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## ***“Novel Characteristics of Oxidative Dyes: Location in Hair”***

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### **1. Introduction**

Hair coloring is one of the most important actions that affect personal impressions. Therefore, hair-coloring products are among the most important products in the beauty industry. Among these products, permanent hair dyes are the most widely used because of their ability to provide a relatively long-lasting color. Permanent hair dyes are mixtures of two separate components: an alkaline agent containing precursors and couplers, and an oxidizing agent. When mixed, the precursors and couplers undergo oxidative coupling reactions to form colored molecules (oxidative dyes). The reactivities of the precursors and couplers and their reaction products have been extensively researched [1-4].

However, the demand of people dyeing hair has evolved to encompass not only dyeing but also color fading. Research has been conducted to meet these demands. Fading caused by washing with shampoos and exposure to ultraviolet light has been reported [5,6]. However, although countermeasures against fading have been proposed, the fading behavior of oxidative dyes has been insufficiently explained.

Therefore, the relationship between oxidative dyes and hair must be clarified. To represent this relationship, we used imaging mass spectrometry (IMS), which facilitates the visualization of the location of chemical compounds by overlaying microscopic images with mass spectrometry data obtained from each pixel. Recently, IMS has been widely used in fields such as medicine [7] and drug discovery [8], and its application in hair analysis has attracted attention [9].

In this study, we attempted to determine the locations of oxidative dyes in hair by using IMS. Moreover, we investigated how these locations influence the fading behavior.

### **2. Materials and Methods**

#### **2.1. Materials**

Hair strands were prepared using commercially available Chinese human hair.

#### **2.2. Methods**

##### **(1) Dyeing condition**

The precursor and coupler were dissolved at a concentration of 5 mM each, along with ammonia (0.4 M) and hydrogen peroxide (0.8 M), in a methanol/water (1/2) solvent. The pH of this solution ranged between 10.0 and 10.5. The hair strands were immersed in this solution and incubated at 20-25 °C for 20 min. Subsequently, the hair strands were washed and dried.

## (2) IMS

Sample were randomly selected from the dyed hair, and tissue sections having a thickness of 7  $\mu\text{m}$  were prepared after rapidly freezing using liquid nitrogen and embedding using a 4% carboxymethyl cellulose sodium aqueous solution. The tissue sections were mounted on 100  $\Omega$  slide glass coated with indium tin oxide. Matrix deposition was performed using an iMLayer system (Shimadzu, Japan), with  $\alpha$ -cyano-4-hydroxycinnamic acid as the matrix compound. After matrix deposition, IMS was performed using an iMScope QT instrument (Shimadzu, Japan). The data obtained were analyzed using IMAGEREVEAL MS (version 1.30.0.12570). These procedures were performed with the support of the Tokyo Metropolitan Industrial Technology Research Institute.

## (3) Bleaching process

The hair strands were bleached at room temperature for 30 min. This procedure was repeated after washing and drying.

## (4) Washing–fading process

The colored hair strands were immersed in 30 g of 28% polyoxyethylene alkyl (12, 13) ether sulfate sodium (3E.O.) for 10 min at 40  $^{\circ}\text{C}$ . Subsequently, the hair strands were washed and dried.

## (5) Calculation of color difference ( $\Delta E^*$ )

The color difference ( $\Delta E^*$ ) was calculated by applying Equation (1) using the  $L^*$ ,  $a^*$ , and  $b^*$  values measured with an NF555 instrument (Nippon Denshoku, Japan). The results for each sample were based on the average of three measurement points.

$$\Delta E^* = ((L_x^* - L_0^*)^2 + (a_x^* - a_0^*)^2 + (b_x^* - b_0^*)^2)^{(1/2)} \quad (1)$$

## (6) Calculation of Hansen distance ( $R_a$ )

The Hansen solubility parameters ( $\delta D$ ,  $\delta P$ , and  $\delta H$ ) were calculated using a computational software named HSPiP (version 5.4.02). The Hansen distance was then calculated by applying Equation (2) using the solubility parameters obtained for the oxidative dyes and solvents [10,11].

