J. Soc. Cosmet. Chem., 28, 259–271 (May 1977)

Thin-layer chromatography (TLC) of redox reaction products of oxidative hair dyes

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Received August 26, 1976. Presented October 13, 1975, Joint AOAC-SCC Symposium on Cosmetic Analytical Techniques, Washington, D.C.

Synopsis

OXIDATIVE or permanent HAIR DYES are based on ALKALINE PEROXIDE OXIDATION of PHENYLENEDIAMINE (PDA) or related AROMATIC AMINES. These amines, when oxidized alone or in combination with other phenolic and aromatic amino compounds (couplers), yield a mixture of colored oxidation products. This paper describes the use of thin-layer chromatography (TLC) for the qualitative analysis of these complex mixtures of oxidation products. Effects of variables including the nature of adsorbency, layer thickness, water content, development of chromatograms, and sample application techniques are presented. Scopes and limitations of chromatography for the isolation and the identification of these dyes are discussed.

INTRODUCTION

Permanent hair dyes, which are presently available commercially, are mostly oxidative dyes which contain 2 main ingredients. One ingredient is a dye precursor, while the other ingredient is a developer or oxidizer, usually hydrogen peroxide. The dyeing process involves mixing the dye precursors with the dye developer in an alkaline medium generally around pH 9 to 10. The dye precursors and oxidizer diffuse in the hair fibers where chemical reactions leading to color development take place inside the hair fiber.

Dye precursors contain 2 main ingredients: primary intermediates and couplers. Primary intermediates used in oxidative dyes are mainly ortho- and para-aromatic diamines or aminophenols, which are colorless, but, upon oxidation, give colored oxidation products. The most commonly used primary intermediates are p-phenylenediamine (PDA), p-toluenediamine (TDA), and p-aminophenols (PAP).

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Color couplers are compounds which produce little or no color when oxidized alone in the hair fiber, but which produce new colored species when used in the presence of primary intermediates. These color couplers include m-diamines, m-aminophenols, polyhydric phenols, etc. The most commonly used couplers are resorcinol (R) and 2,4 diaminoanisole (DAA), which when oxidized with p-phenylenediamine give green and blue colored products, respectively, along with many other minor products.

When studying these dyes, it is important to ascertain the primary intermediates and couplers used in a particular dye, and secondly, the colored oxidation products formed from the dye precursors with the dye developer, i.e., hydrogen peroxide. To study these aspects, improved methods are needed for the separation and identification of oxidative hair dyes.

Much work has been done in developing separation techniques to identify dye intermediates and other components present in oxidative hair dyes. These methods include the use of paper chromatography (PC) (1–6), gas chromatography (GC) (7–10), thin-layer chromatography (TLC) (11–19), and/or combinations of these chromatographic techniques (11,16), and other methods (20–23). In order to understand the mechanisms of the color-forming reaction, it is vital to identify the colored species produced during the hair dyeing process. Application of chromatography of the oxidation products of primary intermediates alone or in the presence of various couplers is essential. Chromatography separation of the oxidation products of oxidative hair dyes has been reported (24–31), which emphasizes the importance of understanding the redox reaction products of the dye intermediates.

In the field of separation methods, chromatography occupies a rather unique position, and TLC provides the best answer to this problem in many cases. This paper describes a comprehensive study of TLC of the oxidation products and their application to oxidative hair dye analysis. It presents the effect of 3 main factors of chromatographic separations, i.e., solutes (nature and amount); sorbents (quality and nature, thickness and uniformity, activation and sotrage); and solvent (quality and nature, vapor saturation) of the separation of oxidative dyes.

Also, an isolation procedure for the desired component of the oxidation mixture after their separation is shown. This paper should (a) serve as a background of information for those who would be utilizing this technique for identifying oxidation dye products; and (b) establish variables of this analytical method to provide the best separation technique for the multicomponent dye product.

