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Polyphenol-Metal Complex With Dopamine for Dyeing Natural White Hair

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Synopsis

Hair becomes white due to melanin deficiency, and hair dye can be used to restore hair color. Currently, commercial hair dyes are dominated by p-phenylenediamine (PPD) hair dyes, although PPD has been suspected of being carcinogenic. Natural plant polyphenols, when complexed with metals, produce a variety of colors that can be used for hair coloration. Gallic acid and ferrous ions self-assemble to form a network structure, and the addition of dopamine results in a composite hair dye. By using ethanolamine to unfold the natural white hair scales, dye molecules can penetrate through the hair scales to the hair cortex, and the dopamine oxidative self-polymerization in the hair dye forms an adhesion film in the hair cortex layer, thereby fixing the dye molecules. A hair washability test showed that the color difference value remained unchanged after 40 washes. Scanning electron microscope (SEM) analysis of the hair after dyeing showed that the hair dye did not destroy the hair structure. Additionally, results from hair stretch tests displayed good stretch resistance to the hair. This study shows that hair dye based on a polyphenol-metal complexes containing dopamine could be used as an alternative to PPD-based hair dyes.

INTRODUCTION

Natural melanins are important pigments found throughout living organisms, including microorganisms, plants, animals, as well as in human eyes, skin, and hair.[1,2] Melanin has several biological functions, such as UV protection, free radical scavenging, antibiotic function, and neuroprotection.[3,4] Natural hair color is derived from the presence of melanin in the cortex,[5] with its abundance and relative concentration determining hair color. A deficiency of melanin is believed to be responsible for graying or whitening hair, and white hair can be restored to color using hair dyes.[5,6] Modern hair dyes are classified as oxidative or non-oxidative; oxidative hair dye products are often referred to as permanent or semi-permanent, while non-oxidative hair dye products are considered temporary or semi-permanent.[7] A variety of new, permanent oxidative chemical hair dyes dominate the global hair dye market. Permanent hair dyes have the highest market share of all modern hair dyes in Asia, the Americas, and Europe.[8] However, most commercial permanent hair coloring products contain hydrogen peroxide and PPD or PPD-derived compounds as key ingredients. These substances undergo chemical reactions both on the hair's surface and internally, resulting in

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*Address all correspondence to Min Yang, 2578973180@qq.com a permanent alteration of hair color.[5] However, such compounds are sometimes allergenic, toxic, and potentially carcinogenic to humans. For example, PPD, despite being deemed safe for cosmetic use by the Cosmetic Ingredient Review, has been shown to readily cross the skin barrier and has been considered a human allergen and carcinogen.[9] In addition, *in situ*, oxidation reactions in the presence of such compounds may damage hair and scalp,[10] and some alternative hair coloring methods have been proposed to address the potential human health hazards of the aforementioned oxidative permanent hair dyes. For example, hair dyes based on graphene sheets utilize graphene oxide (GO) and its reduced form, r-GO, to create water-based formulations that form a smooth and continuous coating on the hair to achieve a dyeing effect.[11] Another approach involves inducing melanin-like polydopamine (PDA) and brown melanin-like PDA/cysteine co-deposited coatings on the hair's surface using sodium periodate. This process yields melanin-mimicking multicolor hair dyeing with low toxicity.[12] To achieve dyeing, the metal chelation-assisted deposition of polydopamine on human hair results in black coloration of human keratin hairs.[13] This rapid hair coloring of natural white hair is achieved through the formation of metal-phenolic networks (MPNs).[14] Polymerization by laccase-mediated *in situ* oxidation of dopamine produces intensely pigmented polymers and is used for the biological coloration of human hair.[15]

In addition, gallic acid (GA) has been studied for hair coloring. GA is a phenolic compound found in large quantities in quince and pomegranate skins and is used as a food additive.[16,17] GA is also a very important benzoic acid with anti-ulcer, antibacterial, antifungal, and antioxidant properties.[16,18] It has been reported that GA can be complexed with metal ions to form multi-colored dyes for hair dyeing applications. GA has been polymerized with $CuCl_2$ in aqueous sodium chloride solution to form oligomers to produce polyphenolic hair dyes. These dyes result in a dark brown color to the hair. Meanwhile, Cu(II) ions complex with hydroxyl groups to form Cu-O couplings, which can form an ion-channel coating on the surface of the hair to protect the hair. However, hair dye has the problem of thickening the hair and reducing hair stretch.[19] Based on the property that laccase can catalyze the polymerization of phenolic structures to form dimers, Jeon et al. used laccase to catalyze the reaction of GA to produce a brown dye and for coloring white hair.[20] Varsha Panwar et al., used a heat-resistant bacterial laccase from Puccinia agalactiae to react with GA in a coupling reaction to produce hair color, which can be used to dye bleached hair to a dark brown color.[21] Deepak Kumar et al. used a laccase from *Bacillus subtilis* DS to react with GA to produce a golden yellow dye, which can be used as an alternative to synthetic dyes (such as PPD) for hair coloring.[22] In addition to being prepared as a dye for hair coloring, GA was combined with GA and tannic caffeic acid to prepare a hair-coating polyphenol complex that was able to provide UV radiation protection to the hair by coating the hair cuticle, minimizing the electrostatic effect and improving the mechanical strength of the hair.[23] In addition, GA was prepared as a hair supplement for its antioxidant effect on hair.[24] As mentioned earlier, commercial hair dyes (such as PPD) are allergenic, so it is also a matter of consideration when exploring GA for dyeing gray hair. However, GA has been reported to have potent antioxidant, anti-inflammatory, antibacterial, and anticancer activities,[16, 18, 25] and even in studies applied to cosmetics, GA has been reported to have anti-inflammatory, antioxidant, and anti-aging activities.[26-28] Swan et al. reported that a patient with PPD allergy used pure henna, black tea, and indigo powder as an alternative hair dye, but the patient still experienced allergic symptoms. It is well known that pure henna and black tea contain GA, so Swan et al. tested patients and 10 controls for GA allergy, and the results were all negative.[29] Therefore, there's no need for excessive concern about allergies when using GA in hair dye.

