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Iron Gall Ink Revisited: Natural Formulation for Black Hair-Dyeing

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Abstract: Inspired by the iron gall ink that has been used since the Middle Ages, we formulated a hair-dyeing solution for blackening hair. The ingredients in the formulation have been approved as cosmetic ingredients, including tannic acid, gallic acid, and Fe(D-gluconate)₂. The formulation does not require any harmful oxidizing agents, such as hydrogen peroxide—the Fe(II) cations bound to tannins are oxidized spontaneously upon exposure to air and form the blackish Fe(III)-tannin nanocomplex that coats hair firmly. In our study, we show that the dyed color did not fade under sunlight exposure for at least three months and after shampooing. This natural formulation for black hair-dyeing can have great impact in the hair cosmetic industry.

Keywords: gallic acid; hair dyeing; iron gall ink; Fe(D-gluconate)₂; tannic acid

1. Introduction

The coordination complex of Fe(III) ions and tannins [1–5] has been widely used throughout human history, exemplified by the iron gall ink whose use dates back to the Middle Ages in Europe [6]. In the traditional recipe of the iron gall ink, oak galls are the source of tannins (i.e., gallic acid and tannic acid), and green vitriol (iron(II) sulfate, FeSO₄) is typically used as a source of Fe(II) [7]. Iron(III) sulfate or other iron(III) compounds are not directly used presumably because of their low natural abundance and/or accessibility. Therefore, ink blackening, rather pale initially upon mixing, is achieved by the oxidation of Fe(II) to Fe(III) ions and semi-infinite self-assembly of Fe(III)-tannin complexes [8,9]. We have recently reported the use of the Fe(II)-tannin complex, not the final Fe(III)-tannin complex in the iron gall ink, as a reaction precursor for fluidic-interface engineering [10] and coacervate formation [11]. In addition to its water solubility, other advantages of Fe(II)-tannin complex use include reaction controllability, because the oxidation-initiation time and reaction rates could be controlled tightly in the formation of Fe(III)-tannin nanostructures. In this work, we studied the use of the Fe(II)-tannin complex as a precursor for nanomaterial-based hair dyeing, which proved advantageous over the conventional, direct formation of Fe(III)-tannin complex.

Blackening hair color with nanomaterials is not a new concept for hair dyeing. It is in the ancient Egyptian or Greco-Roman period that people precipitated black galena (PbS) crystals (size: ~5 nm) on hair with a water paste of lead oxide (PbO) and slaked lime (Ca(OH)₂) [12]. Recent examples of this dyeing strategy include the use of reduced graphene oxide (r-GO) sheets for spraying- or combing-based hair dyeing [13]. Compared with the previous reports, our dyeing system was composed of the chemicals that are being approved as cosmetic ingredients. Specifically, we used Fe(D-gluconate)₂ (COSING REF No.: 56253), gallic acid (COSING REF No.: 76338), tannic acid

(COSING REF No.: 38472), and L-ascorbic acid (vitamin C, COSING REF No.: 74328) in our formulation (Figure 1).

