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Ferrocene-based chromogenic and fluorogenic derivatives for hair dyeing applications

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A new ferrocene-isoniazid Schiff base has been designed and synthesized for application as a hair dye. In addition, two other Schiff bases of ferrocene with aminopyrene and rhodamine B hydrazide were synthesized, and the three compounds are named **IFC**, **AFC**, and **RFC**, respectively. All the resulting Schiff bases were characterized by NMR, HRMS, and their photophysical properties were further evaluated *via* UV-visible and fluorescence spectroscopy. We anticipated that the incorporation of ferrocenecarboxaldehyde—a well-known organometallic compound—alongside amino counterparts could yield potential non-oxidative hair dyes suitable for dyeing human hair in various colors. All three dyes are found to exhibit effective UV-protection performance, in addition to their durability observed through SEM (scanning electron microscopy), contact angle and confocal studies, which showed negligible effect on the hair surface even after dye removal by multiple washings. Furthermore, a cell viability assay on human embryonic kidney (HEK-293A) and mouse fibroblast (L929) cells showed that **IFC** exhibited excellent biocompatibility, supporting its safety for cosmetic applications in hair dyeing.

1. Introduction

COVID revealed the importance of different industries, which had not received much attention previously.[1,2] One particular industry, which showed exponential growth in COVID and post-COVID times, apart from pharmaceuticals, is hair dye, which is a billion-dollar industry in the current scenario.[3,4] The hair dye industry witnessed significant growth as prolonged salon closures and social restrictions encouraged consumers to adopt do-it-yourself hair coloring at home. In addition to convenience and cost-effectiveness, the growing need for virtual appearance management during remote work, along with the rapid expansion in e-commerce platforms, has made these products more easily accessible.

Generally, the composition of a hair dye comprises three key components: a coupler, an oxidant, and a precursor.[5] The precursor is a molecule that gets oxidized in response to an oxidant, such as hydrogen peroxide (H_2O_2), often employed with *p*-phenylenediamine (PPD)-based dyes.[6] Although there are no direct instructions from the FDA regarding the non-useability of PPD and its analogues or trimers, *viz.* Bandrowski's base (BB), the toxicity of PPD has been documented and has also been claimed in different research articles.[7–9] Although it has been claimed that its oxidative trimer BB is less toxic than PPD, the side effects of BB cannot be fully ignored. In addition to this, the use of oxidizing agents, *viz.*, hydrogen peroxide (H_2O_2) and ammonium hydroxide (NH_4OH), has been linked to elevated hair porosity, hair loss, dermatitis and chemical burns.[10–12]

To avoid the usage of PPD and these harmful oxidizing agents, different but complex strategies have been employed to synthesize new types of systems for hair dye applications. Battistella *et al.* reported an enzymatic approach for the synthesis of artificial melanin for dyeing hair without the use of metal chelators or strong oxidants.[13] Cui and coworkers reported tunable hair colors using metal phenolic networks composed of gallic acid.[14] Tang *et al.* reported silver nanoparticles capped by gallic-acid-encapsulated natural occurring diatomite to be utilized for the coloration of bleached human hair.[15] While all these efforts demonstrate long-lasting effects and noteworthy innovations, they also inspire the exploration of new alternative materials with easy synthesis and cost-effective techniques that can further broaden the range of available colors.

Over decades, the greying of hair has always been associated with ageing, leading to formulations of hair dyes exhibiting black and brown colors.[16–19] However, in present times, their high cosmetic and aesthetic value prompts even young people to use hair dyes of different colors, ranging from orange to red and blue, *etc.*[20,21] Therefore, there is a need for new potential candidates, which can be particularly used for hair dyeing