EXPERIMENTAL AND DISCUSSIONS

The use of TLC for oxidative dyes presents 2 specific problems: first is the limited stability, and second is the problem of closely related structures of the oxidation products (Tables I, II). As mentioned previously, 2 of the primary ingredients are aromatic diamines and polyhydric phenols. The chemical transformation in the reaction system, once initiated, may continue indefinitely. Even the individual fractions isolated are self-reactive and/or react with each other and with foreign agents such as air, moisture, sunlight, proteins, etc.; which are manifested by several types of reactions in solid state or in solution.

Table I

RF Values of the Oxidation Products of p-Phenylenediamine and m-Methoxyphenol[a]

Band Color	Relative Intensity	RF X100
Redish Brown (origin)	Strong	0
Yellow Orange	Strong	12.05
Pink	Strong	17.64
Purple	Light	21.47
Yellow	Strong	26.17
Brown	Strong	29.70
Yellow	Medium	35.58
Green	Light	40.58
Orange	Medium	41.76

[a] 1:1 mixture with hydrogen peroxide oxidant and sodium carbonate base; Chloroform:Ethylacetate:Methanol (6:2:2) solvent system.

There is a great advantage in working with these oxidative dyes. Since the products are colored, it is easy to visualize the progress of the chromatography during development, to obtain useful information on the presence or absence of certain components, and even to make a rough estimate of their relative abundance.

Although, PDA yields a number of color components in its reaction with alkaline peroxide both alone and with the individual couplers, the dominant composite colors are a purplish-brown for PDA, a green for PDA-resorcinol, and a purplish-blue for PDA-diaminoanisole. The oxidative reaction of resorcinol and diaminoanisole alone or in combination produce negligible color compared with their coupling products with PDA. Self-coupling of PAP provides multicomponent products like other products, but gives a major yellow color. The binary coupling with resorcinol provides green color similar to that of PDA-resorcinol R, but with coupling with DAA the predominant product is a vivid red with blues, greens and relatively few brown components either in primary, or in polymeric products. Chemical structures of the major colors are shown in Fig. 1. With prolonged dye development time, all the above compounds increasingly convert to polymeric compositions of brown components, having a very low mobility on thin layer plates.

Table II

RF Values of the Oxidation Products of p-Aminophenol and Resorcinol[a]

Band Color	Relative Intensity	RF x100
Brown (origin)	Strong	0
Blue	Light	7.35
Pink	Strong	18.17
Green	Medium	26.47
Brownish Red	Medium	30.88
Brown	Medium	32.35
Red	Strong	37.64
Yellow	Strong	38.82

[a] 1:1 mixture with hydrogen peroxide oxidant and sodium carbonate base; Chloroform:Ethylacetate:Methanol (6:2:2) solvent system.