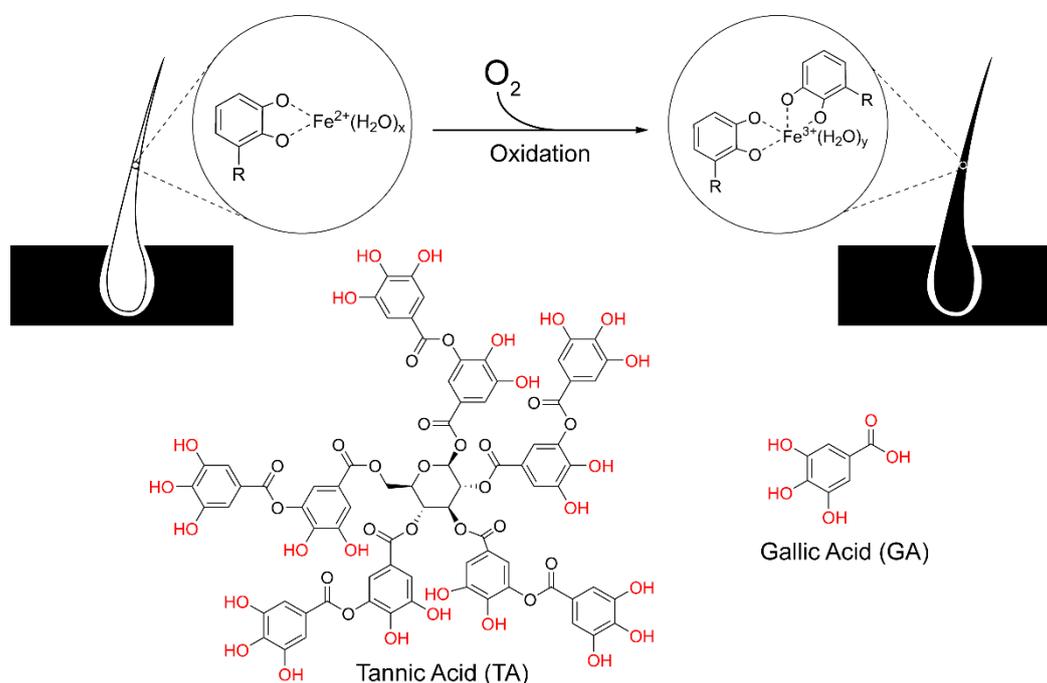


Figure 1. Schematic representation of the air oxidation of soluble Fe(II)-tannin to semi-infinite, dark Fe(III)-tannin complex during hair dyeing. The possible binding sites of tannic acid and gallic acid to Fe(III) are indicated in red.

2. Materials and Methods

Tannic acid (Merck), gallic acid (3,4,5-trihydroxybenzoic acid, $\geq 97.5\%$, Merck), iron(II) gluconate dihydrate ($\text{Fe}(\text{D-gluconate})_2 \cdot 2\text{H}_2\text{O}$, $\geq 98\%$, Merck), iron(II) sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\geq 99\%$, Merck), iron(III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, $\geq 98\%$, Merck), and L-ascorbic acid ($\geq 99\%$, Merck) were used as received. Deionized (DI) water ($18.3 \text{ M}\Omega \cdot \text{cm}$) from Milli-Q Direct 8 (Millipore) was used. To DI water was added tannic acid, gallic acid, and L-ascorbic acid, and the mixture was stirred for complete dissolution, followed by the addition of $\text{Fe}(\text{D-gluconate})_2$. After 1 h, the blonde hair sample was immersed for 30 min in the dyeing solution, dried with a hair dryer, washed thoroughly with tap water, and dried using a hair dryer. The dyed hair was shampooed with a commercially available hair shampoo (Amorepacific) and washed thoroughly with tap water. The L^* values were measured with a colorimeter (TES-135A, TES Electrical Electronics Corp., Taipei, Taiwan).

3. Results and Discussion

The dyeing performance was tested on blond hair samples. Among a multitude of tannins, gallic acid was selected as a main dye ingredient in this study, considering that the blackish color in the iron gall ink originates mainly from gallic acid [7]. Tannic acid was additionally added to enhance the adhesion property of Fe(III)-tannin nanocomplex [10]. In our formulation with Fe(II), the steady O_2 oxidation of Fe(II) to Fe(III) in the Fe(II)-tannin complex, upon application to the hair, led to the continuous coating of Fe(III)-tannin nanocomplex and hair darkening, which could not be achieved by direct use of Fe(III) due to the rapid precipitation of Fe(III)-tannin complex. Prescreening dyeing capability led us to choose 10 mM of $\text{Fe}(\text{D-gluconate})_2$ ($\text{Fe}(\text{glu})_2$). The total concentration of gallic acid (GA) and tannic acid (TA) was set to be 10 mM with different GA mole fractions (χ_{GA} : 0.0 to 1.0 with increment of 0.1). The dyeing efficiency was represented by ΔL^* (ΔL^* : L^* before dyeing minus L^* after dyeing). The lightness value, L^* , in the CIE Lab (Commission Internationale de l'Eclairage) color space

ranges from 0 to 100, with 0 as the darkest black and 100 as the brightest white [14]. The averaged L^* value of blonde hair samples in our study was 81.5 ± 0.2 .