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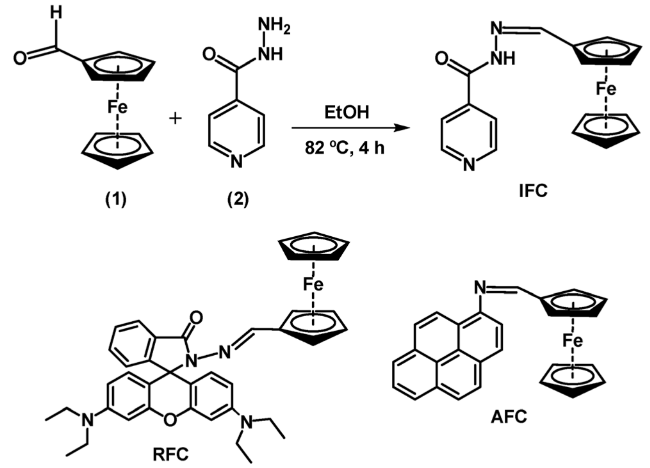
applications. The greying of hair occurs due to a decrease in the formation of melanin in hair with ageing. As melanin has been reported for its redox properties, wherein it can participate in the gaining and losing of electrons,[22–24] we rationalized the selection of some redox-active colored compounds for hair dyeing applications and our investigation ended at the ferrocene unit, which is known for its high redox activity and color changing properties during its oxidation to ferrocenium,[25] so it can serve a similar function to melanin. Ferrocene's redox activity may influence hair dyeing efficiency as its reversible oxidation states alter charge and polarity, which may affect adsorption onto the keratin matrix, and promote stronger interactions with hair proteins with exposed functional groups, such as –COOH, $-NH_2$, –OH, and disulfides, as these provide binding sites for electrostatic, hydrogen-bonding, and π-interactions with redox-active species. This dual binding capacity may not only improve dye adsorption and stability within hair but can also mimic the redox buffering role of melanin.[24] For the selection of its counterpart, we thought of utilizing isoniazid, which is itself a known first-line antibiotic for tuberculosis and is biocompatible and non-cytotoxic.[26] The introduction of isoniazid may also be beneficial, as it can undergo redox changes when required, due to the presence of acidic hydrogen. The other two counterparts, rhodamine B and pyrene, are well-known fluorophores,[27,28] and their hydrazide and amine derivatives, respectively, can readily react with the aldehyde group of ferrocene to form the corresponding Schiff bases.

Recently, Shagun *et al.* reported a dansyl-based fluorogenic system for the detection of antibiotics.[29] Earlier, Dhir *et al.* reported the cocrystal of Bandrowski's base for hair dyeing applications.[30] In the present work, we simultaneously designed and synthesized ferrocene-based imines **IFC**, **RFC** and **AFC** (*vide infra*) to evaluate their efficiency as hair dyes. To the best of our knowledge, the use of these ferrocene imines for hair dyeing applications is unprecedented in the literature.

2. Results and discussion

A condensation reaction between equimolar amounts of ferrocenecarboxaldehyde (**1**) and isoniazid (**2**) was carried out in EtOH. The solution was then refluxed to furnish an orange-colored solid product named **IFC** with 89% yield (Scheme 1). The structure of the compound was confirmed by NMR and HRMS analyses. The ^{1}H NMR spectrum showed a singlet at 8.27 (1H) corresponding to an imine proton, a singlet at 11.70 (1H) for the –NH proton and two singlets corresponding to aliphatic protons of the isoniazid moiety at 7.78 (2H) and 8.76 (2H). In addition, characteristic ferrocene unit proton signals were obtained as three singlets at 4.22 (5H), 4.44 (2H) and 4.65 (2H). A parent ion $[M + H]^+$ peak at *m/z* 334.0826 was obtained in the HRMS spectrum. The NMR and mass spectral data corroborate the structure of **IFC** shown in Scheme 1. The compounds **RFC**[31] and **AFC**[32] were synthesized by already-reported procedures. All three compounds were evaluated for their efficacy in hair dyeing. The spectroscopic data for the three compounds are given in SI (Fig. S1 to S9).



Scheme 1 Synthetic route for compound **IFC** and chemical structures of compounds **RFC** and **AFC**.

One of the main reasons to select ferrocene as a candidate for hair dye application was its redox activity. The retention of the redox nature of all three proposed hair dyes was confirmed by cyclic voltammetry analysis. On an increase in positive potential, **IFC**, **RFC** and **AFC** showed maximum oxidation of ferrocene $[(\eta^5\text{-}C_5H_5)_2\ Fe^{II}]$, to ferrocenium $[(\eta^5\text{-}C_5H_5)_2\ Fe^{III}]^+$ at 0.43 V, 0.45 V and 0.48 V, whereas on a decrease in positive potential, the maximum reduction was observed at 0.54 V, 0.62 V and 0.53 V (Fig. S10, SI). Therefore, the presence of a similar quasi-reversible[33,34] redox cycle to that of the native ferrocene molecule is observed in all three proposed dyes.