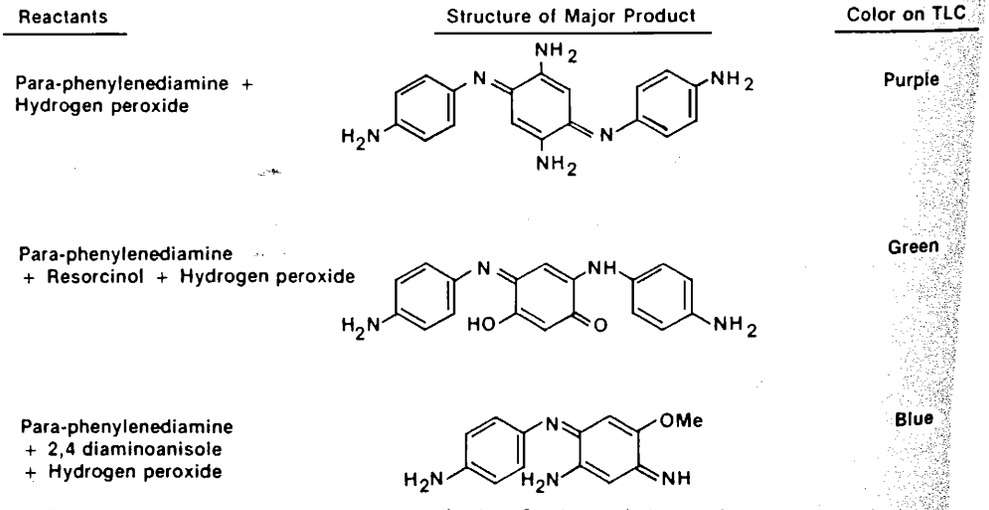

Figure 1. Structure and color of major oxidation products

Table III

THIN LAYER PLATES

No	Source	Adsorbent Coating	Inert Backing
1	Brinkman (Merck)	Slica Gel [a]	Glass plate
2	Mallinckrodt	Slica Gel	Glass fiber web
3	Eastman	Silica Gel	Polyethylene terephthalate
4	Anal. tech.	Alumina Acid	Glass plate
5	Anal. tech.	Alumina Neutral	Glass plate
6	Anal. tech.	Alumina Basic	Glass plate
7	Brinkman	Cellulose	Glass plate
8	Corning	Glass powder	Glass plate
9	Varied	Filter papers	None

[a] Analytical (.25 mm) and preparative (2 mm)

FACTORS OF CHROMATOGRAPHIC SEPARATIONS

Solutes: The nature of the solutes to be analyzed is important to the success of obtaining an effective separation. Upon oxidation, PDA and allied amines give a very complex mixture of products. This is because oxidation of PDA involves the formation of a highly reactive intermediate (32), which can undergo various reactions. Upon oxidation, PDA gives quinonedimine, which on hydrolysis, gives quinone-monoimine, and on further hydrolysis, results in the formation of benzoquinone (33).

Once formed, quinonediimine reacts rapidly with unreacted PDA and gives a major products known as Bandrowski's base. As has been shown before in oxidative dyes, there are may couplers, other than primary intermediates, which undergo competitive reaction with quinonediimine to give a mixture of products (34). These are mostly indophenols and indamines and can be bound through nitrogen or oxygen. These are main products. In addition to these, various forms of one ring, two ring or poly-ring structures can form (25). Thus, selectivity in the reaction is very low. Even when a single precursor is used, one always ends up with 3 to 4 major components, and at least 5 to 6 minor components. While the nature of the solute plays a role, the amount of solvent is also important. In many instances, inversion of the bands was found, with a change in the amount of solute, fast-moving bands becoming slow-moving and vice-versa.

Table IV

Resolution of p-Phenylenediamine and Resorcinol Oxidation Products on Silica Gel and Alumina TLC Plates[a]

Alumina plate[b]		Slica gel plate[b]	
Band color	distance removed, cm.[c]	Band color	distance removed, cm.[d]
Brown	0	Brown	0
Green	1.2	Blue	1.5
Yellow	1.9	Pink	4.0
Blue	3.0	Green	5.0
Yellow	4.5	Yellow	6.0
Pink	5.4	Orange Yellow	8.5
Red	6.7	Yellow	9.5
Yellow	7.1		

[a] Solvent system, Chloroform:Ethylacetate:Methanol (6:2:2)

[b] In the case of alumina plate the solvent front moved 17.0 cm, while in silica gel it moved 17.5 cm.

[c] Result of three development

[d] Result of single development

Adsorbent: In order to find out which adsorbent would best resolve the complicated mixtures, a number of different adsorbents with plastic or glass backing were tried as is shown in Table III.

In all analyses, commercially available thin-plates were used. For these studies, silica gel plates were the most useful in overall separation, up to 8 components were easily resolved in a single elution. Plates with a flexible inert phase, such as plastics or glass fiber web, never equaled the quality of resolution obtained on glass plates—even when identical adsorbent phases were used. Although Chroam AR sheets* carried the same

*Mallinckrodt Inc., St. Louis, MO.