Figure 2a shows the optical photograph of the blonde hair that was dyed at $\chi_{GA} = 0.9$. The hair turned from blonde to black to the naked eyes, and, accordingly, the L^* value decreased significantly to 21.1 ± 0.4 from 80.9 ± 0.5 (ΔL^* : 59.8). The graph of ΔL^* versus χ_{GA} clearly indicated that GA was the active ingredient in blackening hair (Figure 2b and Table S1; for the optical micrographs of dyed hair samples, see Figure S1). The same dyeing characteristics were found, when the concentration of TA ([TA]) was fixed to be 1 mM, and the concentration of GA ([GA]) varied from 0 to 70 mM (Figure 2c and Table S2). However, it was observed that at GA concentration >10 mM there was no increase in dyeing efficiency but rather a decrease.

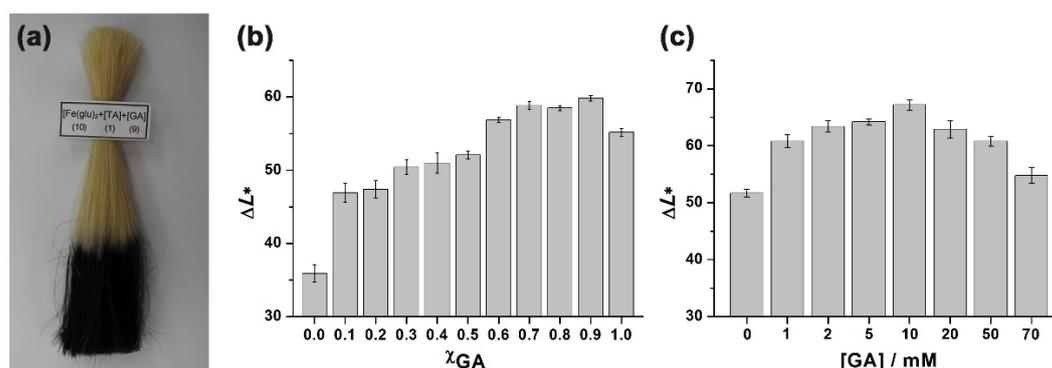


Figure 2. (a) Optical photograph of a dyed hair sample. $[\text{Fe}(\text{glu})_2] = 10$ mM, $[\text{TA}] = 1$ mM, and $[\text{GA}] = 9$ mM; (b,c) graphs of ΔL^* versus (b) χ_{GA} and (c) $[\text{GA}]$ with the fixed $[\text{TA}]$ of 1 mM.

It is to note that the use of only TA (χ_{GA} : 0.0) resulted in nonuniform, unreliable dyeing, and the dyed hair lost its black color to some extent when washed. The hair looked brownish to the naked eyes at $\chi_{GA} = 0.0$. Accordingly, dyeing uniformity increased as χ_{GA} increased. This observation might be explained by facile coordination of multi-armed, dendritic TA with Fe(II), compared to GA, and subsequent oxidation of Fe(II) in the formulation: the facilitated oxidation of Fe(II) to Fe(III) led to the rapid formation of big, water-insoluble Fe(III)-TA complexes, which might have precipitated onto the hair through weak physical interactions [15]. However, TA seemed to act as a binder, because the use of only GA (χ_{GA} : 1.0) resulted in slightly decreased ΔL^* than that at $\chi_{GA} = 0.9$ (ΔL^* : 55.2 vs. 59.8). Take all together, it could be concluded that the optimal molar ratio of TA to GA is around 1 to 10.