We further characterized the three dyes using optical studies. Compound **IFC** is characterized by two absorption bands, *viz.* 300 nm and 206 nm, whereas its emission spectrum showed an emission band at 372 nm at λ_{ex} = 257 nm. The other two compounds, **RFC** and **AFC**, showed absorption bands at 308 nm, 273 nm, 238 nm and 382 nm, 280 nm, 237 nm with emission bands at 567 nm (λ_{ex} = 520 nm) and 430 nm (λ_{ex} = 350 nm), respectively. The absorption and emission spectra are given in SI (Fig. S11 and S12, SI).

To evaluate the dyeing efficiency of all the synthesized compounds (**IFC**, **RFC**, **AFC**), we chose methanol as a solvent, as generally alcohols are used, and we observed bright contrasting colors of our compounds in methanol. 10 mM solutions of all three compounds were prepared in methanol (Fig. S13, SI), and a substantial amount of human hair was dipped in these solutions for 2 h (Fig. 1a to c). After 2 h, the hairs were rinsed with water and washed once with shampoo. The uptake of subsequent colors of the respective methanolic solutions was observed. The hairs dipped in **IFC**, **RFC** and **AFC** showed light brown, orange-red and yellowish-orange colors, respectively (Fig. 1e to g). The results of all three dyes are compared with PPD, a commercial hair color ingredient (Fig. 1h).

The low amounts of melanin in the human hair make them more prone to photoactive damage by UV radiation, leading to

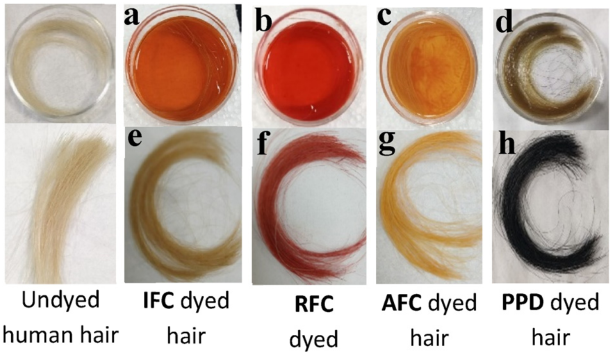


Fig. 1 Photographs of human hair samples dipped in dye solutions (a)–(d) and colors retained by the hair in the respective dye solution (e)–(h) in comparison to undyed hair samples.

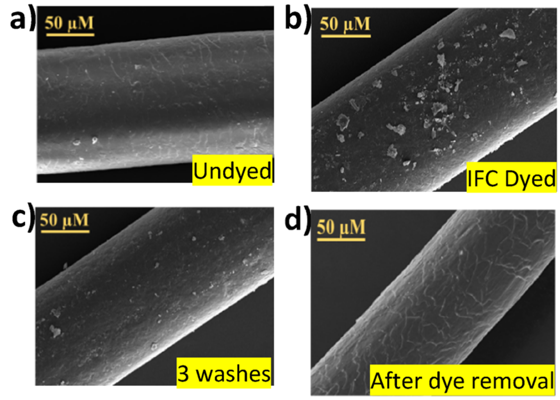


Fig. 2 SEM images of (a) undyed hair, (b) **IFC** dyed hair and (c) **IFC** dyed hairs after 3 washes, (d) hair after dye removal.

decreased mechanical strength of the hair.[35–37] We hypothesized that the presence of ferrocene units in dyes may increase thermal and chemical stability due to the symmetrical sandwich structure of ferrocene, providing inertness to the molecule, making it less prone to all types of degradation.[38–40] To confirm our hypothesis, we performed UV transmittance studies. The transmittance of undyed hairs increased sharply with an increase in wavelength (200–500 nm), indicating that more UV light passes through the undyed hairs. In contrast, the hairs dyed with **IFC**, **RFC** and **AFC** showed a decrease in transmittance with increasing wavelength (Fig. S14, SI), indicating that the dye blocked the UV radiation, protecting the hairs.[41] The opposite trend has been observed in the case of absorbance measurements (Fig. S15, SI). This is because dyed hairs (particularly with darker dyes) contain aromatic compounds and chromophores that absorb UV radiation efficiently. Therefore, a consumer with **IFC**, **RFC** or **AFC** dyed hair will have the additional advantage of protection from sunlight and prevention from the damage that can be caused by prolonged exposure to the sun.