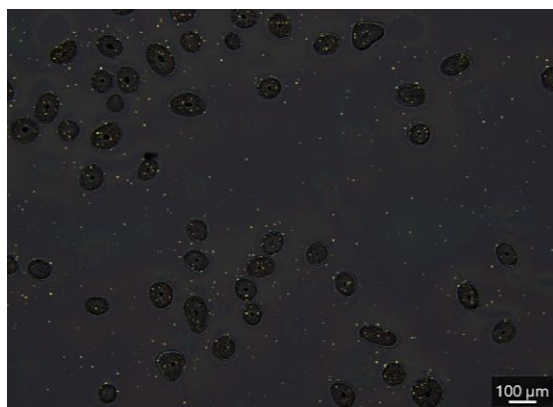
$$R_a = (4(\delta D_x - \delta D_{\text{solv}})^2 + (\delta P_x - \delta P_{\text{solv}})^2 + (\delta H_x - \delta H_{\text{solv}})^2)^{(1/2)} \quad (2)$$

## 3. Results

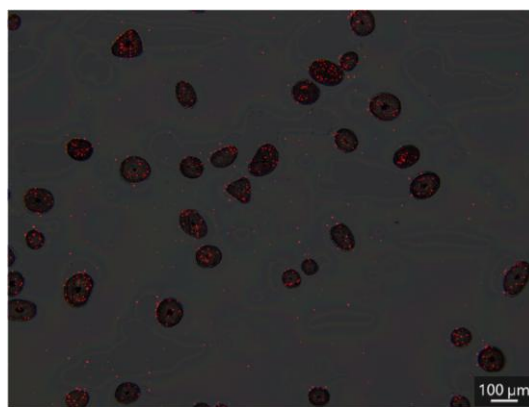
### 3.1. IMS

IMS was used to observe the locations of the products of four precursors (p-phenylenediamine (pPD), p-aminophenol (pAP), 2,5-diaminotoluene (2,5DAT), and N,N-bis(2-hydroxyethyl)-p-phenylenediamine (BHP)) and five couplers (resorcinol (RES), 5-amino-o-cresol (5AOC), 2,4-diaminophenoxyethanol (2,4DAPE), 2,6-diaminopyridine (2,6DAP), and  $\alpha$ -naphthol ( $\alpha\text{N}$ )). The structural formulae of the reaction products used for mass spectrometry were referenced from [1]. Compounds that potentially form both dimers and trimers were individually confirmed by performing mass spectrometry, and only those for which peaks were observed are reported here.

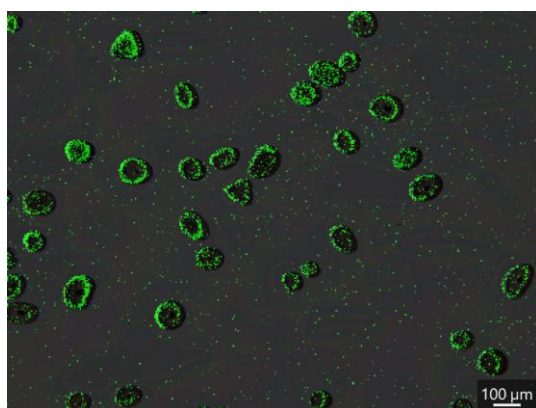
Figures 1–4 show the IMS result of each reaction product in colored hair.