We used FeCl_3 , instead of $\text{Fe}(\text{glu})_2$ —as a source of the Fe(III) cation that had been used in the traditional, century-long dyeing protocol—for comparative control. We observed that a precipitate formed rapidly upon mixing of FeCl_3 and tannins, which would not make the traditional formulation applicable seamlessly to the commercial production due to its solution instability. Moreover, the rapid precipitation of Fe(III)-TA semi-infinite complex led to the unsatisfactory black-dyeing with ΔL^* of 30.2 ± 1.4 . In contrast, in our Fe(II)-based formulation the soluble Fe(II)-tannin complex could be kept stable, leading to the long-term storage without precipitation. This kinetic control of aerial Fe(II) oxidation made the dyeing blackish in our formulation. The control experiment clearly confirmed that our iron-gall-ink-inspired formulation was superior to the conventional protocol with direct use of Fe(III) in the aspects of dyeing efficacy and shelf-life.

The long-term stability of dyeing was tested under sunlight exposure ($\chi_{GA} = 0.0, 0.9$, and 1.0) (Table 1). We did not observe any noticeable increase in the L^* value (i.e., color fading) after 3 months. Shampooing after one week of dyeing did not change the L^* value significantly, additionally confirming the dye stability. The observed color stability in our system was rather unexpected, because most coating-based hair-dyeing systems were color-fading. We thought that the observed durability might be explained two-fold: TA is well known to interact strongly with proteins [16], which makes the first layer of TA nanospecies bound firmly to hair proteins; in addition to the coordination with iron

cations, TA is capable of covalently linking to other TAs and molecules [17], which would increase the coating stability.

Table 1. L^* values before and after dyeing, and after sunlight exposure.

χ_{GA}	Before Dyeing	After Dyeing	Sunlight Exposure			
			1 d	2 d	5 d	90 d
0.0	82.1 ± 0.4	46.2 ± 0.8	46.8 ± 0.7	47.1 ± 0.5	49.3 ± 0.6	49.3 ± 0.5
0.9	80.9 ± 0.5	21.1 ± 0.4	21.3 ± 0.3	21.3 ± 0.3	22.3 ± 0.5	22.5 ± 0.3
1.0	80.0 ± 0.5	24.8 ± 0.5	25.0 ± 0.7	25.4 ± 1.0	25.9 ± 1.2	25.8 ± 0.2

We found that $FeSO_4$, the ingredient in the iron gall ink, did not make the dyed hair as blackish as $Fe(glu)_2$. The blonde hair turned to dark brownish to the naked eyes, and the L^* value was measured to be 26.2 ± 0.5 after dyeing with $FeSO_4$. We thought that the observed difference in the dyeing capability was caused by differences in the anion-dependent oxidation kinetics that had previously been observed [10], but detailed mechanisms remain to be seen at this stage. Nonetheless, this finding is meaningful in the cosmetic formulation, because customers tend to be reluctant to use sulfate-containing cosmetic products. We also observed that L-ascorbic acid (i.e., vitamin C) was beneficial in the black hair-dyeing. The addition of L-ascorbic acid (1 mM) to the formulation increased ΔL^* , although the concentrations of more than 1 mM lowered the dyeing efficiency presumably because of its antioxidizing, inhibitory effect against the oxidation of Fe(II) to Fe(III).