Table V

Solvent Selection

Dye Group	Properties	Solvent
1	Fast moving, low molecular wt. one or two ring structures	Ether: chloroform
2	Two or three ring structures	Chloroform: ethylacetate: methanol Chloroform: DMF
3	Immobile polymeric	Alcohol, DMF, DMSO

solid phase as the thin-layer plates, they did not have the sharpness of the resolution of the analytical plates. All of the components diffused much more, not only in the direction of the elution, but also perpendicular to it as well, showing channeling of the solvents along the fibers which form an isotroric web. This resulted in more extensive component spreading.

Alumina was less efficient in performing the total analysis of all product-compositions studies. In some instances, alumina gave much sharper or cleaner separations for specific components. Specificity was not a characteristic of pH, but of the adsorbent itself, and it was qualitative for most components in the form of no adsorption or extremely strong adsorption.

Development time was slightly reduced when a glass adsorbent was used, but there was poor resolution, especially for the fast-moving and slow-moving components. Good resolution was never achieved with cellulose solid phase either in the form of papers or TLC plate. Table IV shows the resolution of PDA and resorcinol oxidation products on silica gel and alumina plates. On silica gel plates, the product is well resolved and there is not much specificity of strongly adsorbed and nonadsorbed components. On alumina plates, the fast-moving pink and many other components stayed close to the origin.

Solvent: A difficult task in this study was the choice of a proper solvent or solvents, as no one system was found which resolved all components simultaneously. From the point of view of solvent requirement, all dye components can be divided into 3 groups (Table V).

The dyes belonging to Group 1 are low molecular weight, one-ring or two-ring structures (such as nitro aniline) and are compounds which are mostly orange, yellow, or red. These components are usually water soluble. This group of dyes can be separated by using chloroform and ether in different ratios.

The dyes in Group 2 include all the specific dye components, which are of primary importance for the shade and intensity of hair color. They are components containing 2 or 3 rings bound by nitrogen or oxygen. The elution system for the resolution of these components must contain significant amounts of highly polar and hydrophilic components such as dimethylformamide (DMF), dimethylsulfoxide (DMSO), and alcohols, as well as low-activity solvents, such as halogenated hydrocarbons.

In an arbitrary way, all dye components, which did not elute with the above solvent systems, were grouped as polymeric materials (Group 3). They are mostly immobile and can be resolved partially using DMF, DMSO, or methanol.

Oxidation products are sensitive to both acids and bases used in the solvent system and produce irreversible destruction of a number of components of the product. Their use in solvent systems was avoided.

Moisture: In the resolution of oxidative dyes, a trace amount of moisture is more detrimental for the reproducibility than other chromatographic separations. It was observed that the resolution of certain components can be eliminated completely by allowing moisture in the system through the plates or from the atmosphere.

Activation: Developed plates, on which oxidative dyes cannot be reactivated for a second elution in a similar fashion, because they undergo irreproducible changes or cannot be controlled. Dyes to be applied on the activated plate were dissolved in DMF, since the polymeric component of the dye had a limited solubility in other less polar solvent. When preparing a sample of a solvent, that solution should never be warmed, and fresh solution should always be prepared just before applying it to the plate.

Application to plate: With oxidative dyes, it is preferable to apply the products to the plate as a sharp band, rather than as a spot, for 2 reasons. One, the left-over unreacted dye precursors, which developed during the elution process, causes heavy trailing and interferes with the chromatography of an already formed oxidized product. Second, the oxidized products undergo secondary coupling reactions on silica gel, giving grayish-brown polymeric trailing. When the dye is applied as a band, visual identification of the components present in smaller amounts can be achieved.

Evaluation and documentation: Evaluation and documentation of the thin-layer plates should be done immediately after elution to record the presence or absence of components and the relative strength of color of individual components. This is required because 3 events may occur on the developed plate. One, a new color may form from untreated precursors; two, color may fade from the intensely colored products; and three, color may change. For example, green color changes to gray, blue changes to purple, and so on.