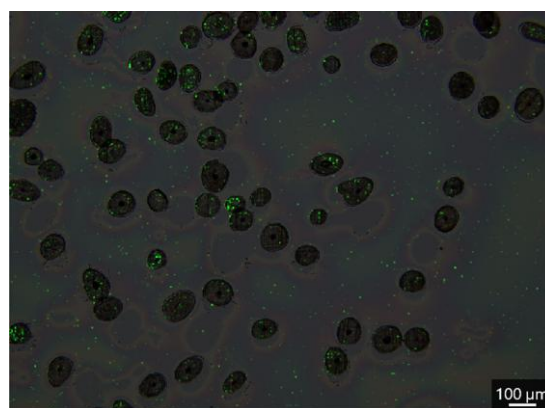
**(1-a)** Trimer of pPD and RES



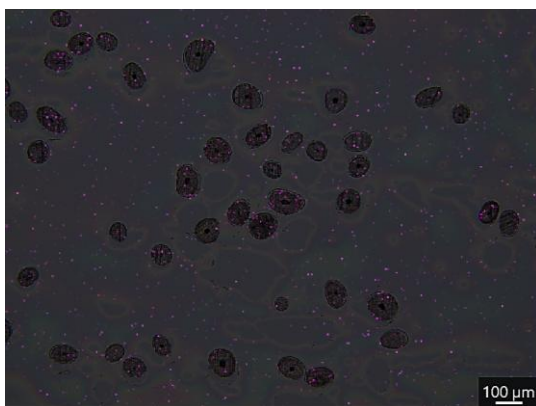
**(1-b)** Dimer of pPD and 5AOC



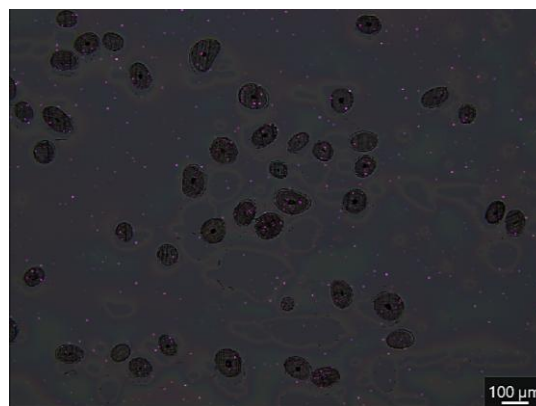
**(1-c)** Dimer of pPD and 2,4DAPE



**(1-d)** Dimer of pPD and 2,6DAP

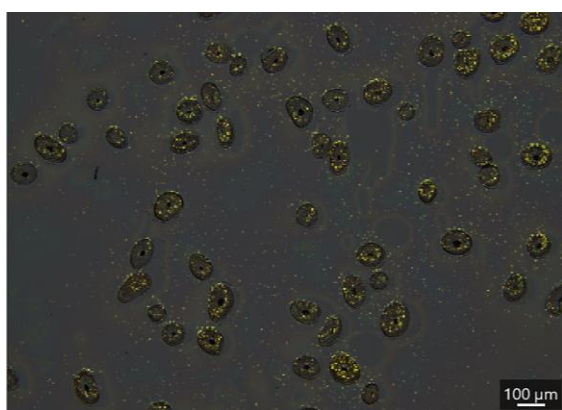


**(1-e)** Dimer of pPD and αN

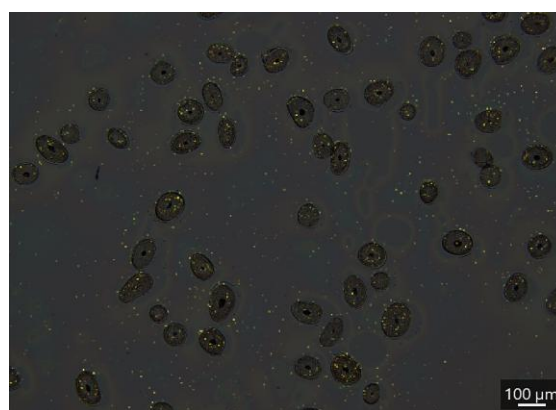


**(1-f)** Trimer of pPD and αN

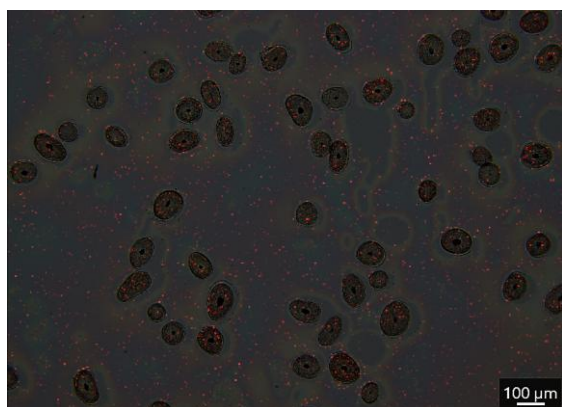
**Figure-1.** IMS result of reaction products using pPD as the precursor