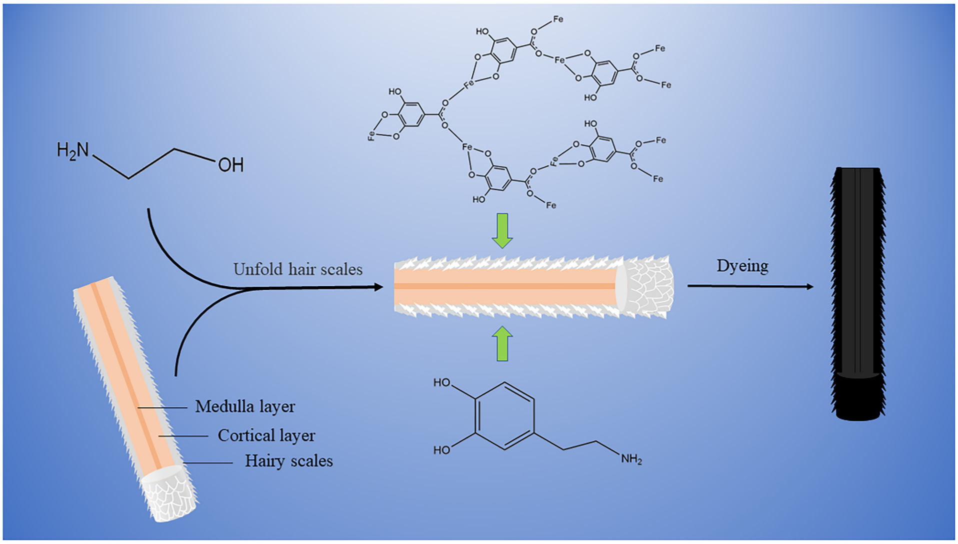

Figure 1. Schematic diagram of polyphenol-metal coordination structure compound dopamine dyeing natural white hair.

In this paper, to obtain safer and more environmentally friendly polyphenol-based hair dyes, the effects of different parameters (i.e., nine polyphenols, Fe(II) or Fe(III), pH, time) on the coloration of the dyed gray hair were investigated. When ethanolamine (EA) was used as an alkalizing substance to open the hair cuticle, natural white hair could be dyed by using the complex of GA and Fe(II), and the dyed hair fibers showed good durability in washing with the aid of dopamine hydrochloride (DA) (Figure 1).

MATERIALS AND METHODS

MATERIALS

GA, DA, Tannic acid (TA), Caffeic acid (CA), Resorcinol (RE), ascorbic acid, sodium benzoate, glycerol, and tween-80 were purchased from Shanghai Aladdin Biochemical Technology (Shanghai, China). 3, 4-Dihydroxybenzaldehyde (Protocatechualdehyde; PA), Catechin hydrate (CH), Tris (hydroxymethyl)aminomethane (Tris), ethanolamine (EA), oleic acid, and sodium dodecyl sulfate were purchased from Shanghai Macklin Biochemical. (Shanghai, China). Salicylic acid (SA) from Yuanye Bio-Technology (Shanghai, China). Pyrogallol (PG) and Ethanol absolute from Energy Chemical (Shanghai, China). Ferrous chloride tetrahydrate from Meyer (Shanghai, China). 1,10-Phenanthroline from Leyan Technologies (Shanghai, China). Sodium acetate from Jinshan Chemical Test (Chengdu, China). Hydroxylamine hydrochloride, Anhydrous sodium sulfite, and isopropanol from Tianjin Kermel Chemical Reagent Co. (Tianjin, China). Propylene glycol from Tianjin Fuyu Fine Chemical Co. (Tianjin, China). Sodium thiosulfate from Youpu (Tianjin, China). Commercial white hair (9° platinum blonde) was purchased from YNE (China), human natural white hair (non-commercial) samples (provided by volunteers), and pure water (ELGA) prepared by PURELAB Flex ultrapure water system was used for the experiments.

HAIR DYEING PRE-TREATMENT

Before hair coloring, commercial human white hair and human natural white hair samples were soaked in commercial shampoo (Ruwei Cosmetics Co., RNW, Product Standard No. GB/T 29679) and repeatedly rubbed for 5 minutes. After washing, the hair was rinsed with water and left to dry naturally in the air.

POLYPHENOL SCREENING

0.035 g GA, TA, PA, PG, CH, RE, CA, and SA were dissolved in 30 mm and 5 mL Tris solution to prepare polyphenol solution; 0.035 g DA was dissolved in 30 mm, 5 mL Tris-HCl solution to prepare DA solution; 0.035 g DA was dissolved in 30 mm and 5 mL Tris solution to prepare PDA solution. The above solution was mixed with 10 parts of ferrous chloride tetrahydrate dissolved in 3 mL of water according to the ratio of polyphenols to Fe(II) at a ratio of 1:5, and mixed with vortex vibration for 10 seconds, and then centrifuged (2,000 g, 5 min) to remove the precipitate to prepare the polyphenol-Fe (II) solution. We took 10 parts of 0.2 g commercial white hair and immersed it in polyphenol-Fe(II) solution for 30 minutes. We then removed it and washed the residual solution with clean water and dried it with absorbent paper. Next the samples were allowed to dry naturally. Finally, we measured the color difference after natural drying, and screened the polyphenols with the best dyeing effect.

