ISSN 1996-3424

Asian Journal of Dermatology

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

OPEN ACCESS Asian Journal of Dermatology

ISSN 1996-3424
DOI: 10.3923/ajd.2017.7.22

Research Article
Establishing the Safety of Powder Henna Hair Colour Through Identification of Bandrowski's Base by HPLC Method

Jayaganesh Sankar, Sudhir Sawarkar, Zulfiqar Ali Muhammad Anwar and Jainendra Malakar

Dabur Research and Development, International Business Development, Dubai, P.O. Box 6399, United Arab Emirates

Abstract

Background and Objective: The p-phenylenediamine (PPD) used as a coloring agent in hair colour and it is known to be allergic due to formation of trimer Bandrowski's base. At favorable condition the p-phenylenediamine molecule convert in to dimer and trimer configuration, trimer configuration is called as a Bandrowski's Base. The objective of study was identification of Bandrowski's Base through the HPLC estimation. This estimation will helpful to find out the safety aspects of henna based hair colours. Estimate the Bandrowski's Base formation with various pH and it help to draw a optimum pH range for the Bandrowski's Base formation and prevent the formation with adjusted the pH range. Therefore, main objective of the study was formulating the safer henna based hair colour without the formation of sensitive potential of Bandrowski's Base and develop the safer henna based hair colour for the end user. **Materials and Methods:** Bandrowski's Base (BB) was synthesized by the method described by Ritter and Schmitz. BB base formation depends upon the pH condition (>10.0), high alkaline and hydrogen peroxide concentration. Various concentrations of standard PPD and BB base injected and identified the retention time. Samples are kept injected at 24 and 48 h time interval interest to monitor the BB base formation and PPD self -coupling. **Results:** In this combination of standard materials of PPD and BB standard injected and the peaks showed the different standard retention time, PPD eluted at 2.352 min. Retention time and BB base eluted at 4.696 min. Addition of resorcinol plays a crucial role to prevent the BB base formation. Quinone-diimine react with coupler resorcinol to form the indophenol complex (green component) further it under goes coupling with PPD or resorcinol or co-oxidation coupling it forms a poly indophenol complex. **Conclusion:** Henna based hair color with pH 10-12.0 with 30 min developmental time does not contribute the formation of Bandrowski Base. However, henna based hair colour products pH fall between 7.0-8.0, it further confirms that there is no BB formation and safe to use. No evidence for the formation of Bandrowski base after 24 h with the treatment of higher ammonia and hydrogen peroxide.

Key words: p-phenylenediamine (PPD), bandrowski's (BB) base, HPLC estimation, henna based hair colour, coupler, alkaline pH

Citation: Jayaganesh Sankar, Sudhir Sawarkar, Zulfiqar Ali Muhammad and Jainendra Malakar, 2017. By HPLC method established powder henna's safety through identification of Bandrowski's Base. Asian J. Dermatol., 9: 7-22.

Corresponding Author: Jayaganesh Sankar, Dabur Research and Development, International Business Development, Dubai, P.O. Box 6399, United Arab Emirates Tel: +971-528769864

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The p-phenylenediamine used as a coloring agent in hair colour. At favorable condition the PPD molecule convert into dimer and trimer configuration, trimer configuration is called as a Bandrowski's Base and it was pronounced from 18th century onwards. Many scientists examined the PPD toxicity with respect to Bandrowski's Base formation[1-3]. Bandrowski's Base formation depends upon the alkaline pH condition, initial PPD concentration, higher concentration of hydrogen peroxide and alkalizing agent concentration. Higher pH favors the p-phenylenediamine dimer and trimer formation. Polymerization of PPD molecule by and large happen at higher alkaline pH i.e., greater than 10.0. The molecular formula of Bandrowski's Base is $C_{18}H_{18}N_6$ and CAS number is 20048-27-5. PPD allergy potential with respect to hair colour category exclusively studied by Krasteva *et al*.[4]. Azo dyes synthesis process started since 18th century onwards, however, in recent years also PPD used as a intermediate in printing ink preparation[1,2]. Many scientists exclusively studied about the aryl diamines (PPD), transformation, toxicity of aryl diamines (PPD), transformation, toxicity and *in vitro* mutagenic study etc completed[3,5,6]. Wu *et al*.[7] found that the allergic potential effect of PPD and Jerschow *et al*.[8] and Koopmans and Bruynzeel[9] exclusively found that the allergic potential of PPD. Henna has been used as a cosmetic hair dye for 6,000 years. The leaves are reduced to fine powder and mixed with water, to form a paste, which was applied to the hair to deliver the colour and conditioning to the hair. In recent years, the dye intermediate and oxidizing agent like sodium perborate (banned recently to be used in cosmetic)/barium peroxide also mixed with henna powder to deliver the desired colour to the hair[10-12]. According to SCCP opinion[13] and SCCS[14], para-phenylenediamine as a coloring agent for hair dyes and maximum concentration of on head concentration was 2%. Toxicological potential not caused by the pure form of aryl amines, but the toxicological potential raised due to the higher pH and oxidation agent[15]. In general peroxide oxidizing agents promote the PPD timer and dimer formation. Couplers are aromatic compound and it derived from benzene, substituted by atleast 2 electron donor groups such as NH_2 and OH in para or ortho positions to confer the property of easy oxidation, acting as a color developer[16]. Coulter *et al*.[17] studied the coupler component they found that addition of coupler prevent the toxic byproducts from aryl diamines. PPD transformation mechanism not clearly explained till date and topic remain open and research was ongoing. The pH of the dye mixture plays a crucial role in a permanent color mechanism, higher pH/alkaline medium promotes the opening of the cuticle and it beneficial to the penetration of dye molecules into the cortex in a fast manner. However, the initial reaction was triggered by the oxidizing agent and the reaction occurs in the cortex of the hair. Some reaction also takes place in the outer layer of the hair i.e., cuticle and it was easily removed by the first washing with shampoo[18,19]. Ammonium hydroxide, amino ethyl propanol and ethanolamines are regularly used as alkalizing agents. Similarly hydrogen peroxide, calcium dioxide, sodium per borate, barium peroxide act as an oxidizing agent. Hydrogen peroxide directly releases the oxygen molecule. However, the powder form of oxidizing agents like, calcium peroxide, barium peroxide, etc., releases the oxygen molecule whenever it reacts with water. Review of literature revealed that the formation of Bandrowski's Base formation depends upon the pH, alkaline condition, reaction times and concentration of alkali. Many scientists tested as an allergy indicator of hair coloring products in different formula. The European Surveillance System on Contact Allergies (ESSCA) collected the data in Europe region with respect to the PPD allergy potential. Survey results indicate that, the prevalence of contact allergy to PPD was 0.8% in 5 European countries. Present study aims to find out the formation of Bandrowski's Base in henna based powder hair colours. Present study aims to derive the optimum pH, alkaline condition and dosage, suitable oxidation agent and period to form a Bandrowski's Base. Many researchers studied out the influence of pH, influence of coupler, influence of alkali with respect to Bandrowski's Base. However, there was no compendium about the Bandrowski's Base study with respect to the henna based hair colour mechanism. This article will patch the grey area with respect to Bandrowski's Base formation in henna based powder form of oxidation hair dyes. This would helpful for establishing the allergy potential of Bandrowski's Base in henna based powder hair colour category. Main objective was to optimize the suitable pH to prevent the Bandrowski's Base formation and find out the optimum time for the Bandrowski's Base formation. It could be immense useful to formulate the safer henna based hair colour for the consumer usage purpose.

