THE ANALYSIS OF ARYLAMINES AND PHENOLS IN OXIDATION-TYPE HAIR DYES BY PAPER CHROMATOGRAPHY

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In the course of examining various types of cosmetic preparations in this laboratory, the need for a simple method for the separation and identification of the colouring agents of oxidation-type (*p*-phenylenediamine) hair dyes became evident. Although specific procedures exist[1] for many of the major hair dye components, no overall analytical method for colouring agents has been reported. The colouring agents most commonly used are the phenylenediamines, diaminotoluenes, nitrophenylenediamines, aminophenols, nitroaminophenols, and di- and trihydroxybenzenes. Many compounds are in use and therefore paper partition chromatography, with its simplicity and high separating power, was the logical choice as the analytical technique to be used in this work.

Little has been published on the paper chromatographic separation of compounds of this type. The sole publication specifically devoted to colouring agents of hair dyes was by IACOBELLI-TURI[2]. This worker accomplished the separation of *p*-phenylenediamine, 2,5-diaminotoluene, *p*-aminophenol, resorcinol and pyrogallol, and also studied the behaviour of these compounds toward a variety of detecting reagents. ŠIMEK[3] and also PANNELL AND LUVALLE[4] have chromatographed photographic developers and some of these compounds are the same as those used as colouring agents of hair dyes. SUNDT[5] has studied the three isomeric aminophenols but, under his chromatographic conditions, these compounds failed to move from the starting line. BATE-SMITH[6] has reported the separation of the isomeric dihydroxybenzenes and pyrogallol.

This paper is concerned with the paper partition chromatography of 29 compounds, all of which have been used as colouring agents of hair dyes. A mixture of *n*-butanol, ethanol and water containing acetic acid and sodium sulphite was used as the developing solvent. This solvent system gave a good distribution of R_F values. All compounds could be identified by their R_F values together with their behaviour toward the detecting reagents used in this work.

EXPERIMENTAL

1. *Materials*

All of the reference compounds were obtained from chemical supply houses and were of the best quality that was available. These materials were used without further purification.

2. Pretreatment of hair dye samples prior to spotting chromatograms

When the hair dye preparation was a liquid and completely miscible with water, a 1–2 ml portion was diluted with an equal volume of water and the diluted solution was spotted directly on the chromatograms.

For a preparation that was a liquid, but not completely miscible with water, a 2 ml portion was diluted with 20 ml of *n*-hexane in a separatory funnel. This mixture was then shaken gently with 10 ml of dilute aqueous acetic acid (1:9). When the two phases had separated, the lower aqueous acid layer was run off and used to spot the chromatograms.

For a hair dye preparation that was either a dry solid or a paste, approximately 1 g was stirred vigorously with 5 ml of dilute aqueous acetic acid (1:19). The resulting mixture was filtered through glass wool and the filtrate was spotted on the chromatograms.

3. Detecting reagents

Ammoniacal silver nitrate. Two grams of silver nitrate were dissolved in 100 ml of distilled water. To this solution was added concentrated aqueous ammonia, dropwise with vigorous swirling, until the precipitate that formed initially just redissolved. An excess of 1 ml of aqueous ammonia was then added.

p-Dimethylaminobenzaldehyde. To a solution containing 1 g of *p*-dimethylaminobenzaldehyde in 100 ml of absolute ethanol was added 1 ml of concentrated hydrochloric acid.

Sodium 1,2-naphthoquinone-4-sulphonate. A solution containing 0.5 g of sodium 1,2-naphthoquinone-4-sulphonate in 95 ml of distilled water was mixed with 5 ml of glacial acetic acid. If undissolved matter was present, it was removed by filtration through glass wool.

4. Paper chromatography

The paper chromatograms were developed in a cylindrical glass tank (24 in. high and 12 in. in diameter) with a plate glass cover. Within the tank was a stainless steel rack that supported glass troughs positioned for downward flow of the developing solvent. The paper used for the chromatograms was Whatman No. 3 MM cut into strips 6 × 22 in. A starting line was drawn about 3 in. from one end of each paper and marked at intervals of $^3/_4$ in. in order to position the spottings. Standard solutions were prepared by dissolving approximately 25 mg of the amine or phenol reference compound in 5 ml of a (1:19) solution of glacial acetic acid in water. When working with the amines, fresh solutions were prepared each day because these compounds degraded rapidly when in solution.

Solutions of the hair dye samples were prepared as described previously and spotted along with *p*-phenylenediamine and other appropriate reference compounds on three papers, one for each of the detecting reagents. A 5 λ volume of an unknown or a standard solution usually produced a satisfactory chromatogram. The spots were dried and the three chromatograms were developed simultaneously. The developing solvent was the upper phase of a mixture of 500 ml of *n*-butanol, 100 ml of ethanol, 400 ml of distilled water, 10 ml of glacial acetic acid, and 10 g of anhydrous sodium sulphite. The chromatograms were developed until the solvent front had travelled 10–12 in. from the starting line; this required 4–5 h. The chromatograms were then

removed from the developing tank and dried in air for about 30 min. Each paper was then sprayed with one of the three detecting reagents. No heating of the chromatograms was required for colour development. After allowing at least 30 min for full development of the spots, the chromatograms were evaluated. Table I lists the R_F values of the amines and phenols which were studied and data on their behaviour toward the three detecting reagents. Nitro compounds, being yellow in colour, usually could be seen on the chromatograms before spraying with the detecting reagents.