4. Conclusions

In summary, we successfully demonstrated the hair-dyeing capability of water-soluble Fe(II)-tannin metal-organic complex, inspired by the iron gall ink, that does not require any hair-oxidizing step in the dyeing process. New findings in this study include the optimal molar ratio of tannic acid and gallic acid (~1:10) for black hair-dyeing, better dyeing performance of $Fe(D\text{-gluconate})_2$ over $FeSO_4$, inefficiency of the direct use of Fe(III) cations, and additive role of L-ascorbic acid. The use of Fe(II) complex provides further benefit in product development, because the direct formation of Fe(III)-tannin complex has a huge problem in rapid precipitation upon mixing of Fe(III) and tannins. The ingredients in our formulation are all approved as cosmetic ingredients and even as food additives, relieving the health concern on cosmetic dyes [18]. In addition, the dyeing process is simple, and the color lasts long, which would make our formulation advantageous over the conventional permanent or oxidative hair dyes [19]. The formulation would be functional in the UV protection against hair damage, because the Fe(III)-tannin nanocomplex is UV-protective [20]. We also envision that our formulation could be combined with the synthetic melanogenesis in hair dyeing [21], because 5,6-dihydroxyindole has a capability of making a coordination complex with Fe(II). One of the drawbacks in our current formulation would be the solution's pH, which is ~3.9 upon mixing and decreases to 3.1 after 90 days of storage. Considering that the average pH of the skin is 4.9 and the pH of forehead is 4.4 [22], the pH values of lower than 4 might have damaging effect on the hair and skin. Our preliminary results show that the pH value of the dyeing solution does not affect the dyeing efficiency significantly (unpublished results), and the pH optimization of our formulation is currently underway.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2079-9284/6/2/23/s1>, Figure S1: Optical photographs of the hair samples after dyeing (χ_{GA} : 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0); Table S1: L^* values before and after hair dyeing. [TA + GA] = 10 mM; [Fe(glu)₂] = 10 mM; Table S2: L^* values before and after hair dyeing. [Fe(glu)₂] = 10 mM; [TA] = 1 mM.