MATERIALS AND METHODS

Entire study and thought process was carried out between 2015 and 2017 at Dabur International Research and Development, Dubai, UAE and HPLC estimation was carried out at PSN Life Sciences International Lab, Dubai.

Synthesis of Bandrowski's Base: Bandrowski's Base was synthesized by the method described by Ritter and Schmitz[20]. About 5 g of PPD was dissolved in 375 mL of water and 1.5 mL of 28% ammonium hydroxide added and adjusted the pH to 9.5 and adds 62.5 mL of hydrogen peroxide (3%) and kept the solution under room condition for 24 h later. After 24 h filtered the solution and collected the crystalline form of Bandrowski's Base and confirmed through the melting point.

HPLC chromatograph peak evaluation: Synthesized BB base and standard PPD was estimated through HPLC with the combination of methanol Sorenson's buffer (pH = 8.0) ratio of 4:6 with C8 column. Standard and samples were prepared with methanol Sorenson's buffer (pH = 8.0) and injected.

Henna powder sample preparation: Powder based henna hair colour formula containing the dye intermediates, conditioning agent, oxidising agent, thickener and buffering agent and formulation details furnished in Table 1.

Initially heat the henna powder and claim herbal materials to 80-85°C and remove the moisture content then add the buffering agent, filler and antioxidant and mix for 5-10 min. Further add the dye intermediates and mix for 10 min. Finally add the oxidizing agent and mix for another 30 min.

Identification of Bandrowski's Base through HPLC method: Injected sample details are explained in the Table 2 and 3, sample A is an absolute control sample, as per pack instruction sample dissolved in water and further sample diluted with regular buffer solution and injected. Similarly the sample B was prepared with addition of hydrogen peroxide. pH of the sample C is 10.0 and it adjusted by ammonia and sample D pH is 12.00 and pH value adjusted with help of ammonia solution. Sample E and sample F prepared with higher concentration of ammonia and hydrogen peroxide solutions.

Pure analytical grade chemicals are used for the standard and Millipore water used for the dilution purpose.

Table 1: Formulation details of henna based hair colour

Materials	Functions of each raw materials
Henna powder	Conditioning the hair
Para phenylenediamine	Dye intermediates
Para amino phenol	Dye intermediates
Resorcinol	Coupler
Calcium dioxide	Oxidizing agent
Tartaric acid	Buffering agent
Sodium sulphite	Antioxidant
Magnesium carbonate	Adsorbent
Sodium carboxymethyl cellulose	Thickener
Claim herbal materials	Hair conditioning agent

Table 2: Injected and prepared sample details-henna based hair colour-black shade

Sample code	Sample and formulation details	pH	Sample developmental time (h)
Sample A	Henna based hair colour-BLACK+40 mL water	7.5	24, 48
Sample B	Henna hair colour-black+6 mL of H_2O_2 (3%)+34 mL water	7.7	24, 48
Sample C	Henna hair colour-black+6 mL of H_2O_2 (3%)+30 mL water and adjusted pH with NH_3 solution	10.0	24, 48
Sample D	Henna hair colour-black+6 mL of H_2O_2 (3%)+30 mL water and adjusted pH with NH_3 solution	12.0	24, 48
Sample E	Henna hair colour-black+12 mL of H_2O_2 (3%)+20 mL water and adjusted pH with NH_3 solution	10.0	24, 48
Sample F	Henna hair colour-black+12 mL of H_2O_2 (3%)+20 mL water and adjusted pH with NH_3 solution	12.0	24, 48