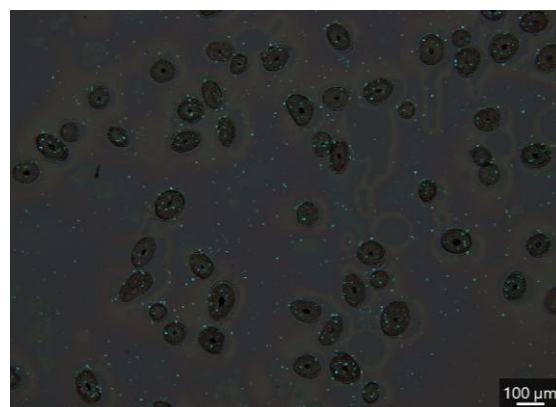
**(2-a)** Dimer of pAP and RES



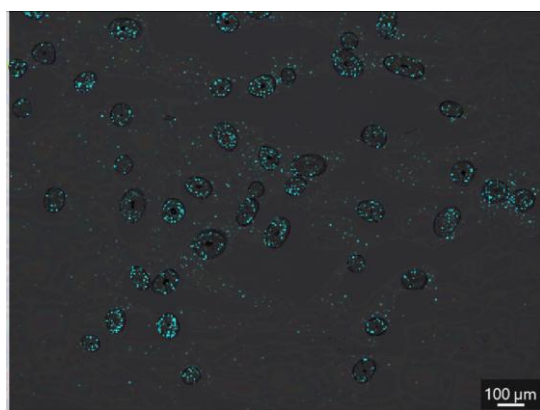
**(2-b)** Trimer of pAP and RES



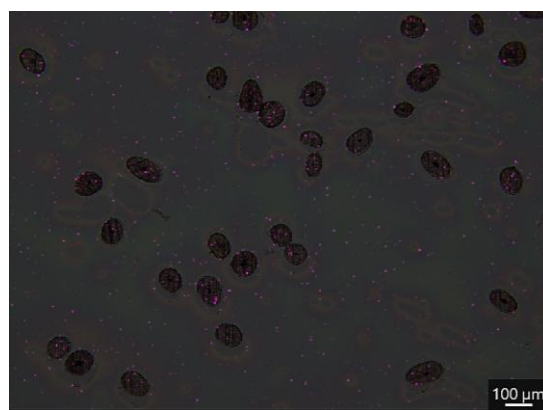
**(2-c)** Dimer of pAP and 5AOC



**(2-d)** Dimer of pAP and 2,4DAPE



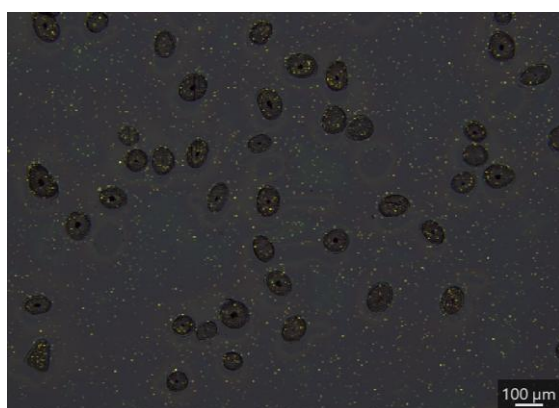
**(2-e)** Dimer of pAP and 2,6DAP



**(2-f)** Dimer of pAP and αN

**Figure-2.** IMS result of reaction products using pAP as the precursor

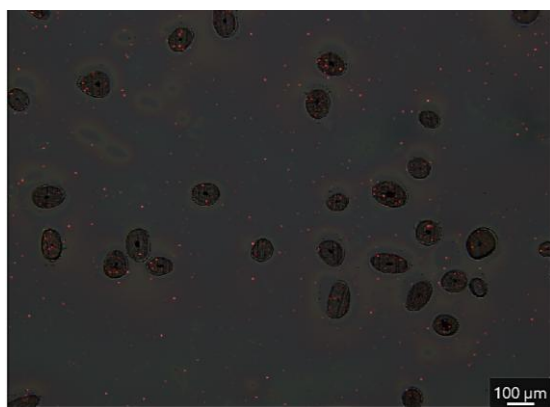




