauty salons by liquid chromatography-tandem mass spectrometry-selected reaction monitoring (LC-MS/MS-SRM), Anal. Methods 12 (2020) 5415–5423.
[9] A. Towns, A review of developments in industrial hair colorant actives for oxidative dyes, Color. Technol. 137 (2021) 301–335.
[10] G. Zhang, R.L. Mcmullen, Investigation of hair dye deposition, hair color loss, and hair damage during multiple oxidative dyeing and shampooing cycles, J. Cosmet. Sci. 67 (2016) 1–11.
[11] P. Ghosh, A.K. Sinha, Hair colors: classification, chemistry and a review of chromatographic and electrophoretic methods for analysis, Anal. Lett. 41 (2008) 2291–2321.
[12] O.J.X. Morel, R.M. Christie, Current trends in the chemistry of permanent hair dyeing, Chem. Rev. 111 (2011) 2537–2561.
[13] T.B. Zanoni, F. Hudari, A. Munnia, M. Peluso, R.W. Godschalk, M.V.B. Zanoni, G.J. M. den Hartog, A. Bast, S.B.M. Barros, S.S. Maria-Engler, G.J. Hageman, D. Palma de Oliveira, The oxidation of p-phenylenediamine, an ingredient used for permanent hair dyeing purposes, leads to the formation of hydroxyl radicals: Oxidative stress and DNA damage in human immortalized keratinocytes, Toxicol. Lett. 239 (2015) 194–204.
[14] R.C. Garner, C.A. Nutman, Testing of some azo dyes and their reduction products for mutagenicity using *Salmonella typhimurium* TA 1538, Mutat. Res. 44 (1977) 9–19.
[15] A. Aktaş Şüküroğlu, S. Burgaz, Occupational Exposure to the Chemicals in Hairdressing Salons and Health Risk, Turk Hij Den Biyol Derg 75 (2018) 195–212.
[16] M. Chatterjee, V. Mallela, G. Bhaskar, T.S. Vidya, B. Mohod, A. Jaiswal, A real-world data analysis on the usage of paraphenylenediamine and ammonia free hair dye, Int J Res Dermatol 9 (2023) 170–174.
[17] Z.D. Draelos, A clinical evaluation of a permanent hair dye designed to reduce allergic contact dermatitis and hair damage, J. Cosmet. Dermatol. 21 (2022) 3925–3928.
[18] A.D. Bailey, B.P. Murphy, H. Guan, Mechanistic insights into oxidative oligomerization of p-phenylenediamine and resorcinol, J. Phys. Chem. A 120 (2016) 8512–8520.
[19] S.C. Rastogi, A method for the measurement of intermediates of oxidative hair dyes in cosmetic products, J. Sep. Sci. 24 (2001) 173–178.
[20] Y.Z. Hessefort, B.T. Holland, R.W. Cloud, True porosity measurement of hair: A new way to study hair damage mechanisms, J. Cosmet. Sci. 59 (2008) 303–315.
[21] E.O. Alegbe, Uthman TO, A review of history, properties, classification, applications and challenges of natural and synthetic dyes, Heliyon 10 (2024) e33646.
[22] N.M. George, A. Potlapati, Shampoo, conditioner and hair washing, Int J Res Dermatol 8 (2021) 185–191.
[23] A. Schlosser, Silicones used in permanent and semi-permanent hair dyes to reduce the fading and color change process of dyed hair occurred by wash-out or UV radiation, J. Cosmet. Sci. 55 (2004) S123–S131.
[24] A.F. Hamel, J.S. Meyer, E. Henchey, A.M. Dettmer, S.J. Suomi, M.A. Novak, Effects of shampoo and water washing on hair cortisol concentrations, Clin. Chim. Acta 412 (2011) 382–385.
[25] S.K. Kristensen, S.C. Larsen, N.J. Olsen, J. Fahrenkrug, B.L. Heitmann, Hair dyeing, hair washing and hair cortisol concentrations among women from the healthy start study, Psychoneuroendocrinology 77 (2017) 182–185.
[26] K. Hetherington, A. Tidder, B.J. Tack, M. Benohoud, D. Nowlan, A. Zahar, X. Li, D. Prater, J.C. Zguris, T. Tokle, C.M. Rayner, R.S. Blackburn, Method to analyse and quantify the propensity of hair dyes to desorb from hu-man hair fibre, Heliyon 11 (2025) e43528.
[27] C. Solazzo, Characterizing historical textiles and clothing with proteomics. Characterizing historical textiles and clothing with proteomics, Conservar Património 31 (2019) 97–114.
[28] L. Ford, R.L. Henderson, C.M. Rayner, R.S. Blackburn, Mild extraction methods using aqueous glucose solution for the analysis of natural dyes in textile artefacts dyed with Dyer's madder, J. Chromatogr. A 1487 (2017) 36–46.
[29] D. Mantzouris, I. Karapanagiotis, L. Valianou, C. Panayiotou, HPLC-DAD-MS analysis of dyes identified in textiles from Mount Athos, Anal. Bioanal. Chem. 399 (2011) 3065–3079.
[30] C. Mouri, R. Laursen, Identification of anthraquinone markers for distinguishing Rubia species in madder-dyed textiles by HPLC, Microchim. Acta 179 (2012) 105–113.
[31] I. Serafini, G. Favero, R. Curini, G.M. Kavich, T.P. Cleland, Development of a Combined Protein and Dye Extraction Approach for the Analysis of Keratin-Based Textiles, J. Proteome Res. 23 (2024) 3890–3903.
[32] M.S. Jeong, C.M. Lee, W.J. Jeong, S.J. Kim, K.Y. Lee, Significant damage of the skin and hair following hair bleaching, J. Dermatol. 37 (2010) 882–887.
[33] L. Sargysan, V. Vill, T. Hippe, Investigation of vegetable tannins as hair dyes and their interaction with pre-bleached hair fibres, Int J of Cosmet Sci 42 (2020) 320–327.
[34] P. Gimeno, C. Bousquet, N. Lassu, A.-F. Maggio, C. Civade, C. Brenier, L. Lempereur, High-performance liquid chromatography method for the determination of hydrogen peroxide present or released in teeth bleaching kits and hair cosmetic products, J. Pharm. Biomed. Anal. 107 (2015) 386–393.
[35] M.-S. Jeong, C.-M. Lee, W.-J. Jeong, S.-J. Kim, K.-Y. Lee, Significant damage of the skin and hair following hair bleaching, J. Dermatol. 37 (2010) 882–887.
[36] T.N. Williams, F.I. Vacchi, A. dos Santos, Umbuzeiro G. de Aragão, H.S. Freeman, Metal-complexed monoazo dyes as sustainable permanent hair dye alternatives – Toxicological and durability properties, Dyes Pigments 197 (2022) 09819.