In addition, we assisted in the screening of the best polyphenol materials by determining the chelation rate of polyphenols and Fe(II). The first is to establish a standard curve. We added 1 mL and 1 mg/mL of ferrous chloride tetrahydrate solutions, 0.1 mL hydroxylamine hydrochloride (10%), 0.2 mL o-diazepine (0.15%), and 0.5 mL sodium acetate (1 mol/mL). Water was added to 10 mL, and 0.1 mg/mL Fe(II) solution was prepared. The above 0.1 mg/mL Fe(II) solution was prepared into Fe(II) standard solutions of 0.025 mg/mL, 0.05 mg/mL, 0.07 mg/mL, 0.085 mg/mL, and 0.095 mg/mL, respectively. The absorbance of each group of solutions was measured at a wavelength of 510 nm, and the concentration C (mg/mL) of Fe(II) standard solution was used as the abscissa, and absorbance A (nm) was used as the ordinate to establish a standard curve. We took 1 mL of the above polyphenol-Fe(II) solution, added absolute ethanol to 10 mL, sonicated for 15 minutes, centrifuged (2,000 g, 5 min), and took two parts of 1 mL supernatant, numbers 1 and 2, respectively. Tube 1 added 0.1 mL of hydroxylamine hydrochloride, 0.2 mL of phenanthrene hydrochloride, 0.5 mL of sodium acetate, and added water to set the volume to 10 mL; Tube 2 added 0.1 mL of hydroxylamine hydrochloride, 0.5 mL of sodium acetate, and added water to set the volume to 10 mL. The absorbance at 510 nm wavelength was determined separately, and the chelation rate of polyphenol-Fe(II) was calculated.

ETHANOLAMINE CONDITIONS WERE SCREENED WITH GA-FE(II) CONDITIONS

A single-factor test was employed to investigate the pH value, concentration, and ethanolamine immersion time of natural gray hair after various ethanolamine soaking durations. Subsequently, the hair was stained in a GA-Fe(II) solution with a molar ratio of GA to Fe(II) of 1:5 and a pH range of 4–5 for 2 hours. After post-processing, the color difference value was measured under different ethanolamine conditions to identify the optimal conditions for staining natural gray hair with ethanol.

Natural gray hair was treated in 20% ethanolamine solution at pH 9–10 for 1 hour, followed by removal of excess liquid using absorbent paper and allowing it to dry naturally. In this study, the single-factor test was conducted to access the dyeing time of GA-Fe(II)-stained natural white hair, the pH of GA-Fe(II) solution, and the molar ratio of GA-Fe(II) to Fe(II). The optimal dyeing conditions for GA-Fe(II)-stained natural white hair were determined by measuring the color difference value of stained white hair.

ETHANOLAMINE EMULSION FOR UNFOLDING NATURAL WHITE HAIR SCALES

First, 2 mL of ethanolamine was added to 8 mL of water and mixed, and the pH was adjusted to 9.2–9.4. Then, 0.8 mL of glycerol, 0.4 mL of Tween-80, 0.2 g of sodium dodecyl sulfate, 0.1 g of sodium sulfite, 0.2 g of sodium benzoate, 2 mL of propylene glycol, and 2.3 mL of water were added to the ethanolamine solution and mixed at 65°C for 15 minutes to obtain the aqueous phase solution. Additionally, 2 mL of isopropanol and 2 mL of oleic acid were heated and stirred at 65°C for 15 minutes to create the oil phase solution. The oil phase was added to the aqueous phase solution while heating and stirring at 65°C for 10 minutes, resulting in the formation of the oil-in-water ethanolamine emulsion. The emulsion was allowed to cool to room temperature, and 0.2 g of natural white hair was immersed in the emulsion for 1 hour. Subsequently, the emulsion was removed, and the residual solution on the white hair was wiped off with absorbent paper to be set aside.

GA-FE(II)+DA HAIR DYE FOR DYEING NATURAL WHITE HAIR

0.035 g GA was dissolved in 30 mm of 5 mL Tris solution to obtain GA solution. Next, 0.2088 g of ferrous chloride tetrahydrate was dissolved in 3 mL of water to create the Fe(II) solution (with a molar ratio of GA to Fe(II) at 1:5). The two solutions were mixed by vortex vibration for 10 seconds. Then, 0.035 g of DA was dissolved in the mixture, and the pH was adjusted to 5.0–5.2. Afterward, it was centrifuged (2,000 g for 5 minutes) to remove the precipitate, resulting in the preparation of the GA-Fe(II)+DA solution.

To prepare the aqueous phase solution, 2 mL of propylene glycol, 0.4 mL of Tween-80, 0.2 g of sodium dodecyl sulfate, 0.1 g of sodium thiosulfate, 0.1 g of sodium sulfite, 0.1 g of ascorbic acid, 0.2 g of sodium benzoate, 0.8 mL of glycerol, and 3.8 mL of water at 65°C with heating and stirring for 15 minutes. The GA-Fe(II)+DA solution was then added under the same conditions and further heated and stirred for 10 minutes.

The oil phase solution was prepared by mixing 2 mL of isopropanol and 2 mL of oleic acid at 65°C with heating and stirring for 15 minutes. The oil phase was added in a fine stream to the aqueous phase solution while heating and stirring at 65°C, and the mixture was stirred for an additional for 10 minutes to obtain the oil-in-water emulsion. The emulsion was cooled to room temperature to yield GA-Fe(II)+DA hair dye. The natural white hair, unfolded by ethanolamine, was dipped into the hair dye for 2 hours. Then the residual solution was washed off with water and dried with a hair dryer.

MEASUREMENTS OF COLOR PARAMETERS

Color parameters, including L* (luminance), a* (red/green coordinates), and b* (yellow/blue coordinates), were obtained using a colorimeter (HP-2136, Shanghai HanPat Optoelectronics

Technology Company Limited, Shanghai, China). The measurements were repeated three times in different areas of the hair sample. The averages of the measured parameters (L*, a*, b*) represent the averages of three repeated measurements in different areas. Color difference (ΔE) is calculated by the formula: $\Delta E = \left[(100 - L^*)^2 + (a^*)^2 + (b^*)^2\right]^{1/2}$.

COLOR DURABILITY TEST

The dyed hair samples were completely immersed in 50 mL of shampoo solution, soaked, and rubbed for 1 minute, then dried with a hair dryer for one wash. The washing procedure was repeated to determine the color parameters for 1, 10, 20, and 40 washes, allowing the determination of the color durability of the dyed hair samples.