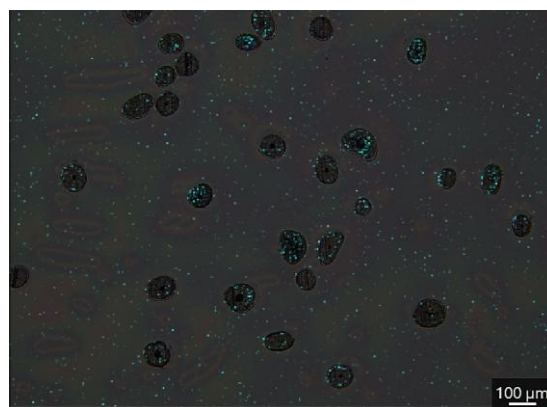
**(3-a)** Dimer of 2,5DAT and RES



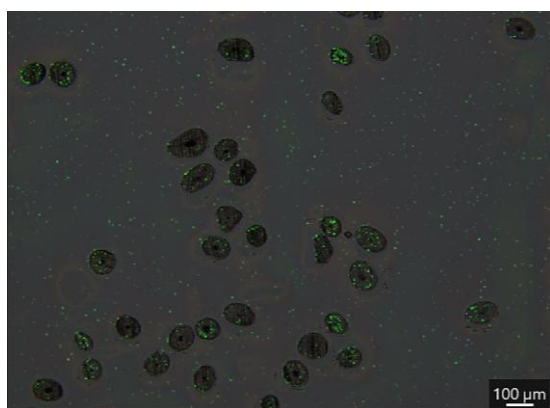
**(3-b)** Trimer of 2,5DAT and RES



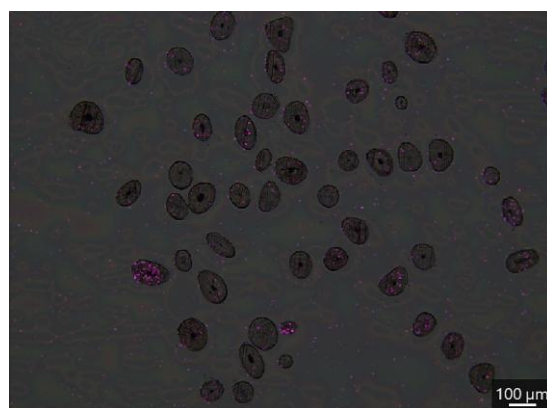
**(3-c)** Dimer of 2,5DAT and 5AOC



**(3-d)** Dimer of 2,5DAT and 2,4DAPE

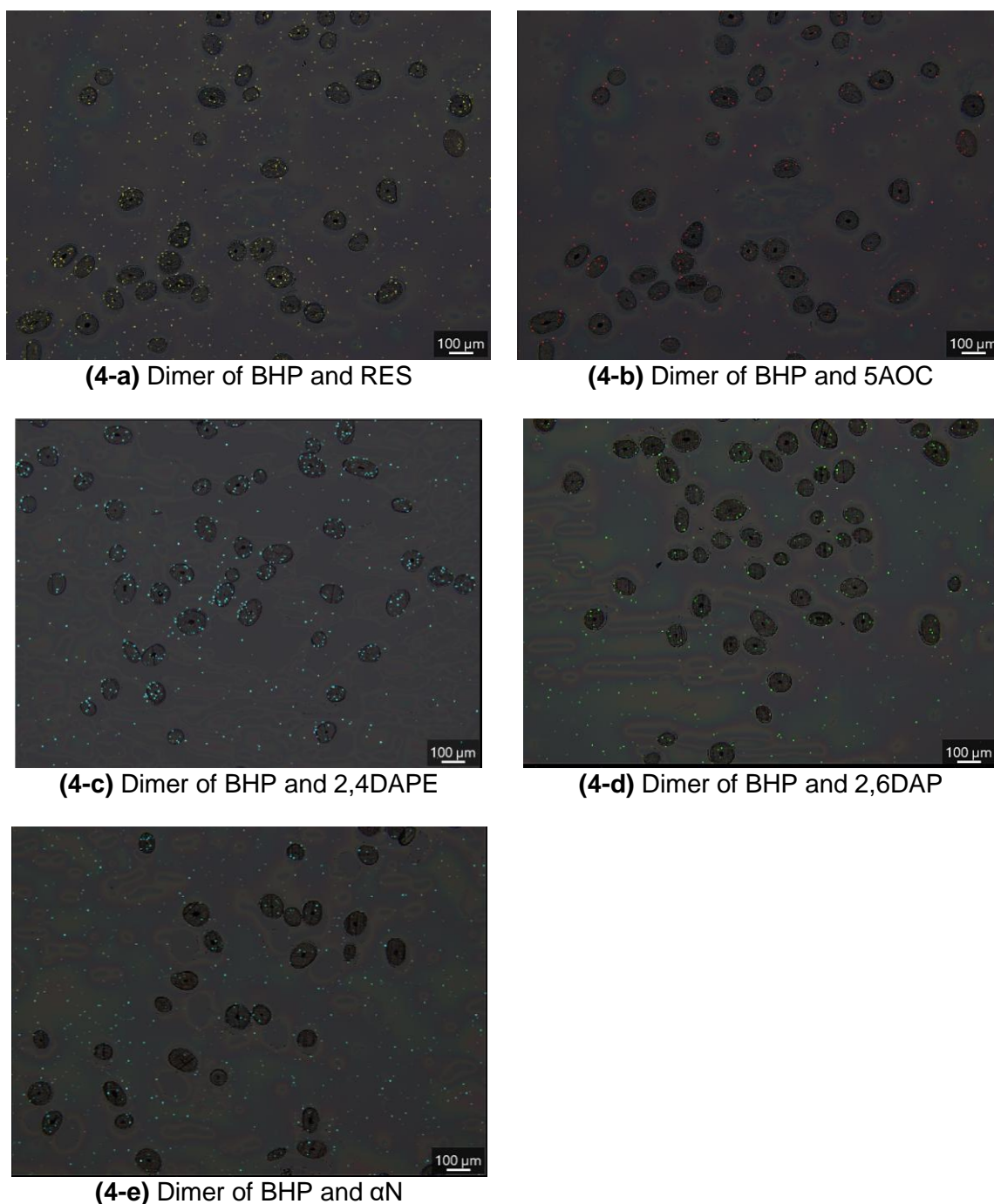


**(3-e)** Dimer of 2,5DAT and 2,6DAP



**(3-f)** Dimer of 2,5-DAT and αN

**Figure-3.** IMS result of reaction products using 2,5DAT as the precursor



**Figure-4.