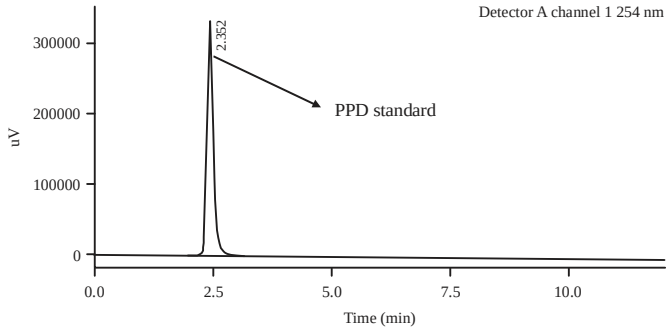
Table 3: Injected and prepared sample details-Henna based hair colour-dark brown

Sample code	Sample and formulation details	pH	Sample developmental time (h)
Sample A	Henna based hair colour-dark brown+40 mL water	7.5	24, 48
Sample B	Henna hair colour-dark brown+6 mL of H_2O_2 (3%)+34 mL water	7.7	24, 48
Sample C	Henna hair colour-dark brown+6 mL of H_2O_2 (3%)+30 mL water and adjusted pH with NH_3 solution	10.0	24, 48
Sample D	Henna hair colour-dark brown+6 mL of H_2O_2 (3%)+30 mL water and adjusted pH with NH_3 solution	12.0	24, 48
Sample E	Henna hair colour-dark brown+12 mL of H_2O_2 (3%)+20 mL water and adjusted pH with NH_3 solution	10.0	24, 48
Sample F	Henna hair colour-dark brown+12 mL of H_2O_2 (3%)+20 mL water and adjusted pH with NH_3 solution	12.0	24, 48

RESULTS

Various concentrations of standard PPD and BB base injected and identified the retention time. Combination of PPD and BB standard injected and the peaks showed the different retention time, PPD eluted at 2.352 min (Fig. 1). Retention time and BB base eluted at 4.696 min (Fig. 2). Combination of standard PPD and BB base injection further confirmed the different retention time. Similarly one more standard samples the combination of PPD and BB base injected for reconfirmation of BB base and PPD retention time (Fig. 3). In chromatograph 3, PPD peek observed at 2.352 min and BB base peak observed at 4.656 min. Linearity study will be carried out with higher concentration of PPD and BB base, the same retention time noticed in higher concentration and quantification results also matched with the theoretical values.

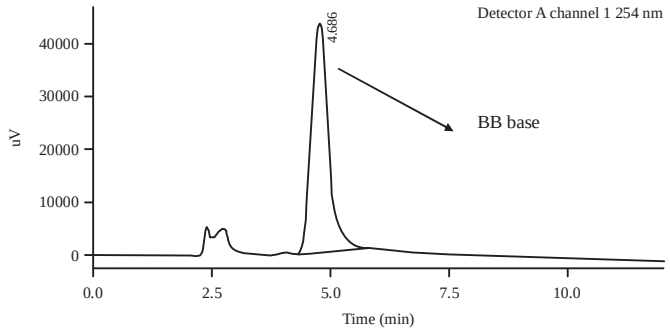
Review of literature revealed that the BB base formation depends upon the pH condition, alkali and hydrogen peroxide concentration. Sample B and sample F prepared with different concentrations of ammonia and hydrogen peroxide, prepared samples are kept up to 24 h. After 24 h the samples examined through HPLC for identification of Bandrowski's Base formation (Fig. 4a-f). Identification of BB base study confirms that henna based hair colour not promote the BB base formation up to 24 h. Similar kind of trend was noticed in dark brown shade also (Fig. 5a-f), however, in dark brown shade is the combination of PPD, para-amino phenol and resorcinol combination, it further helps to prevent the BB base formation. Samples are kept up to 48 h and estimated the BB base formation and results are summarized in the Fig. 6a-f (black shade) and Fig. 7a-f (dark brown shade).



Detector A channel 1 254 nm Peak Table

Peak#	Ret. time	Area	Height	Concentration	Unit	Name	Area (%)
1	2.352	3143617	332996	0.000	mg L^{-1}	PPD	100.000
Total		3143617	332996				100.000

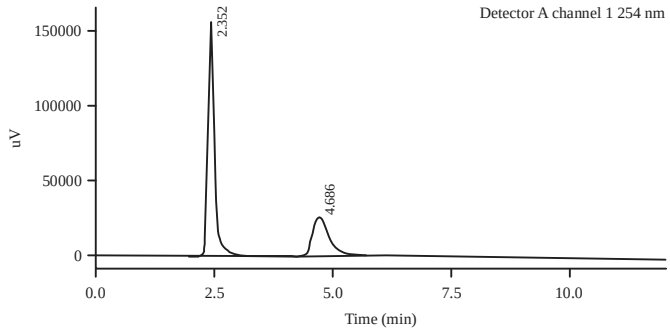
Fig. 1: HPLC estimation of p-phenylenediamine standard retention time



Detector A channel 1 254 nm Peak Table

Peak#	Ret. time	Area	Height	Concentration	Unit	Name	Area (%)
1	4.686	1150526	44665	0.000	mg L^{-1}	BB	100.000
Total		1150526	44665				100.000

Fig. 2: HPLC estimation of Bandrowski's Base (BB) standard retention time



Detector A channel 1 254 nm Peak Table

Peak#	Ret. time	Area	Height	Concentration	Unit	Name	Area (%)
1	2.352	1548886	157440	0.000	mg L^{-1}	PPD	70.631
2	4.686	644045	25713	0.000	mg L^{-1}	BB	29.369
Total		2192931	183153				100.000

Fig. 3: HPLC estimation of combination of Bandrowski's Base and p-phenylenediamine standard retention time

DISCUSSION

Study results revealed that there was no Bandrowski's Base peak observed across the samples and the reason was powder based henna hair colour containing the coupler the coupler may prevent the PPD dimer and trimer formation reaction. Similar kind of results already noticed by Coulter *et al.*[1], they exclusively studied the influence of coupler on prevention of aryl diamines. However, many authors found that[1,16,17,21] electron donating group presented in the para position prevent the oxidation of dye intermediate. Similar kind of observations noticed in this article. Regular application of henna based hair colour does not show any Bandrowski's Base peak by HPLC estimation study. It confirms that incorporation of coupler prevent the Bandrowski's Base formation.