All of these processes can be slowed down, immediately following elution, if the plates are wrapped tightly to exclude air in polyethylene bags (preferably black) or are kept in a dark place.

ELUTION TECHNIQUES

Single elution: Single elution of the spotted plates should be done as soon as the spotting solvent has evaporated. A spotted plate cannot be dried at an elevated temperature or even at low temperature for very long. It should be developed immediately after drying.

If not completely removed, DMF moved all the components until it was sufficiently diluted by the weaker eluting solvent. Aging at room temperature resulted in a disproportionate loss of specific components and caused the formation of polymeric material. Complete drying caused the destruction of some components. So it was necessary to choose between complete drying and leaving spotting solvent on the plate. These studies indicated that it was harmful to leave some spotting solvent rather then to dry completely.

Two dimensional: Two-dimensional chromatography was most useful for compositions in which individual members differ qualitatively in their response to solvents, e.g., acidic and basic amino acids. This was not the case with oxidative dyes. Any increase in solvent activity increased the mobility of all the components. No solvent compositions was found which specifically favored slow-moving components.

Continuous elution: This method was used in order to obtain single components in sufficient amount for structural determination. Continuous elution was made in an apparatus* (Fig. 2). Oxidation product was applied as a band at the bottom of the plates. The lids on the developing tank were positioned to form slots 4 nm wide. Plates were allowed to stand in the solvent with the upper ends projecting into the free atmosphere. The solvent moved and evaporated at slot levels as a continuous process, so that all components, except the undesirable polymeric uneluted product, were redeposited at lid level as a narrow line. The plate was reversed so that the streak became the origin and developed again. Two separate functions can be achieved using this method. First, broad areas of sample can be converted into hairline streaks; thus, subsequent runs provide better separation. Second, the running length of a plate is multiplied many times, which enables even the most slow-moving fractions to be separated effectively.

Continuous elution was found to be more advantageous than the repeated elution technique. In addition to eliminating time-wasting multiple developments, it required no continuous attention. Furthermore, in repeated elution technique, the plate had to be dried after each step. During each exposure to air and humidity, some components undergo polymerization, which leaves a brown residue.

ISOLATION PROCEDURE

From the complex mixtures, a few components were isolated and purified for structural determination by spectroscopic methods. The procedure used is shown in Fig. 3.

The total dye mixture, obtained as a solid material after filtration, was applied as a band on a 2-mm preparative plate and developed a number of times. After maximum resolution was obtained, single component bands were scraped from the plate and were extracted with methanol at room temperature. This extract was centrifuged to remove silica gel and then filtered to remove any remaining silica. The extracts were evaporated immediately to reduce the extent of "polymerization," and then were purified by spotting on analytical plates. This process was repeated several times. Despite all precautions, single components after 3 elution and recovery cycles consistently showed the same nonmoving residue at the origin, demonstrating the chemical sensitivity of the dyes.

Although column chromatography can be used for the separation of the components in large amounts, trailing after the first few components was more severe than with thin-layer plates. For this reason, column chromatography was useful as a preliminary enrichment of components for subsequent thin-layer separation.

*Shandon Southern Instrument Co., Inc., 515 Broad Str, Sewickley, PA 15143. Cat. #SAB-2852.