** IMS result of reaction products using BHP as the precursor

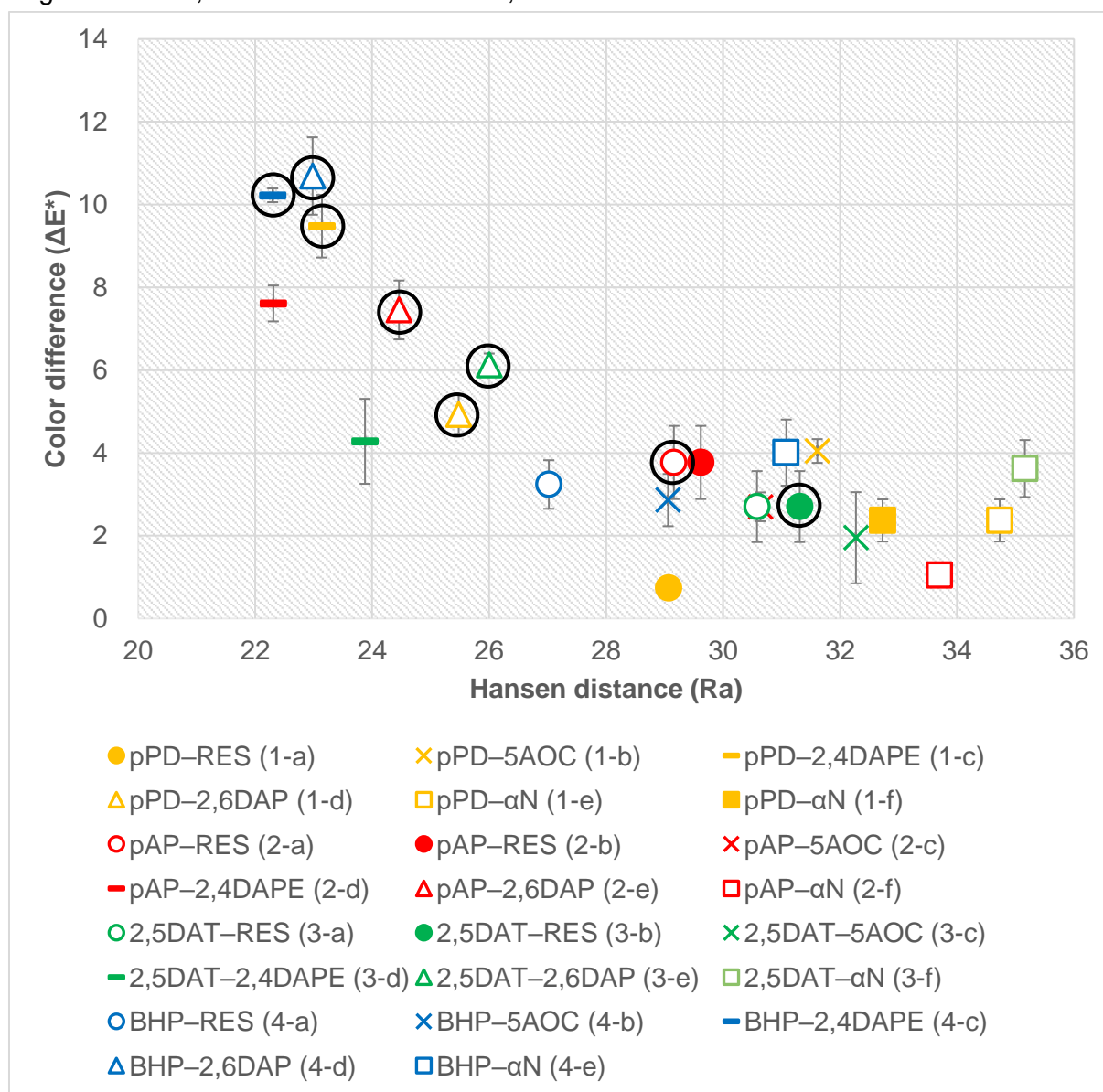
The IMS results reveal that oxidative dyes are either ubiquitous in the hair or localized on the inner edges of the hair. The oxidative dyes ubiquitous in the hair are displayed in Figures 1-a, 1-b, 1-e, 1-f, 2-b, 2-c, 2-d, 2-f, 3-a, 3-c, 3-d, 3-f, 4-a, 4-b, and 4-e. The oxidative dyes localized on the inner edge of the hair are depicted in Figures 1-c, 1-d, 2-a, 2-e, 3-b, 3-e, 4-c, and 4-d.