HAIR DYE UV-VIS, FT-IR ANALYSIS, AND SEM CHARACTERIZATION OF HAIR SAMPLES

The UV-visible absorption spectra of GA, DA, GA-Fe(II)+DA were determined using a UV-visible spectrophotometer (Shanghai Metash Instruments Co., Ltd., UV-5500PC). The infrared spectra of dopamine hydrochloride, GA, ferrous chloride tetrahydrate, GA-Fe(II) solution, and GA-Fe(II)+DA solution were determined by FT-IR infrared spectrophotometer (PerkinElmer, Spectrum Two). The hair morphology was observed using a field emission SEM; Zeiss, Sigma 500).

HAIR STRETCHING TEST

Hair's tensile strength was measured using an electronic universal material testing machine (Shimadzu, AGS-X50N AGS-10KN) with a 200 mm scale and a stretching speed of 20 mm/min.

RESULTS AND DISCUSSION

POLYPHENOL STAINING EFFECT AND CHELATION RATE

Phenolic compounds contain many dihydroxyphenyl or trihydroxyphenyl groups with high π electron content and aromatics, which provide an effective negative binding site for cation formation cation-π interactions. These interactions, which dominate non-covalent cation-π interactions in the presence of other competing motifs, form stable polyphenol-metal structures. During staining, polyphenol-Fe(II) first comes into contact with the outer layer of hair scales and then develops color by adsorption, diffusion to the cortex, and accumulation in its interior to form a polyphenol-Fe(II) network structure. Different polyphenols react with ferrous ions to form structures that result in different colors and varying dyeing effects on white hair. We selected eight polyphenol materials, dopamine, and polydopamine to stain commercial gray hair and measured the color difference value (Figure 2A). In addition, we used ultraviolet spectrophotometry to establish a standard curve (Figure 2B), measured the absorbance value (A) of each polyphenol material chelated to Fe(II) at 510 nm, and calculated the chelation rate according to the standard curve and equation (1)(2)(3)(4)(5) to further screen for the best polyphenol materials.

$$A = 6.7356c_3 + 0.0077 \quad \text{(eq. 1)}$$

$$c_2 = 10c_3 \quad \text{(eq. 2)}$$

$$m_2 = c_2V \quad \text{(eq. 3)}$$

$$m_1 = M_{Fe} \div M \times m_0 \quad \text{(eq. 4)}$$

$$w = (m_1 - m_2) \div m_1 \times 100\% \quad \text{(eq. 5)}$$

Among them, c_3 is used to measure the concentration of Fe(II) in the solution during absorbance; c_2 is the concentration of Fe(II) in the centrifuged supernatant after the reaction; m_2 is the mass of Fe(II) in the centrifuged supernatant after the reaction; V is the reaction volume, which is 10 mL; m_1 is the total mass of Fe(II) in the reaction system; m_0 is the mass of weighed ferrous chloride tetrahydrate; M_{Fe} is the molar mass of iron (M_{Fe} = 56 g/mol); M is the molar mass of ferrous chloride tetrahydrate (M = 198.71 g/mol); w is the chelation rate.

Among the color difference results of 10 polyphenols reacted with Fe (II) to prepare hair dyeing solution to dye natural white hair, the color difference values of GA, PA, CH, and CA were all above 60, indicating that these four materials had a better dyeing effect on

Table I
Absorbance Value and Chelation Rate of Various Polyphenols and Fe(II)

Polyphenol	m_0 (g)	A (mL/ mol/cm)	c_3 (mg/mL)	m_2 (mg)	m_1 (mg)	w (%)
GA	0.2050	0.3517	0.0511	5.1100	57.7726	91.15
TA	0.2302	0.6199	0.0909	9.0900	64.8744	85.99
PA	0.2520	0.4892	0.0715	7.1500	71.0181	89.93
PG	0.2742	0.6644	0.0975	9.7500	77.2744	87.38
CH	0.1205	0.7068	0.1038	10.3800	33.9590	69.43
SA	0.1941	0.6633	0.0973	9.7300	71.0744	86.31
CA	0.2522	0.6535	0.0959	9.5900	54.7008	82.47
DA	0.2266	0.6031	0.0884	8.8400	63.8599	86.16
PDA	0.2286	0.6015	0.0882	8.8200	64.4423	86.31

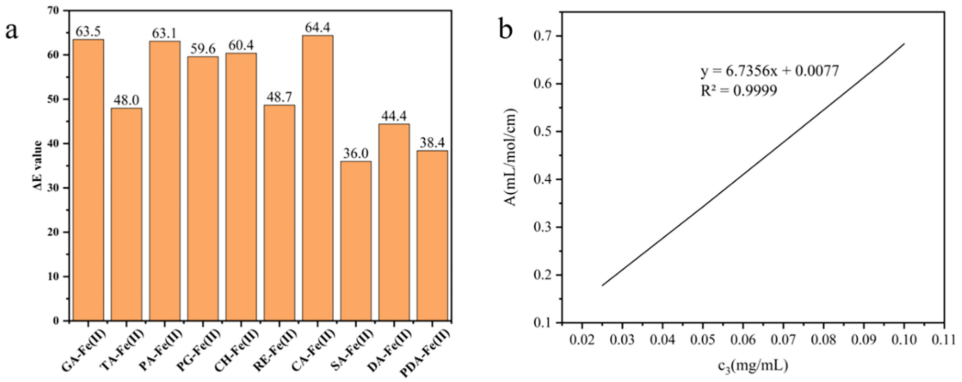

Figure 2. (A) Color difference value of polyphenol and Fe(II) stained natural white hair and (B) standard curve.

natural white hair. Among these four polyphenol materials, GA, PA, and Fe(II) had the highest chelation rates, all of which were above 89%, while the chelation rate of CH and CA was lower. CH had a chelation rate of only 69.43%, which showed that GA, PA, and Fe(II) were better combinations. However, considering the toxicity of polyphenol materials, GA was ultimately selected for the study of dyeing natural gray hair.