It may assumes that henna based powder hair colours are considered as a safer when compared to the cream based oxidation hair colour, because cream based hair colours are containing the ammonia and hydrogen peroxide. In powder form of hair colour containing the powder oxidation releaser and it reacts with water slowly, release the oxygen molecules helps to oxidise the PPD molecule and electron donating molecule/modifier. According to the formula (Table 1), there was no alkali source in the formula and pH of the product was between 6.5 and 7.5. Reaction mechanisms are as below:

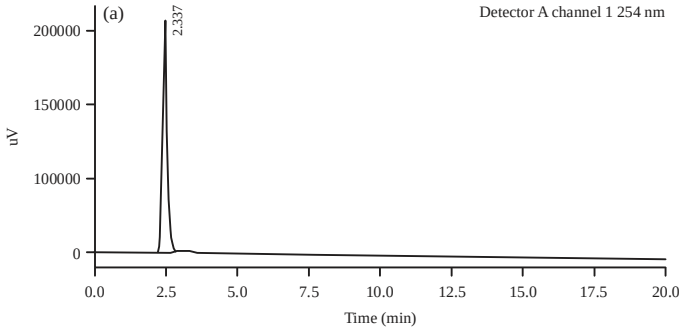
$$2CaO_2+2H_2O \longrightarrow 2Ca(OH)_2+O_2\uparrow$$

Many scientists proved that the pH plays a crucial role in the Bandrowski's Base formation. Sosted *et al.* [22,23] exclusively studied that the severe allergen hair dye with various pH interval and authors concluded that the higher pH accelerate the allergic reaction because in higher pH, favourable the coupling or polymerisation of primary dye intermediate happens faster than the reaction of coupler.

General hair colour (Black) reaction mechanism furnished in Fig. 8. In Step 1, paraphenylenediamine under-going oxidation to form a diamine derivative further it reacts with resorcinol (Step 2) and forms an indophenol complex. In Step 2, electrophilic species attacks a resorcinol anion, para to the phenolic group and forming the compound, which was under go oxidation and form a indophenol complex in step 3. Indo phenol further reacts with another one indophenol complex form a brown polymeric poly indophenol derivatives, similarly indophenols react with paraphenylenediamine and form a trinuclear green pigment and it further reacts with another mole of indophenol and delivers a brown polymeric poly indophenols.

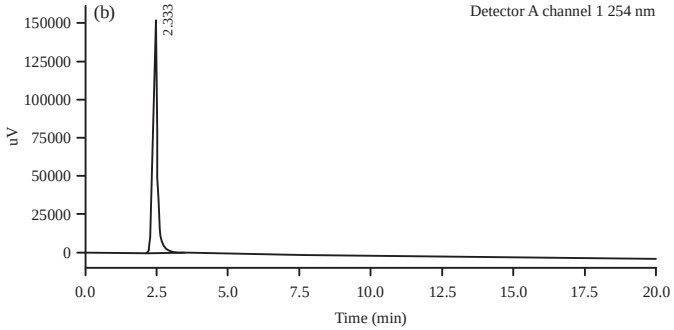
Entire reaction mentioned above mechanism desired by the concentration of paraphenylenediamine, resorcinol, the oxidizing agent and pH. The pH plays a crucial role in above-mentioned chemical reaction. Overall HPLC estimation study revealed that henna based powder hair colour does not contributing any Bandrowski's Base peak formation at the pH between 7.0 and 8.00 with 24 and 48 h developmental time. It confirms that neutral pH prevent the self coupling of PPD. Meyer and Fischer[24] reported that reactivity of PPD, colour change/oxidation of PPD are pH dependent. The reaction found that aerial oxidation (self) of PPD takes long time, initially it turns from brown into dark brown colour and continuously turns into the black brown colour. Similar kind of observations noticed by the many scientists[21,25,26] however, in henna based powder colour also observed the same trend in

this study, i.e., up to 48 h there was no formation of Bandrowski's Base formation. In addition to that addition of resorcinol also plays a crucial role to prevent the BB base formation. Quinone-diimine react with coupler resorcinol to form the indophenol complex (green component) further it under go coupling with PPD or resorcinol or co-oxidation



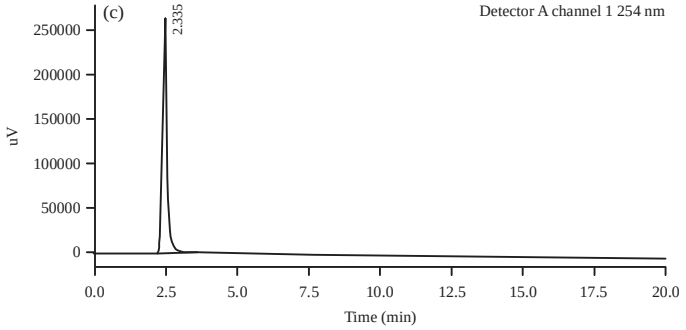
Detector A channel 1 254 nm Peak Table

Peak#	Ret. time	Area	Height	Concentration	Unit	Makr	Name
1	2.337	2170856	209645	0.000	mg L^{-1}	M	PPD
Total		2170856	209645				