### 3.2. Washing–fading tests

To determine whether the localization of oxidative dyes in hair is related to the properties of the dyes, we performed washing–fading tests on bleached and subsequently dyed hair strands. The washing–fading process was expressed in terms of the color difference ( $\Delta E^*$ ).

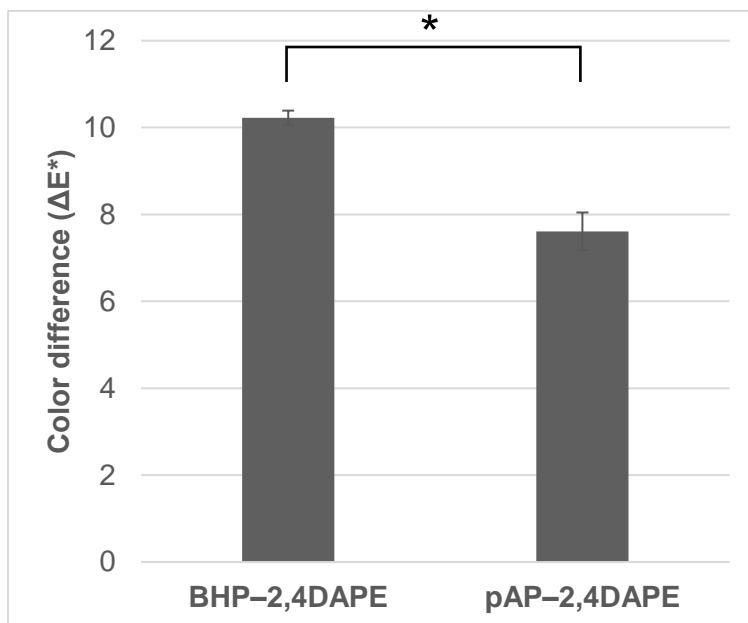
If the affinity to water is assumed to play a major role in washing and fading, the affinity is represented by the Hansen distance to the solvent ( $R_a$ ), calculated based on the Hansen solubility parameters.

Figure 5 shows the Hansen distance to the solvent ( $R_a$ ) and the color difference ( $\Delta E^*$ ) due to washing for each oxidative dye. The color differences between the dimers and trimers cannot be distinguished. Additionally, the plots of the oxidative dyes localized on the inner edge of the hair, based on the IMS results, are enclosed in circles.



**Figure-5.** Color difference of dyed hair strands after washing and the Hansen distance between the oxidative dye and water.

The Hansen solubility parameters help to clearly distinguish between dyes having high ( $R_a \geq 26$ ) and low ( $R_a < 26$ ) wash fastness. However, among the dyes having a similar high affinity to water (i.e., similar Hansen distance,  $R_a < 26$ ), oxidative dyes localized on the inner edge of the hair exhibit greater fading upon washing than those ubiquitous in the hair (Figure 6).