POLYPHENOL-METAL COMPLEX WITH DOPAMINE FOR DYEING NATURAL WHITE HAIR

Natural plant polyphenol GA and metal ferrous chloride tetrahydrate in solution undergo a coordination reaction to form a polyphenol-metal complex with a black solution, which is then prepared by adding dopamine to form a complex solution for dyeing commercial white hair and dyed black (Figure 3C). Based on this principle, we prepared GA-Fe(II)+DA hair dye (Figure 3F) to dye natural white hair. However, during the study, we found that we could not directly dye natural white hair with GA-Fe(II)+DA hair dye, and we could not successfully dye natural white hair even after modifying the dyeing time, the concentration of hair dye, or the pH of hair dye. Upon comparing scanning electron microscopy images of commercial white hair and natural white hair (Figure 3G, H), we observed that the epidermal layer of commercial white hair had no ordered overlapping scales, indicating that the hair scales were completely damaged, and the hair structure was destroyed.

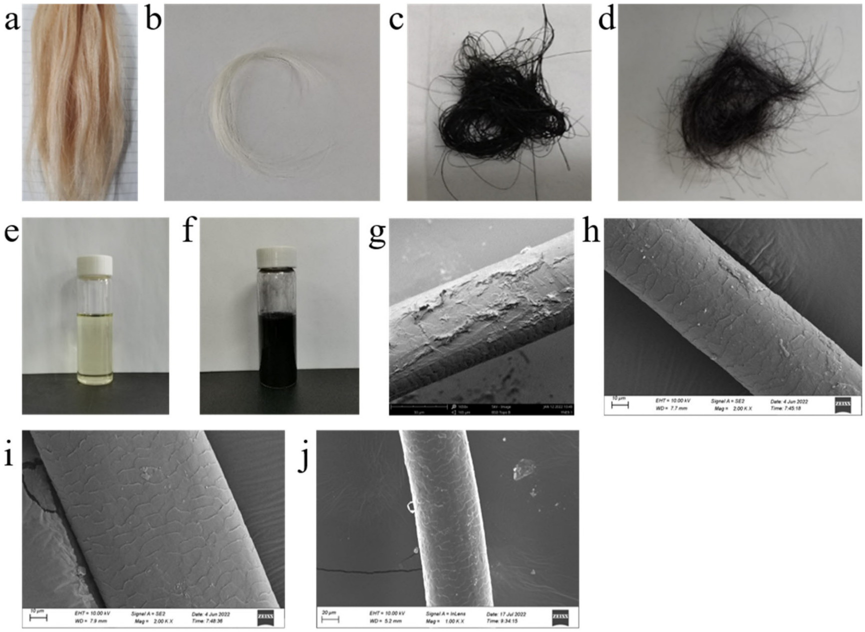

Figure 3. (A) Commercial white hair samples. (B) Natural white hair sample. (C) Commercial white hair dyed with GA-Fe(II)+DA solution. (D) GA-Fe(II)+DA hair dye dyed natural white hair. (E) Ethanolamine emulsion and (F) GA-Fe(II)+DA hair dye. (G) SEM image of commercial white hair (undyed) and (H) SEM image of natural white hair (undyed). (I) SEM image of natural white hair with ethanolamine emulsion unfolded hair scales. (J) SEM image of natural white hair with ethanolamine emulsion unfolded hair scales dyed by GA-Fe(II)+DA hair dye.

Consequently, dye molecules could easily penetrate the hair cortical layer and achieve successful dyeing.

In contrast, natural white hair retained intact hair scales and an undisturbed hair structure, preventing dye molecules from directly passing through the scales into the hair cortex for effective dyeing. Therefore, we prepared an ethanolamine emulsion (Figure 3E) in an alkaline environment to unfold the hair scales of natural white hair so that the dye molecules of GA-Fe(II)+DA hair dye could directly pass through the hair scales into the hair cortex for deposition to achieve dyeing of natural white hair (Figure 3D). SEM images of natural white hair treated with ethanolamine emulsion and natural white hair dyed with GA-Fe(II)+DA hair dye after unfolding the hair scales with ethanol emulsion showed (Figure 3I, J) that the hair surface was smooth without the presence of dye molecule adhesion films. This indicated that both dye molecules and dopamine entered the hair cortical layer. Furthermore, the hair scales in the epidermal layer of dyed natural white hair exhibited no debonding, layering, or flexing, and the hair surface remained smooth and structurally intact. These findings were consistent with the results obtained from SEM images of untreated natural white hair. They indicated that both the ethanolamine emulsion and the GA-Fe(II)+DA hair dye did not adversely affect the hair structure and that the intact hair scales helped retain moisture in the hair more effectively.[30]

The raw materials used in both the ethanolamine emulsion and hair dye are cosmetic ingredients approved for use by the China Food & Drug Administration. In addition, ferrous ions are relatively non-toxic compared to other heavy metals. According to the regulatory guidelines of the Korean Ministry of Food and Drug Safety, a certain concentration of ferrous ions is allowed to be used on human hair and scalp.[13] Therefore, GA-Fe(II)+DA hair dye may be a potential safer alternative to traditional hair dyes.

In addition, the effects of ethanolamine solution pH (Figures 4A, 4B, 4C), ethanolamine concentration (Figure 4D), and the time of ethanolamine soaking natural gray hair (Figure 4E) on hair scaling, and consequently, the hair coloring effect, were evaluated. Optimal conditions for achieving the desired dyeing effect with ethanolamine were determined based on the ΔE value. The best results were observed when the pH of the ethanolamine solution was within the range of 9.2–9.4, with the most effective dyeing of natural white hair occurring in this pH range. Deviating from this pH range resulted in a decrease in the color difference during dyeing. It's important to note that excessively high or low pH levels can be detrimental to human hair and the scalp. Thus, maintaining a pH of 9.2–9.4 is considered more suitable for this process.

Moreover, soaking natural gray hair in ethanolamine for 1 hour proved to be the most reasonable duration in the context of the hair coloring industry. Lastly, an ethanolamine concentration of 20%,was found to be the most effective for coloring natural gray hair.