TABLE I

CHROMATOGRAPHIC DATA FOR SOME ARYLAMINES AND PHENOLS USED AS COLOURING AGENTS IN OXIDATION-TYPE HAIR DYES

Solvent: *n*-Butanol–ethanol–water–acetic acid–sodium sulphite (50:10:40:1:1, upper layer).
Paper: Whatman No. 3 MM.
Detection: D_1 = Ammoniacal silver nitrate.
D_2 = Ethanolic *p*-dimethylaminobenzaldehyde plus hydrochloric acid.
D_3 = Sodium 1,2-naphthoquinone-4-sulphonate plus acetic acid.

Compound	R_F	D_1*	*Colour***	
			D_2	D_3
2-Amino-1-phenol-4-sulphonic acid	0.08	5 sec	Y	O
2,4-Diaminophenol	0.34	5 sec	B-O	Bl-P
p-Phenylenediamine	0.36	5 sec	R	R-P
m-Phenylenediamine	0.46	30 min	O-Y	P
4-Methoxy-*m*-phenylenediamine	0.49	5 min	O	P
2,5-Diaminotoluene	0.49	5 min	R	P
2,4-Diaminotoluene	0.58	30 min	Y	P
p-Aminophenol	0.59	5 sec	Y	P
p-Methylaminophenol	0.62	5 sec	Y	P
o-Phenylenediamine	0.63	5 min	O	P
2-Nitro-*p*-phenylenediamine	0.64	5 sec	B	P
p-Diethylaminoaniline	0.68	5 sec	Y	P
4,4′-Diaminodiphenylmethane	0.68	30 min	Y	R
m-Aminophenol	0.71	5 min	G-Y	P
p-Anisidine	0.72	5 min	Y	P
4-Nitro-*o*-phenylenediamine	0.73	5 sec	B-Y	B
Picramic acid	0.75	5 sec	O	P
Pyrogallol	0.75	5 sec	P	P
o-Aminophenol	0.77	5 sec	Y	O
N-Phenyl-*p*-phenylenediamine	0.79	5 sec	B-R	Bl
4-Amino-2-nitrophenol	0.82	5 sec	Y	P
4-Chloro-*o*-phenylenediamine	0.83	5 min	O-Y	B-R
Hydroquinone	0.86	5 sec	neg.	neg.
Catechol	0.87	5 sec	neg.	neg.
2-Amino-4-nitrophenol	0.88	5 sec.	Y	O
o-Anisidine	0.89	30 min	Y	R
Resorcinol	0.90	5 min	P	neg.
1,5-Naphthalenediol	0.91	5 sec	G	neg.
p,*p*′-Methylene-bis-(N,N-dimethyl-aniline)	0.92	30 min	Y	G

* Approximate time required for development of spot.
** Y = yellow; B = brown; O = orange; R = red; G = green; P = purple; Bl = blue; neg. = negative.

RESULTS AND DISCUSSION

Oxidation-type hair dyes are usually aqueous solutions containing 1–5 % of a mixture of colouring agents. In addition ammonia, surfactants, hair conditioners, perfume

and sodium sulphite may be present. These other materials did not interfere with the identification of the colouring agents. Products of this type were found to be quite viscous and direct spotting usually did not produce satisfactory chromatograms. This appeared to be due to the physical nature of these preparations in that they did not readily "wet" and penetrate the paper on spotting. For most products, this difficulty was overcome by the simple expediency of diluting the sample with water. For those liquid samples that produced two phases when mixed with water, the alternative extraction procedure using dilute acetic acid gave satisfactory chromatograms without significant losses of colouring agents. Paste and solid samples presented no problem in that the colouring agents could readily be extracted with dilute acetic acid. All of the commercial preparations that were examined in this laboratory gave satisfactory chromatograms after application of the appropriate pretreatment procedure.

Twenty-nine compounds were investigated in this work and it was found that not all of the possible combinations could be separated with the single developing solvent. Investigation of the use of several developing solvents to achieve a unique R_F value for each compound met with little success. In general, the developing solvents separated these compounds in the same order but gave different ranges of R_F values. The alternative of employing several detecting reagents in order to identify compounds having similar R_F values proved to be quite successful. However, there were a few cases where compounds having the same R_F values could be identified only if they were present singly.