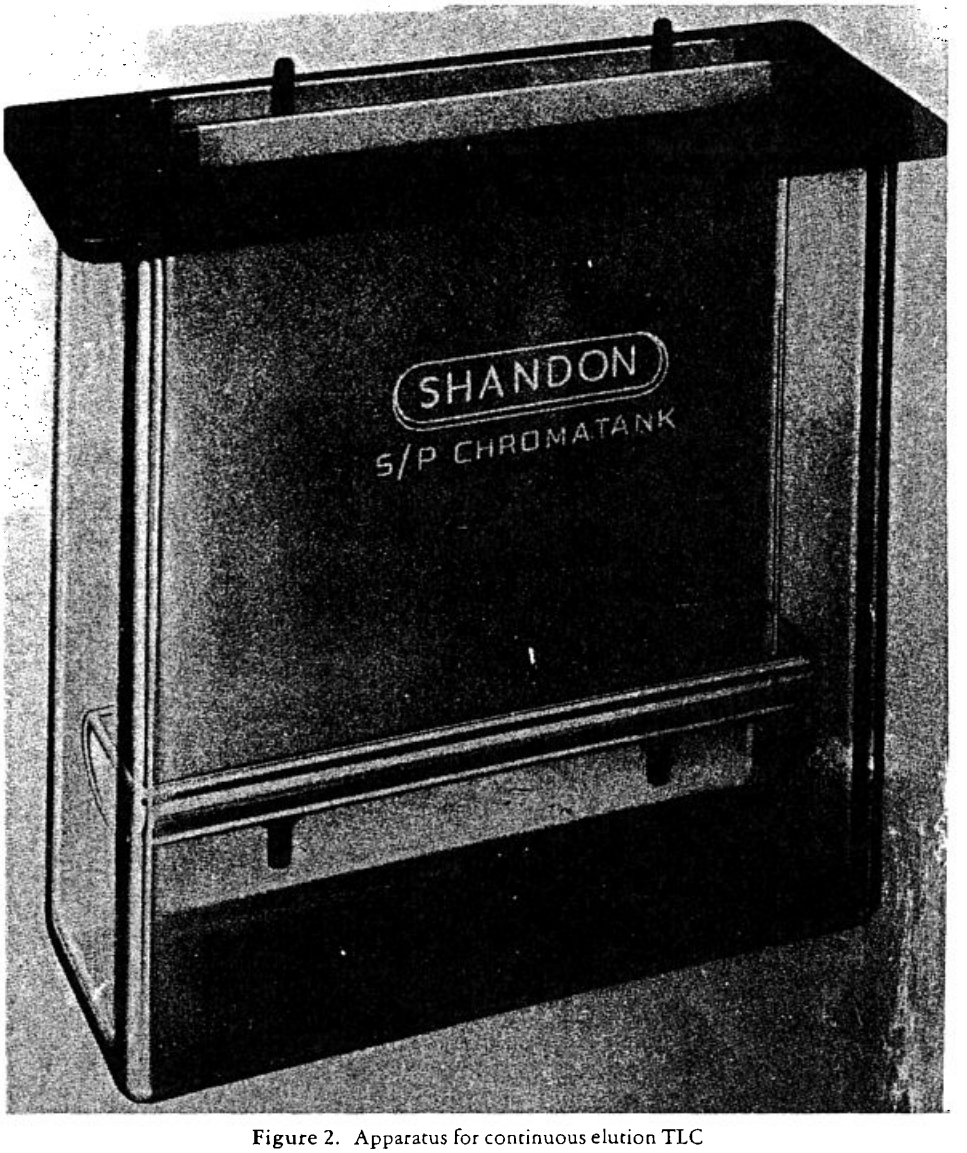

Figure 2. Apparatus for continuous elution TLC

Figure 3. Isolation procedure

CONCLUSION

TLC of oxidative dyes is a less than perfect answer for the dye chemist because of problems associated with the chemical composition of the dyes. However, to date, TLC has been the single most effective method for providing pure components for structural determinations. It is the only standard analytical technique able to identify the composition of the already-formed dyes, simply because it indicates colors and the range of mobilities of the different product components whch can form from specific precursors. The chromatographic separation of the oxidative products will make it possible to deduce trends in the pattern of dye developments.

ACKNOWLEDGMENT

This work was carried out at Gillette Research Institute. I wish to acknowledge my gratitude to the late Dr. William Tolgyesi for his many helpful suggestions.

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