We also screened the effects of GA-Fe(II)+DA impregnation time on natural gray hair (Figure 5A), the molar ratio of GA to Fe(II) (Figure 5B), and the pH of GA-Fe(II)+DA (Figure 5C-D) on the effect of natural gray hair coloring. Our findings revealed that when the molar ratio of GA to Fe(II) was 1:5, the time of GA-Fe(II)+DA impregnating natural white hair was 2 hours, and the pH of GA-Fe(II)+DA hair dye was 5.0–5.2. When the pH of GA-Fe(II)+DA was screened, we found that if the pH of GA-Fe(II)+DA is alkaline, Fe(II) in the solution will be precipitated as $Fe(OH)_2$, which cannot be prepared as a hair dye. In addition, hair dyes with an acidic pH can effectively promote the closure of hair scales and prevent the loss of hair coloring components in the hair. Of course, the dyeing

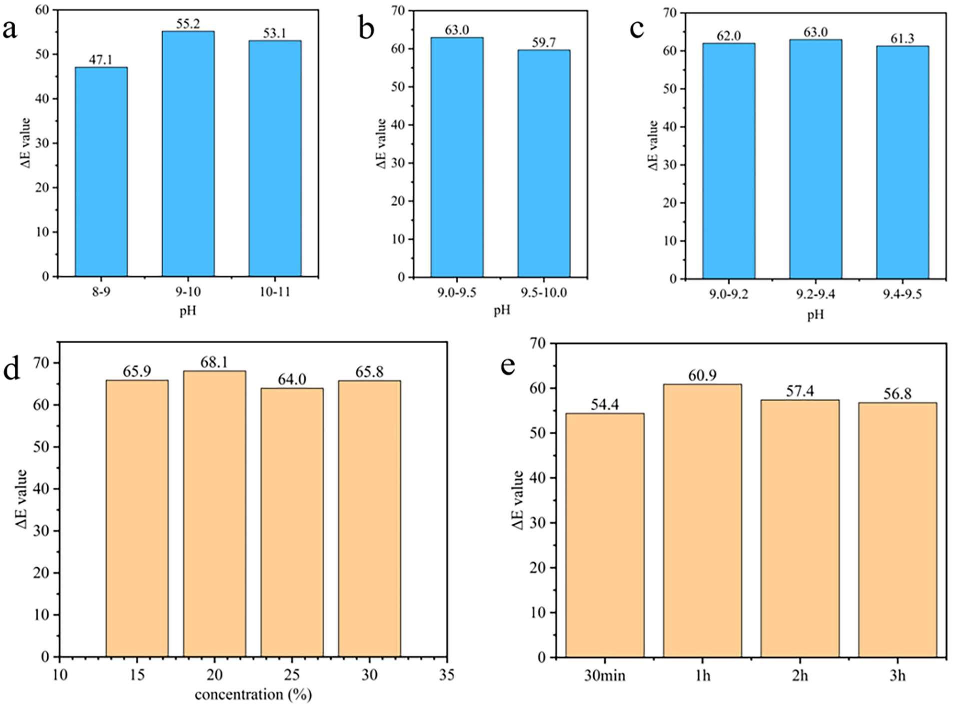

Figure 4. (A), (B), (C) ethanolamine optimal pH screening. (D) Screening for optimal concentration of ethanolamine. (E) The time for which ethanolamine soaks natural gray hair.

time can still be achieved when the natural white hair time of ethanolamine soaking is 30 minutes, and the dyeing time of GA-Fe(II)+DA hair dye is 30 minutes. The dyeing time can be adjusted according to the dyeing needs in the actual application process.

HAIR DYEING MECHANISM AND ANALYTICAL CHARACTERIZATION

To gain a deeper understanding of the self-assembly process involving GA and ferrous chloride tetrahydrate, which undergo a coordination reaction in solution to form a polyphenol-metal complex, and to confirm that dopamine remains independent in the solution without participating in the coordination reaction, we prepared GA-Fe(II)+DA solutions and analyzed their UV-Vis absorption spectra. This analysis aimed to illustrate that GA-Fe(II) successfully reacts to complete the self-assembly process, while dopamine does not.

The UV-Vis absorption spectrum of GA-Fe(II)+DA solution has a broad absorption ranging from 800 to 500 nm, along with a coordination-to-metal charge transfer (LMCT) band at 621 nm. These findings indicate the in-situ formation of GA-Fe(II) coordination structure and the predominant presence of bi-type coordination within the polyphenol-metal complex (Figure 6C). The shoulder peak at 230 nm–290 nm demonstrated the intact presence of dopamine. The composite solution of GA-Fe(II)+DA and GA-Fe(II) solution (without DA addition) were centrifuged (2,000g, 5 min) and the supernatant was freeze-dried (SCIENTZ, SCIENTZ-12N, Freeze-dryer) to obtain FR-IT spectra for