Detector A channel 1 254 nm Peak Table

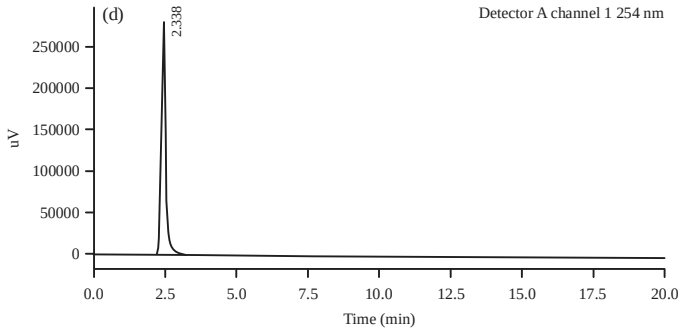
Peak#	Ret. time	Area	Height	Concentration	Unit	Makr	Name
1	2.333	1609888	152578	0.000	mg L^{-1}	M	PPD
Total		1609888	152578				



Detector A channel 1 254 nm Peak Table

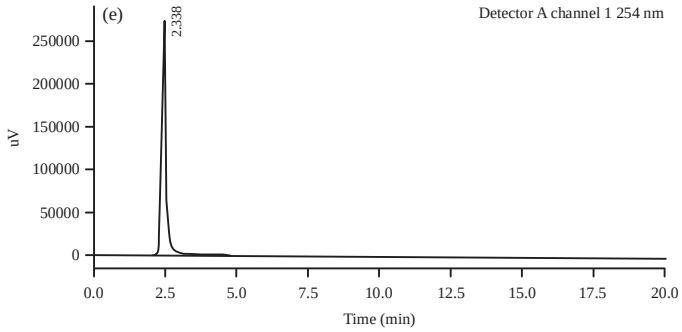
Peak#	Ret. time	Area	Height	Concentration	Unit	Makr	Name
1	2.335	2773899	262094	0.000	mg L^{-1}	M	PPD
Total		2773899	262094				

Fig. 4(a-f): Continue



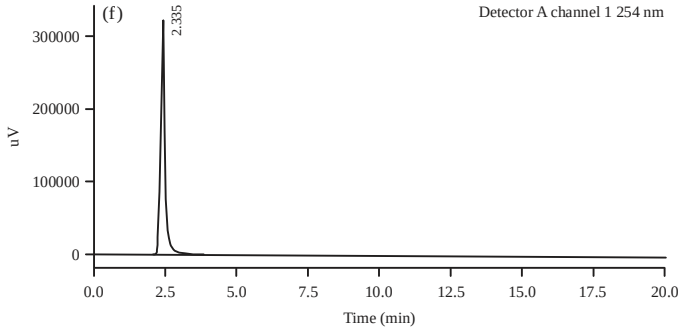
Detector A channel 1 254 nm Peak Table

Peak#	Ret. time	Area	Height	Concentration	Unit	Name
1	2.338	2932758	279022	0.000	mg L^{-1}	PPD
Total		2932758	279022			



Detector A channel 1 254 nm Peak Table

Peak#	Ret. time	Area	Height	Concentration	Unit	Makr	Name
1	2.335	2883042	273263	0.000	mg L^{-1}	M	PPD
Total		2883042	273263				



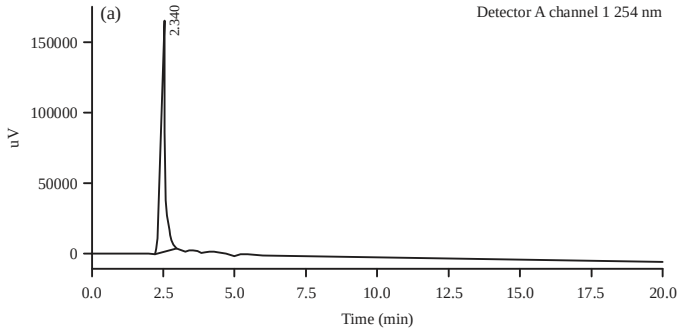
Detector A channel 1 254 nm Peak Table

Peak#	Ret. time	Area	Height	Concentration	Unit	Makr	Name
1	2.335	3471120	323257	0.000	mg L^{-1}	M	PPD
Total		3471120	323257				

Fig. 4(a-f): Identification of Bandrowski's Base formation-after 24 h in henna based hair colour black shade. (a) Henna based hair colour-black+40 mL water. Sample A at pH 7.5, (b) Henna hair colour-black+6 mL of H_2O_2 (3%)+34 mL water. Sample B at pH 7.7, (c) Henna hair colour-black+6 mL of H_2O_2+30 mL water and adjusted with NH_3 solution. Sample C at pH 10.0, (d) Henna hair colour-black+6 mL of H_2O_2 (3%)+30 mL water and adjusted with NH_3 solution. Sample D at pH 12.0, (e) Henna hair colour-black+12 mL of H_2O_2 (3%)+30 mL water and adjusted with NH_3 solution. Sample E at pH 10.0 and (f) Henna hair colour-black+12 mL of H_2O_2 (3%)+30 mL water and adjusted with NH_3 solution. Sample F at pH 12.0