The chromatographic procedure used in this work requires few comments. No conditioning or equilibration of the chromatographic paper was necessary prior to development. In the initial stages of this work, the organic phase of *n*-butanol–ethanol–water (5 : 1 : 4) was chosen as developing solvent on the basis that it produced a wide range of R_F values. However, extensive streaking of the spots occurred with this solvent. In addition, there was a loss of sensitivity toward the detecting reagents which was related to the development time of the chromatograms. In some instances, a compound could be detected easily after 1 h of development but after 4–5 h the compound could not be detected on the chromatogram. Probably, this loss of sensitivity was caused by air oxidation of the compounds. The addition of a small quantity of acetic acid to the developing solvent mixture eliminated the streaking and gave well-defined spots. The amount of acetic acid added was minimal because large amounts of the acid were found to be difficult to remove from the developed chromatograms and reduced the sensitivity of the silver nitrate detecting reagent. The addition of sodium sulphite reduced losses from oxidation during development of the chromatograms and greatly improved the sensitivity of the method. It was observed that R_F values began to change after using a given batch of developing solvent for several days. With freshly prepared solvent, the reproducibility of R_F values was excellent.

The detecting reagents used in this work were selected after investigating a wide variety of potential reagents. Those selected produced permanent spots, reacted with all or most of the reference compounds, and showed good sensitivity. The limit of detection was determined under actual chromatographic conditions, using four of the reference compounds. These were *p*-phenylenediamine, 2,5-diaminotoluene, 2-nitro-*p*-phenylenediamine and pyrogallol. Identical results were obtained with all four

compounds. The limit of detection using *p*-dimethylaminobenzaldehyde was 0.1 γ and for the other reagents the limit was 1 γ. *p*-Dimethylaminobenzaldehyde gave a variety of colours and this was very useful for identification of compounds with similar R_F values. In order to take full advantage of the potential of this detecting reagent, it was necessary to allow sufficient time after spraying the chromatograms for complete development of the colours. Many spots that initially were bright yellow, changed to off-shades of yellow or orange on standing and this was accompanied by an increase in the intensity of the colour. The colours listed in Table I for this reagent are those observed after about 30 min.

Ammoniacal silver nitrate reacted with all of the compounds under investigation and showed a high degree of sensitivity. With this reagent, all compounds produced a brown or black colour. The variation in colour seemed to be more dependent on the concentration of material in the spot than on its identity. It was important that the paper was dried thoroughly to remove acetic acid before spraying with this reagent, otherwise there was a loss of sensitivity. The time required for development of spots by this reagent was also of value for identification although this varied with the amount of the compound in the spot. With this reagent, the background darkened rapidly in the presence of sunlight and eventually the spots were obliterated. However, this process was not so rapid as to interfere with the detection of those compounds that were slow to form spots provided that the sprayed chromatogram was located away from direct sunlight. When it was desired to preserve chromatograms treated with this reagent, the sprayed chromatogram was immersed in 5% aqueous sodium thiosulphate solution and then washed with water. This treatment resulted in some reduction in intensity of the spots.

The use of sodium 1,2-naphthoquinone-4-sulphonate as a detecting reagent was based on work reported by SCHMIDT[7]. The reagent reacted with all compounds bearing an amino group but the range of colours produced was rather limited. This reagent was used mainly to distinguish between arylamines and polyhydric phenols and also to provide confirmation of identification.

The polyhydric phenols presented the greatest difficulties in identification, particularly hydroquinone and catechol. These two compounds were not separated by the developing solvent and behaved in an identical manner toward the detecting reagents. However, these compounds could be distinguished by developing an additional chromatogram and spraying it with aqueous 1% ferric chloride solution; hydroquinone produced little or no colour whereas catechol gave a black spot. Pyrogallol produced a black spot with this reagent and resorcinol and 1,5-naphthalenediol both gave pale brown spots.

Several dozen hair dye preparations were analyzed using this method. The colouring agents identified in these products were as follows: *o*-phenylenediamine, *p*-phenylenediamine, 2,4-diaminotoluene, 2,5-diaminotoluene, N-phenyl-*p*-phenylenediamine, *o*-aminophenol, *m*-aminophenol, *p*-aminophenol, *p*-anisidine, 2-nitro-*p*-phenylenediamine, 4-nitro-*o*-phenylenediamine, 4-amino-2-nitrophenol, catechol, resorcinol and pyrogallol. Only those preparations producing grey or black shades contained a single colouring agent and this was either *p*-phenylenediamine or 2,5-diaminotoluene. All of the reddish shades contained at least one nitro compound. Most of the preparations contained a complex mixture of colouring agents having 6–12 components.

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SUMMARY

A paper partition chromatographic procedure is described for the separation and identification of aryldiamines, aminophenols and polyhydric phenols used as colouring agents of oxidation-type hair dyes. Twenty-nine compounds were chromatographed on Whatman No. 3 MM paper using, as developing solvent, the top layer of a mixture of 500 ml *n*-butanol, 100 ml ethanol, 400 ml water, 10 ml acetic acid and 10 g sodium sulphite. Good separations of most combinations of compounds were achieved. Where resolutions could not be accomplished, positive identifications could be made through the use of several detecting reagents. The application of this procedure to the analysis of colouring agents of hair dye preparations is described.

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