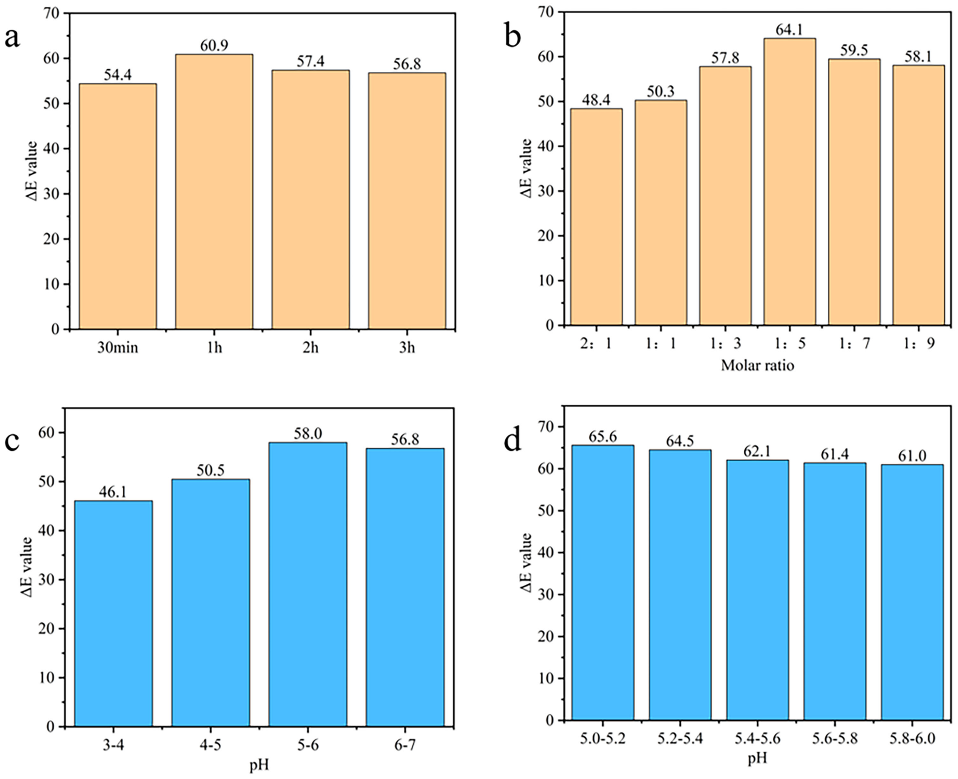

Figure 5. (A) Time screening of GA-Fe(II)+DA impregnated natural gray hair. (B) Molar ratio screening of GA to Fe(II). (C), (D) pH screening of GA-Fe(II)+DA.

solid determination. Figure 6D shows the FR-IT spectra of dopamine hydrochloride, GA, ferrous chloride tetrahydrate, GA-Fe(II) solution, and GA-Fe(II)+DA solution. In the FR-IT spectra of GA-Fe(II)+DA, we found that 1,611 cm^{-1} and 1,522 cm^{-1} are benzene ring skeleton vibrational absorption peaks. Additionally, a peak at 1,286 cm^{-1} indicates carbon-oxygen single bond stretching vibrations. Importantly, the strong absorption peak between 1,100 cm^{-1} and 1,000 cm^{-1}, consistent with ferrous chloride tetrahydrate, confirms the complete formation of the GA-Fe(II) complex structure. In addition, the spectrum of GA-Fe(II)+DA showed no bending vibration peaks of amine and stretching vibration peaks of carbon and nitrogen bonds, which proved that DA was not involved in the complexation in the coordination structure of GA-Fe(II), as DA was removed during the freeze-drying process.

Although GA-Fe(II)+DA hair dye can dye commercial white hair, the dye molecules cannot penetrate the cortical layer of hair through the protective layer of hair scales of natural white hair without the help of external forces. This is because the maximum critical molecular size that can be allowed to pass through the protective layer of hair scales is 1.4 nm. Figure 7A shows the van der Waals sphere model for calculating the molecular sizes of GA, DA, and GA-Fe(II). The results show that the molecular size of GA is 0.965 nm $\leq$ 1.4 nm, and when GA forms a complex with Fe(II), the molecular size increases to $\geq$ 1.4 nm. The larger the molecular volume of the complex, the larger the molecular size, making it impossible

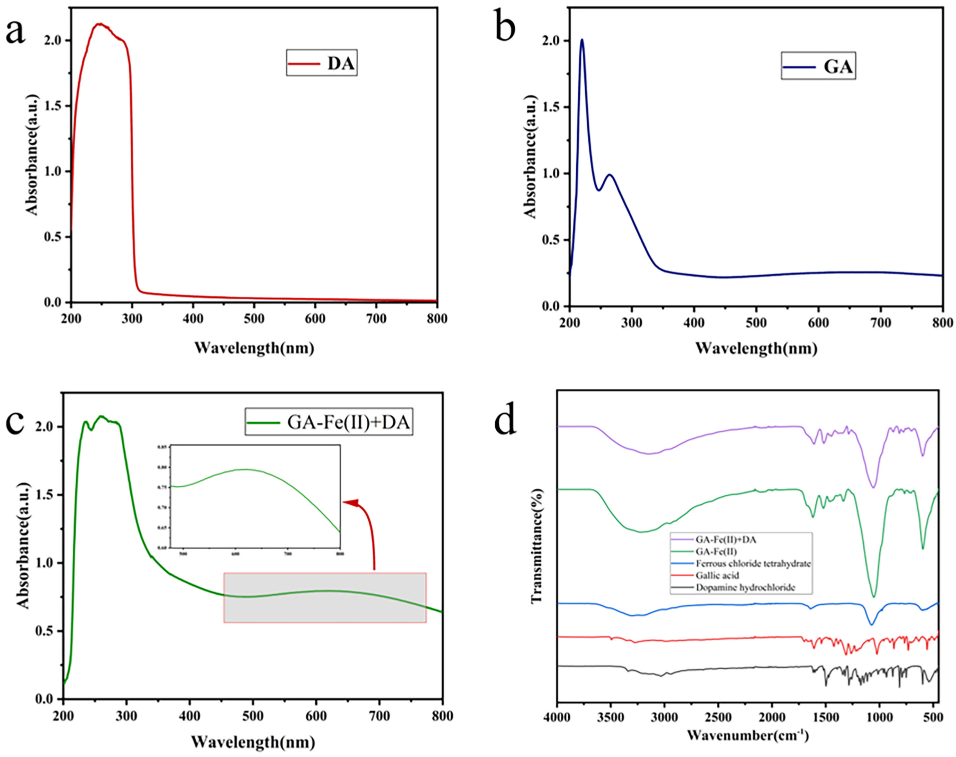

Figure 6. UV-Vis absorption spectra of (A) DA, (B) GA, and (C) GA-Fe(II)+DA. (D) FT-IR spectra of DA, GA, Fe(II), GA-Fe(II)+DA.

for the complex molecules to penetrate the hair cortex layer (Figure 7B). By unfolding the hair scales using ethanolamine emulsion, the maximum critical molecular size that the protective layer of hair scales can allow to pass through increases, and large-size dye molecules can also easily penetrate the hair cortex layer to achieve natural white hair dyeing. Dopamine, which is free in the hair dye, also penetrates the cortical layer of hair and is deposited and autoxidized to polymeric polydopamine, which increases dramatically in size and cannot penetrate from the cortical layer to the hair surface through the hair scales. Its adhesive properties effectively secure the dye molecules within the cortical layer of the hair, accomplishing color fixation, enhancing the hair's resistance to washing, and resulting in a permanent dyeing effect.