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

References

1. Park, T.; Kim, J.Y.; Cho, H.; Moon, H.C.; Kim, B.J.; Park, J.H.; Hong, D.; Park, J.; Choi, I.S. Artificial spores: Immunoprotective nanocoating of red blood cells with supramolecular ferric ion-tannic acid complex. *Polymers* **2017**, *9*, 140. [[CrossRef](#)]
2. Lee, J.; Cho, H.; Choi, J.; Kim, D.; Hong, D.; Park, J.H.; Yang, S.H.; Choi, I.S. Chemical sporulation and germination: Cytoprotective nanocoating of individual mammalian cells with a degradable tannic acid-Fe^{III} complex. *Nanoscale* **2015**, *7*, 18918–18922. [[CrossRef](#)] [[PubMed](#)]
3. Li, W.; Bing, W.; Huang, S.; Ren, J.; Qu, X. Mussel byssus-like reversible metal-chelated supramolecular complex used for dynamic cellular surface engineering and imaging. *Adv. Funct. Mater.* **2015**, *24*, 3775–3784. [[CrossRef](#)]
4. Park, J.H.; Kim, K.; Lee, J.; Choi, J.Y.; Hong, D.; Yang, S.H.; Caruso, F.; Lee, Y.; Choi, I.S. A cytoprotective and degradable metal-polyphenol nanoshell for single-cell encapsulation. *Angew. Chem. Int. Ed.* **2014**, *53*, 12420–12425. [[CrossRef](#)]
5. Ejima, H.; Richardson, J.J.; Liang, K.; Best, J.P.; van Koeverden, M.P.; Such, G.K.; Cui, J.; Caruso, F. One-step assembly of coordination complexes for versatile film and particle engineering. *Science* **2013**, *341*, 154–157. [[CrossRef](#)] [[PubMed](#)]
6. Bat-Yehouda, M.Z. *Les Encres Noires au Moyen Age (jusqu'à 1600) [Black Inks in the Middle Ages (until 1600)]*; Centre National de la Recherche Scientifique: Paris, France, 1983; pp. 96–97.
7. Pone, A.; Brostoff, L.B.; Gibbons, S.K.; Zavalij, P.; Virgah, C.; Hooper, J.; Alnemart, S.; Gaskell, K.J.; Eichhorn, B. Elucidation of the Fe(III) gallate structure in historical iron gall ink. *Anal. Chem.* **2016**, *88*, 5152–5258. [[CrossRef](#)] [[PubMed](#)]
8. Perron, N.R.; Wang, H.C.; DeGuire, S.N.; Jenkins, M.; Lawson, M.; Brumaghim, J.L. Kinetics of iron oxidation upon polyphenol binding. *Dalton Trans.* **2010**, *39*, 9982–9987. [[CrossRef](#)] [[PubMed](#)]
9. Perron, N.R.; Hodges, J.N.; Jenkins, M.; Brumaghim, J.L. Predicting how polyphenol antioxidants prevent DNA damage by binding to iron. *Inorg. Chem.* **2008**, *47*, 6153–6161. [[CrossRef](#)] [[PubMed](#)]
10. Lee, H.; Kim, W.I.; Youn, W.; Park, T.; Lee, S.; Kim, T.-S.; Mano, J.F.; Choi, I.S. Iron gall ink revisited: in situ oxidation of Fe(II)-tannin complex for fluidic-interface engineering. *Adv. Mater.* **2018**, *30*, 1805091. [[CrossRef](#)] [[PubMed](#)]
11. Kim, B.J.; Lee, J.K.; Choi, I.S. Iron gall ink revisited: hierarchical formation of Fe(III)-tannic acid coacervate particles in microdroplets for protein condensation. *Chem. Commun.* **2019**, *55*, 2142–2145. [[CrossRef](#)] [[PubMed](#)]
12. Walter, P.; Welcomme, E.; Hallégot, P.; Zaluzec, N.J.; Deeb, C.; Castaing, J.; Veyssiére, P.; Brénioux, R.; Lévêque, J.L.; Tsoucaris, G. Early use of PbS nanotechnology for an ancient hair dyeing formula. *Nano Lett.* **2006**, *6*, 2215–2219. [[CrossRef](#)] [[PubMed](#)]
13. Luo, C.; Zhou, L.; Chiou, K.; Huang, J. Multifunctional graphene hair dye. *Chem* **2018**, *4*, 784–794. [[CrossRef](#)]
14. Billmeyer, F.W.; Saltzman, M. *Principles of Color Technology*, 2nd ed.; Wiley-Interscience: New York, NY, USA, 1981.
15. Park, T.; Kim, W.I.; Kim, B.J.; Lee, H.; Choi, I.S.; Park, J.H.; Cho, W.K. Salt-induced, continuous deposition of supramolecular iron(III)-tannic acid complex. *Langmuir* **2018**, *34*, 12318–12323. [[CrossRef](#)] [[PubMed](#)]
16. Covington, A.D. Modern tanning chemistry. *Chem. Soc. Rev.* **1997**, *26*, 111–126. [[CrossRef](#)]
17. Andersen, A.; Krogsgaard, M.; Birkedal, H. Mussel-inspired self-healing double-cross-linked hydrogels by controlled combination of metal coordination and covalent cross-linking. *Biomacromolecules* **2018**, *19*, 1402–1409. [[CrossRef](#)] [[PubMed](#)]
18. Cuerra, E.; Llompарт, M.; Garcia-Jares, C. Analysis of dyes in cosmetics: challenges and recent developments. *Cosmetics* **2018**, *5*, 47.
19. Goossens, A. Cosmetic contact allergens. *Cosmetics* **2016**, *3*, 5. [[CrossRef](#)]
20. Park, J.H.; Choi, S.; Moon, H.C.; Seo, H.; Kim, J.Y.; Hong, S.-P.; Lee, B.S.; Kang, E.; Lee, J.; Ryu, D.H.; et al. Antimicrobial spray nanocoating of supramolecular Fe(III)-tannic acid metal-organic coordination complex: applications to shoe insoles and fruits. *Sci. Rep.* **2017**, *7*, 6980. [[CrossRef](#)] [[PubMed](#)]

21. Panzella, L.; Ebato, A.; Napolitano, A.; Koike, K. The late stages of melanogenesis: Exploring the chemical facets and the application opportunities. *Int. J. Mol. Sci.* **2018**, *19*, 1753. [[CrossRef](#)] [[PubMed](#)]
22. Proksch, E. pH in nature, humans and skin. *J. Dermatol.* **2018**, *45*, 1044–1052. [[CrossRef](#)] [[PubMed](#)]



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