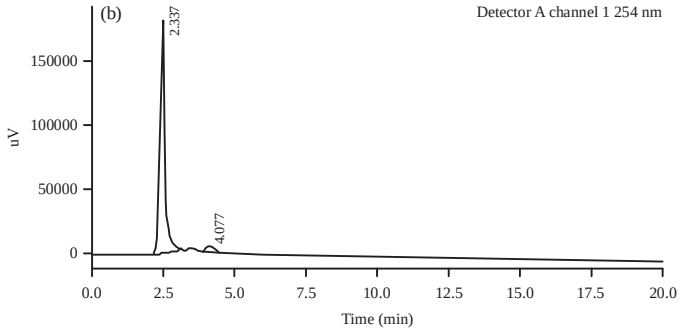
There is no evidence for Bandrowski's Base formation peak after 12 h in black shade treated with different pH condition

coupling it form a poly indophenol complex. It was the brownish black colour polymer and it delivers the brown black colour to the hair. The reaction mechanism clearly indicates that the addition of resorcinol prevents the BB base and promotes poly indophenol complex preparation and the reaction pathway was proved by the HPLC estimation. Entire



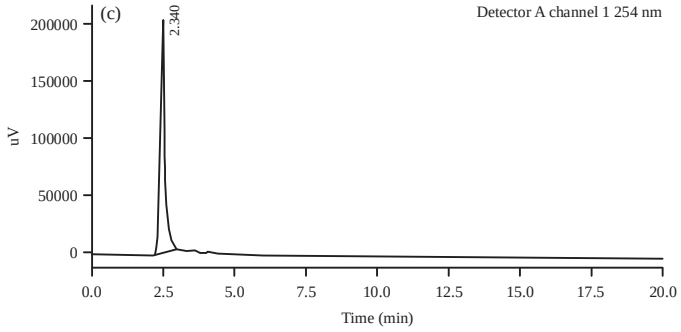
Detector A channel 1 254 nm Peak Table

Peak#	Ret. time	Area	Height	Concentration	Unit	Name
1	2.340	1839473	165908	0.000	mg L^{-1}	PPD
Total		1839473	165908			



Detector A channel 1 254 nm Peak Table

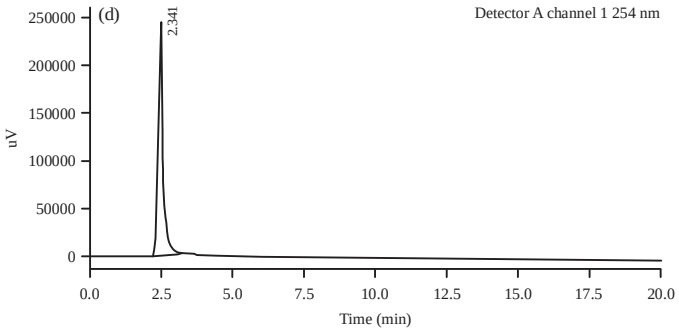
Peak#	Ret. time	Area	Height	Concentration	Unit	Name
1	2.337	2117067	181288	0.000	mg L^{-1}	PPD
2	4.077	99564	4487	0.000	mg L^{-1}	
Total		2216631	185776			



Detector A channel 1 254 nm Peak Table

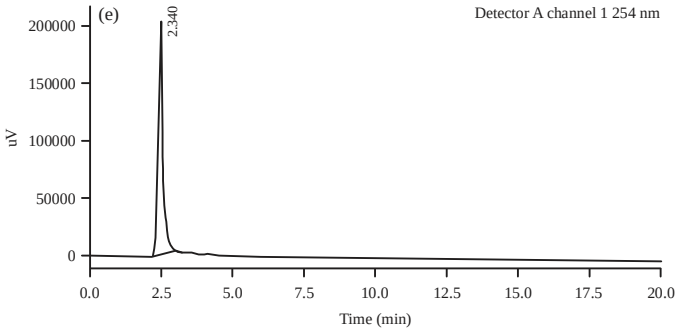
Peak#	Ret. time	Area	Height	Concentration	Unit	Name
1	2.340	2229502	202309	0.000	mg L^{-1}	PPD
Total		2229502	202309			

Fig. 5(a-f): Continue



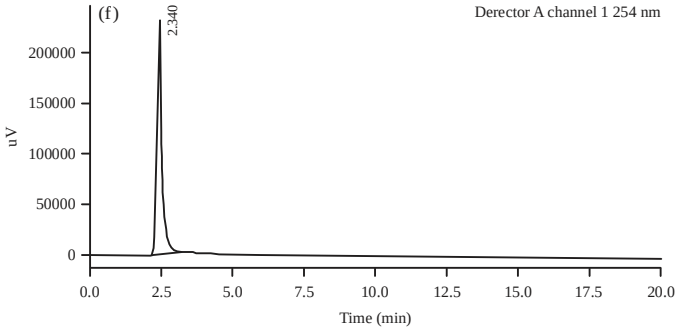
Detector A channel 1 254 nm Peak Table

Peak#	Ret. time	Area	Height	Concentration	Unit	Name
1	2.341	3039842	245597	0.000	mg L^{-1}	PPD
Total		3039842	245597			



Detector A channel 1 254 nm Peak Table

Peak#	Ret. time	Area	Height	Concentration	Unit	Name
1	2.340	2229502	202309	0.000	mg L^{-1}	PPD
Total		2229502	202309			

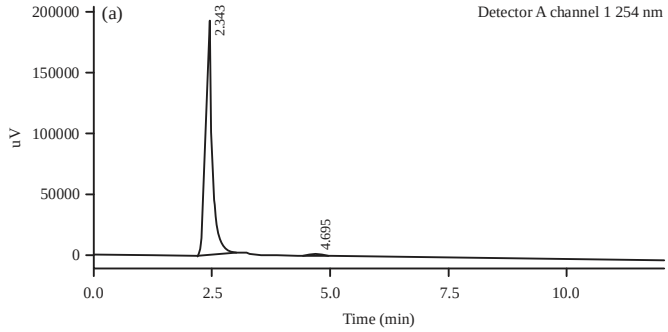


Detector A channel 1 254 nm Peak Table

Peak#	Ret. time	Area	Height	Concentration	Unit	Name
1	2.340	2719885	232600	0.000	mg L^{-1}	PPD
Total		2719885	232600			