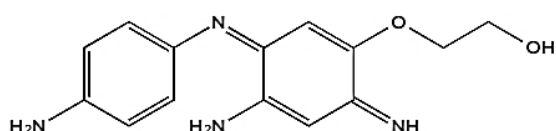


**Figure-6.** Color-difference comparison at equivalent Hansen distance. Mean (SD)  $n = 3$ ;  $t$ -test \*:  $p < 0.05$

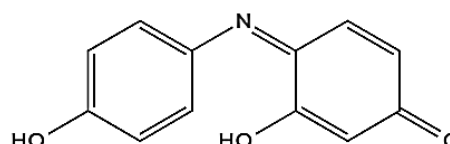
#### 4. Discussion

Oxidative dyes, which are the reaction products of precursors and couplers, are the most important components of permanent hair colors. Although the structures of these compounds have been understood well in previous studies [1,2], their behavior in hair has not yet been thoroughly investigated. Among various behaviors, the localization of oxidative dyes in hair is fundamental [4]. In this study, IMS was used to determine the localization of each oxidative dye. Certain oxidative dyes were found to be ubiquitous in the hair, whereas others were localized on the inner edge.

To explain these characteristics, we focused on the charges of the oxidative dyes. We believe that oxidative dyes exhibiting a tendency toward either a strong positive or negative charge are less likely to penetrate hair because of predominantly ionic electrostatic interactions with the charged side chains of amino acids. For example, dyes containing many amino groups (such as pPD-2,4DAPE in Figure 7-a) or hydroxyl groups (such as pAP-RES in Figure 7-b) fall into this category. By contrast, oxidative dyes having low charge imbalance (such as pPD-5AOC in Figure 7-c) are thought to penetrate the hair without being captured by the amino acid side chains. To verify this hypothesis, the localization of oxidative dyes must be analyzed under different pH conditions and the magnitude of the oxidative dyes' charge should be quantitatively assessed.

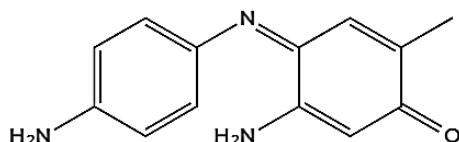


**(7-a)** structure of 2-[6-amino-3-(4-aminophenoxy)imino-4-aminocyclohexa-1,5-dien-1-yl]oxy-ethanol



**(7-b)** structure of 3-hydroxy-4-(4-hydroxyphenyl)iminocyclohexa-2,5-dien-1-one





**(7-c)** structure of 5-amino-4-(4-aminophenyl)imino-2-methylcyclohexa-2,5-dien-1-one

**Figure-7.** Structure of oxidative hair dyes

Moreover, the fact that some oxidative dyes are not ubiquitously distributed in the hair allows us to infer possible locations where the coupling reactions occur. Various locations, such as the vicinity of melanin granules in the matrix layer [4] or within the cuticle layer [3], have been proposed as potential sites for these coupling reactions. However, the localization of oxidative dye polymers revealed in this study suggests that not all coupling reactions occur exclusively within the matrix layer. Previous studies and the findings of this study indicate that even during the coupling-reaction stage in the cuticle layer, certain oxidative dyes may become localized on the inner edge of the hair, owing to predominantly ionic electrostatic interactions with the side chains of amino acids in the hair. By contrast, oxidative dyes that do not exhibit such ionic interactions are presumed to diffuse more freely into the interior of the hair and are distributed more widely.

Subsequently, to investigate whether the localization of oxidative dyes in the hair is related to the behavior of dyes, we focused on color fading caused by washing, which is one of the most significant issues after dyeing. Although measures against fading caused by washing have been reported [5], the detailed behaviors of individual oxidative dyes have not been thoroughly explained. This study suggests that in addition to the affinity between oxidative dyes and water, the localization of these dyes within hair is a contributing factor.

Evidently, oxidative dyes localized on the inner edge of the hair are more susceptible to physical effects during washing than those distributed throughout the hair interior. Furthermore, the increased fading resulting from washing may be attributed to the cleavage of ionic electrostatic interactions between the amino acid side chains in the hair and oxidative dyes caused by the intervention of water.

Based on these results, the localization of oxidative dyes in hair is considered to be a factor that can affect both the scheme up to dyeing and the behavior after dyeing.

## 5. Conclusion

We determined the localization of various oxidative dyes in colored hair by using IMS. This localization can also be used as an index for the differences in the washing–fading process. This index will be used to understand the characteristics of various hair dyes. The localization of oxidative dyes in hair may provide new insights into reaction mechanisms. Consequently, we may be able to identify new features of oxidative dyes and create oxidative hair dyes having new value.

## 6. Reference

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