Further, to evaluate the effect of chemical damage by our ferrocene imines on hair, we performed scanning electron microscopy (SEM) studies to observe the surface morphology of undyed and dyed hair samples.[42–44] SEM images of undyed hairs were compared with freshly dyed hairs. The dyed hairs were washed three times, and finally, the dyed hairs were subjected to multiple washes with shampoo for complete removal of dye. These studies were performed with all three ferrocene imines. SEM images of undyed hairs showed a smooth, consistently well-aligned cuticle layer of healthy hairs (Fig. 2a). After dyeing with **IFC**, the SEM image (Fig. 2b) showed the presence of dye pigments deposited on the hair sample, confirming its deposition. Hair samples that were washed three times (Fig. 2c) after dyeing exhibited some residual dye pigments on the surface, whereas, in the case of multiple washes (Fig. 2d) with shampoo, the SEM image showed the complete removal of surface dye pigments, and the hair surface appeared as smooth and uniform as natural undyed hair. Therefore, this confirms that there is no significant change in the surface properties of the hairs after dyeing with **IFC** and it did not cause any structural damage to the hair surface. Similar results were obtained with **RFC** and **AFC** (Fig. S16, SI).

Modern-day hair dyes are more customer-specific, and a specific dye type is recommended to a customer based on their hair type. The surface properties of hair, *e.g.*, hydrophobicity, are prime factors in deciding the hair type and subsequently the dye to be used. The contact angle measurement of a dye indicates its wetting behavior and takes into account the hydrophobicity of human hair. This measurement can provide insights into the durability of hair color by inferring how the dye might interact with the hair surface. Durability is further dependent upon how well the dye adheres to a hair's cuticle layer. A contact angle of around 70° suggests moderate wettability, inferring the dye solution is neither highly hydrophilic (spreading easily, $<30°$) nor strongly hydrophobic ($>90°$). Therefore, we conducted water contact angle measurement studies of all three proposed ferrocene-based hair dyes in comparison to the conventional hair color ingredients generally used in hair dye formulations, *i.e.*, *p*-phenylenediamine (PPD). The contact angle values 'θ' of **IFC**, **RFC** and **AFC** were observed to be 72.48°, 77.37° and 77.75°, respectively, which are almost comparable to the contact angle of PPD (78.96°). Thus, our proposed hair dyes have almost equal potency to PPD for hair wettability[45,46] (Fig. S17, SI), although PPD is reported for its various side effects.[6,7]

A key factor determining the long-term retention and durability of hair dye is its adherence to the hair surface. Two of the three proposed hair dyes are decorated with well-known fluorophores, *i.e.*, rhodamine B and pyrene.[27,28] Therefore, to observe the adherence of the proposed dyes on the hair surface, we performed confocal microscopic studies. The confocal images (Fig. 3) displayed strong adherence and uniform distribution of dyes on the hair surface, as observed under suitable excitation wavelengths.[47]

To gain insight into the process of releasing dye from hair, we monitored the amount of dye released from hair as a function of rinse cycles, recorded *via* UV-vis spectroscopy. Freshly dyed hair samples (100 mg each) corresponding to IFC, RFC, and AFC were individually suspended in Petri dishes

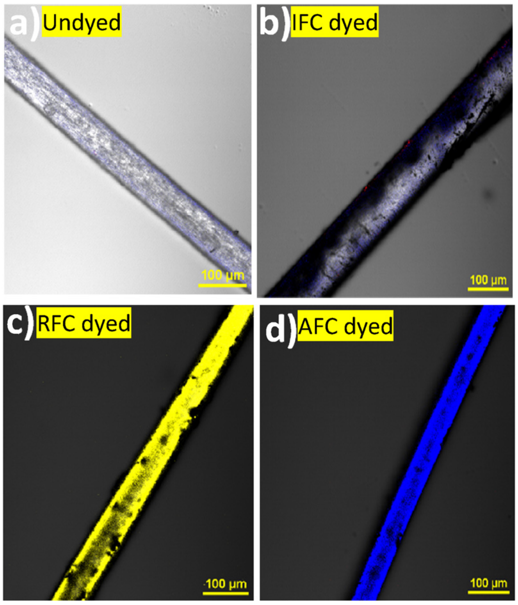


Fig. 3 Confocal imaging of hair strands (a) undyed hair seen in the blue channel; (100 μm, λ_{ex} = 402 nm collected from 425 to 75 nm); (b) **IFC**-dyed hair seen in blue channel; (100 μm, λ_{ex} = 402 nm collected from 425 to 475 nm), (c) **RFC**-dyed hair seen in the yellow channel (100 μm, λ_{ex} = 561 nm collected from 570 to 620 nm), (d) **AFC-**dyed hair seen in the blue channel; (100 μm, λ_{ex} = 402 nm collected from 425 to 475 nm).