In our study, we observed that natural white hair dyed solely with GA complexed with Fe(II) dye molecules exhibited poor resistance to washing, with a significant color difference occurring even after just 40 washes. Dopamine can adhere to the surface of the hair and form an adhesive film that acts as a color fixation.[31] Therefore, we introduced dopamine to prepare a composite hair dye for improving the problem of poor washing resistance and achieving permanent hair dyeing. However, in our study, we found that dopamine did not adhere to the hair surface, but rather entered the hair cortical layer through the hair scales. Based on the property that dopamine is prone to spontaneous oxidation and polymerization into polydopamine,[32] we hypothesized that dopamine accumulates in the cortical layer and undergoes autoxidation and polymerization into polydopamine, which adheres to fix

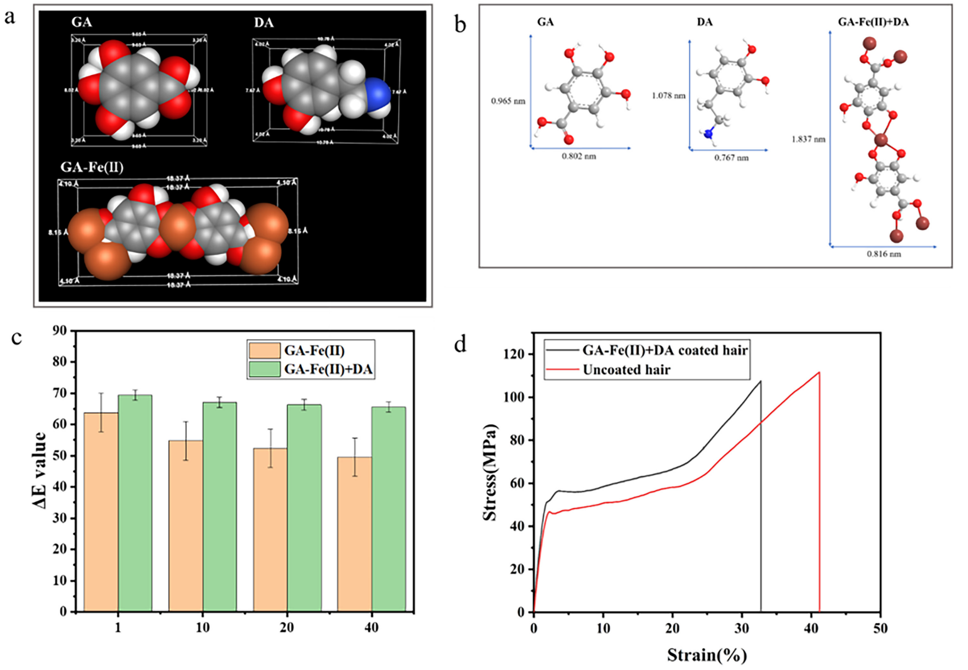

Figure 7. (A) Van der Waals ball model of DA, GA, and GA-Fe(II). (B) Ball-and-stick model of DA, GA with GA-Fe(II). (C) ΔE values of GA-Fe(II) and GA-Fe(II)+DA for washing resistance test. (D) Stress-strain curves of natural white hair (untreated) and GA-Fe(II)+DA dyed hair.

the dye molecules, making them less susceptible to washing and enhancing the washing resistance of dyeing (Figure 7C). We determined that permanent hair dyeing could be achieved using GA-Fe(II)+DA hair dye. In addition, the introduction of DA resulted in a subsequent increase in the color difference value of the hair, i.e., a better dyeing effect. In addition, we did a tensile test on dyed hair versus hair without dyeing treatment, and according to the results in Figure 7D, the dyed hair was able to withstand greater force within a period of strain value (approximately $< 32\%$), and the hair broke when this strain value was exceeded. However, both dyed and undyed hair can withstand similar stress values, which means that the dyed hair can withstand the same force as undyed natural white hair. However, it can withstand less strain in the stress range, i.e., the dyed hair has slightly poorer tensile resistance, which we speculate is caused by the unfolded hair scales not closing in time by ethanolamine. Nevertheless, over time, the unfolded hair scales gradually close, and through oxidation with the deposited dopamine in the cortical layer, they self-polymerize into polymeric polydopamine. This polymeric substance forms an adhesive film within the cortical layer, encapsulating and securing the materials within the hair to prevent loss. Moreover, it enhances the tensile properties of the hair.

CONCLUSIONS

In this study, we developed a method for dyeing natural white hair by creating a complex formed through the interaction of polyphenols with metals, followed by the addition of dopamine to formulate a complex hair dye. With the help of the alkaline environment of ethanolamine emulsion to unfold the hair scales, the dye molecules were able to quickly penetrate the hair cortex layer to accumulate color development. The oxidative self-polymerization property of dopamine allows the dye molecules to be adhered to the cortical layer, fixing the dye molecules to achieve a permanent dyeing effect. Also, the effects of different parameter conditions (i.e., pH, the molar ratio of GA to Fe(II), dyeing time, and ethanolamine concentration) on the dyeing effect of natural white hair were investigated. In addition, the replacement of different polyphenol raw materials or metal raw materials was able to achieve multiple color dying. The ΔE values, SEM characterization results, and stress-strain curves of the hair indicate the hair dye's safe and fast dyeing performance. Our formulation offers a faster and more environmentally friendly alternative to commercial PPD hair dyes while maintaining safety standards. GA-Fe(II)+DA hair dye has the potential to replace conventional commercial PPD hair dyes.

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