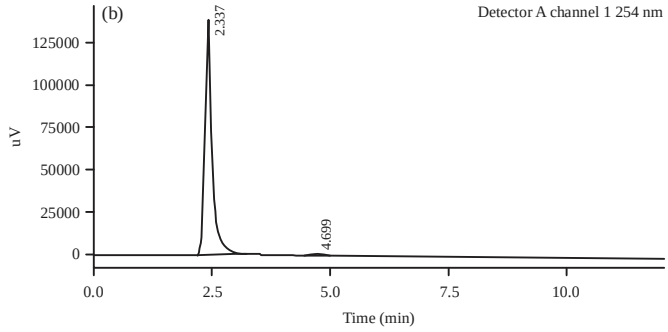
Fig. 5(a-f): Identification of Bandrowski's Base formation after 24 h in henna based hair colour dark brown. (a) There is no Bandrowski's Base formation peak. Henna based hair colour-dark brown+40 mL water. Sample A at pH 7.5, (b) Henna hair colour-dark brown+6 mL of H_2O_2 (3%)+34 mL water. Sample B at pH 7.7, (c) Henna hair colour-dark brown+6 mL of H_2O_2 (3%)+30 mL water and adjusted with NH_3 solution. Sample C at pH 10.0, (d) Henna hair colour-dark brown+ 6 mL of H_2O_2 (3%)+30 mL water and adjusted with NH_3 solution, adjusted pH at 12.0, (e) Henna hair colour-dark brown+12 mL of H_2O_2 (3%)+30 mL water and adjusted with NH_3 solution. Sample E at pH 10.0 and (f) Henna hair colour-dark brown+12 mL of H_2O_2 (3%)+30 mL water and adjusted with NH_3 solution. Sample F at pH 12.0
There is no Bandrowski's Base formation peak after 12 h in dark brown sample treated with different pH condition

study revealed that up to 24 h developmental time there was no BB base formation with a higher amount of alkali, presence system also, however, the henna based powder hair colour does not containing ammonia and hydrogen peroxide, it confirms that the products are safe to use.



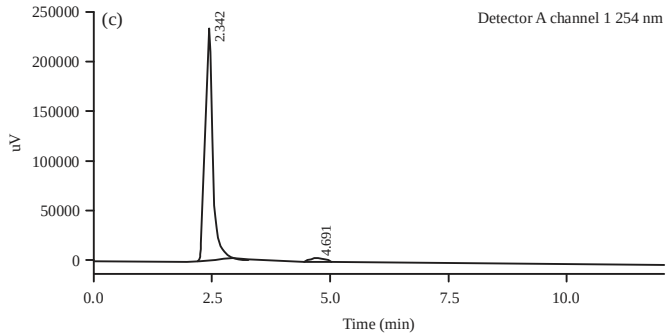
Detector A channel 1 254 nm Peak Table

Peak#	Ret. time	Area	Height	Concentration	Unit	Name	Area (%)
1	2.343	2022907	191789	0.000	mg L^{-1}	PPD	98.112
2	4.695	38923	1757	0.000	mg L^{-1}		1.888
Total		2061830	193546				100.000



Detector A channel 1 254 nm Peak Table

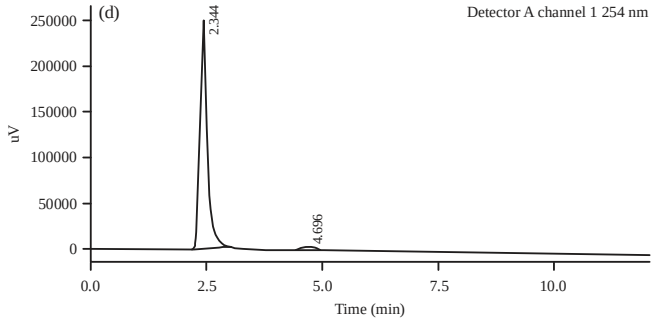
Peak#	Ret. time	Area	Height	Concentration	Unit	Name	Area (%)
1	2.343	1511661	137705	0.000	mg L^{-1}	PPD	98.751
2	4.695	19115	853	0.000	mg L^{-1}	BB	1.249
Total		1530776	138558				100.000



Detector A channel 1 254 nm Peak Table

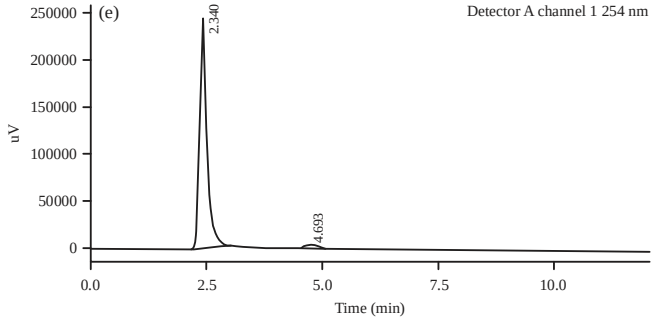
Peak#	Ret. time	Area	Height	Concentration	Unit	Name	Area (%)
1	2.342	2478217	235642	0.000	mg L^{-1}	PPD	96.926
2	4.691	78603	3423	0.000	mg L^{-1}	BB	3.074
Total		2556820	239066				100.000