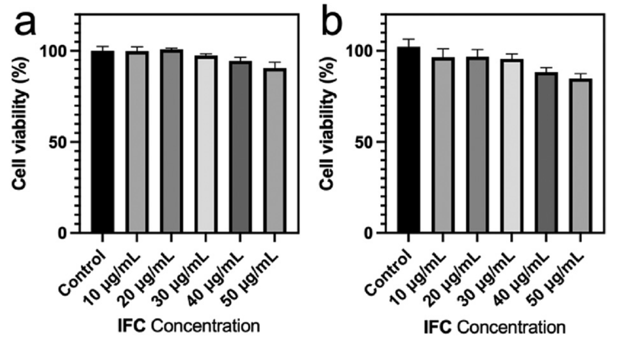


Fig. 4 Cell viability assay showing the effect of different **IFC** dye concentrations on (a) HEK-293A cells and (b) L929 cell line.

containing 10 mL of water. We avoided surfactants as they may interfere with dyes. The dyed hairs were washed 6 times over a 30 min-period, with washes performed at 5-minute intervals. Further, the percentage of dye released in each cycle was calculated by taking the ratio of the absorbance of dye samples from each interval to the total absorbance of all intervals. The loss of each dye **IFC**, **RFC** and **AFC** in the first cycle is 54%, 39% and 35%, respectively. However, the removal of 80–90% of the total dye took another 5 cycles (Fig. S18, SI). The performance of these dyes in terms of sustainability thus matches the performance of semi-permanent hair dyes.[48]

In addition to the aforementioned studies, the color efficacy of the dyed hair samples was further evaluated using the *K*/*S* values, which serve as a critical parameter, reflecting the balance between the absorption (*K*) and scattering (*S*) of light by the dyed fibres.[49] A higher *K*/*S* value is indicative of an increased concentration of dye molecules within the hair structure, corresponding to deeper, more intense coloration. Conversely, a lower *K*/*S* value signifies reduced dye uptake and distribution.[49] For a comprehensive evaluation of the wash-fastness and retention of dye molecules, *K*/*S* values were measured for all dyed samples after the 3rd and 9th wash cycles. The results are presented in Table S1, encompassing a range of colorimetric parameters, including lightness (L*), redness (a*), yellowness (b*), color saturation (C*), hue angle (h°), and the absorption/scattering coefficients (*K*/*S*). Collectively, these parameters provide an understanding of coloring attributes and the stability of the coloration under repeated washing conditions.

Finally, to evaluate the cytotoxicity of **IFC**, our newly designed and synthesized compound, human embryonic kidney (HEK-293A) and mouse fibroblast (L929) cells were cultured in complete cell culture Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% pen-strep under standard cell culture conditions (37 °C, 5% CO_2). The cytotoxicity of the **IFC** dye was evaluated on both cell lines using an Alamar Blue assay. A primary stock solution of **IFC** was prepared in DMSO, and working solutions were subsequently prepared by dilution in complete media. The cells were seeded in 96-well culture plates at a density of 10 000 cells per well and incubated overnight for adhesion, then treated with varying concentrations of **IFC** (0–50 μg mL^{-1}) for 24 h. After treatment, Alamar Blue reagent was added, and cell viability was determined as per the manufacturer's protocol. As shown in Fig. 4a, the assay results confirm that HEK-293A cells maintained high viability after 24 h of exposure to **IFC** dye at concentrations up to 50 μg mL^{-1}. Furthermore, assessment of the dye's biocompatibility with the mouse fibroblast L929 cell line (Fig. 4b) revealed more than 82% cell viability even at 50 μg mL^{-1}, reinforcing the overall cytocompatibility of the system. Collectively, these findings demonstrate that **IFC** dye shows excellent biocompatibility, supporting its potential applicability in hair dye formulations.

3. Conclusions

The present work reports the utilization of ferrocene imines for hair dyeing applications. Ferrocene, because of its redox properties, exhibits color changing behavior, which can be further regulated by functionalizing it with other suitable fluorophores, which excludes the usage of harmful chemicals like PPD and H_2O_2. The surface properties of the proposed dyes are in line with commercial hair dye ingredients. The hair dye industry is already thriving and is well poised for future growth, and the exploration of new biocompatible candidates will arouse the interest of researchers working in the area of chromophoric dyes to reinvestigate and look again into candidates with the most potential.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article has been included in the supplementary information (SI). Supplementary information includes spectroscopic and microscopic data. See DOI: https://doi.org/10.1039/d5nj02724a.

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