Fig. 6(a-f): Continue



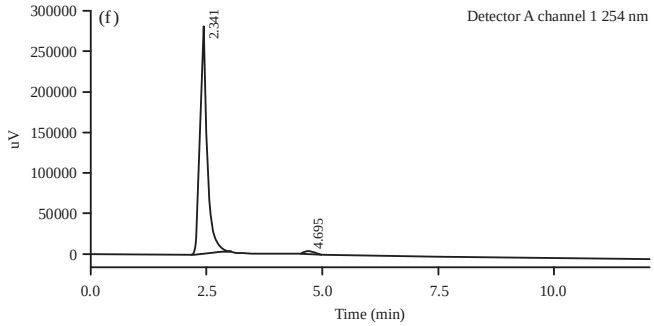
Detector A channel 1 254 nm Peak Table

Peak#	Ret. time	Area	Height	Concentration	Unit	Name	Area (%)
1	2.344	2656426	249166	0.000	mg L^{-1}	PPD	96.477
2	4.696	97002	4125	0.000	mg L^{-1}		3.523
Total		2753428	253291				100.000



Detector A channel 1 254 nm Peak Table

Peak#	Ret. time	Area	Height	Concentration	Unit	Name	Area (%)
1	2.340	2579612	244713	0.000	mg L^{-1}	PPD	96.488
2	4693	93895	4071	0.000	mg L^{-1}	BB	3.512
Total		2673507	248784				100.000



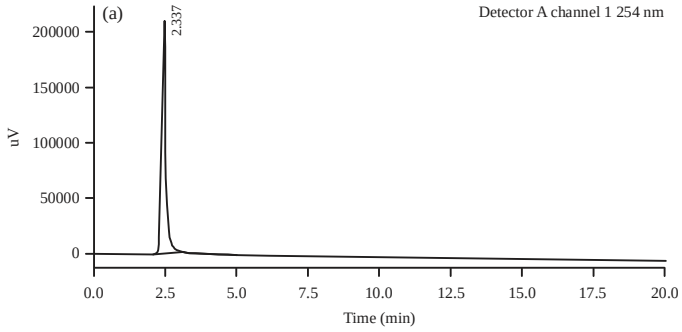
Detector A channel 1 254 nm Peak Table

Peak#	Ret. time	Area	Height	Concentration	Unit	Name	Area (%)
1	2.341	3024478	284637	0.000	mg L^{-1}	PPD	96.575
2	4.695	107274	4626	0.000	mg L^{-1}	BB	3.425
Total		3131750	289263				100.000

Fig. 6(a-f): Identification of Bandrowski's Base formation after 48 h in henna based hair colour black shade, (a) Henna based hair colour-black+40 mL water. Sample A at pH 7.5, (b) Henna hair colour-black+6 mL of H_2O_2 (3%)+34 mL water. Sample B at pH 7.7, (c) Henna hair colour-black+6 mL of H_2O_2 (3%)+30 mL water and adjusted with NH_3 solution. Sample C at pH 10.0, (d) Henna hair colour-black+6 mL of H_2O_2 (3%)+30 mL water and adjusted with NH_3 solution. Sample D at pH 12.0, (e) Henna hair colour-Black+12 mL of H_2O_2(3%)+30 mL water and adjusted with NH_3 solution. Sample E at pH 10.0 and (f) Henna hair colour-black+12 mL of H_2O_2 (3%)+30 mL water and adjusted with NH_3 solution. Sample F at pH 12.0

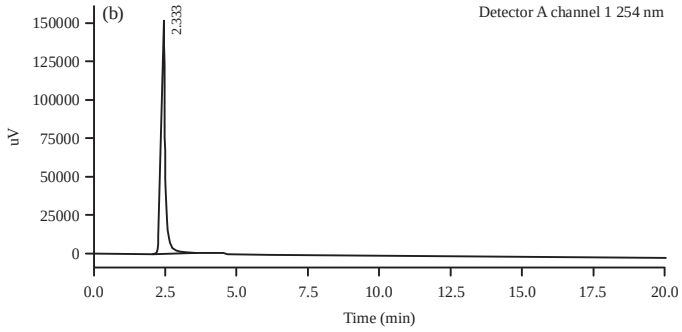
(a) Trace quantity of BB base observed in HPLC peak, it revealed that samples kept up to 48 h with at pH7, itself promote the BB base formation, (b) Trace quantity of BB base observed in HPLC peak, it revealed that samples kept up to 48 h with at pH 7 with presence of hydrogen peroxide alone itself promote the BB base formation, (c) Trace quantity of BB base observed in HPLC peak, it revealed that samples kept up to 48 h with at pH 10 with presence of hydrogen peroxide and ammonia, (d) Trace quantity of BB base observed in HPLC peak, it revealed that samples kept up to 48 h with at pH 12 with presence of hydrogen peroxide and ammonia, (e) Trace quantity of BB base observed in HPLC peak, it revealed that samples kept up to 48 h with at pH 10 with higher concentration presence of hydrogen peroxide and ammonia and (f) Trace quantity of BB base observed in HPLC peak, it revealed that samples kept up to 48 h with at pH 10 with higher concentration presence of hydrogen peroxide and ammonia

About 48 h injected samples results revealed that, traces of BB base peak observed in black sample with respect to the all treatments including control samples. Coutler *et al.*[17] studied the coupler component they found that addition of coupler prevent the toxic by-products from aryl diamines. However, the addition of coupler does not completely scavenge the toxic potential and it scavenged to some extent. Study results revealed that BB base formation depends upon



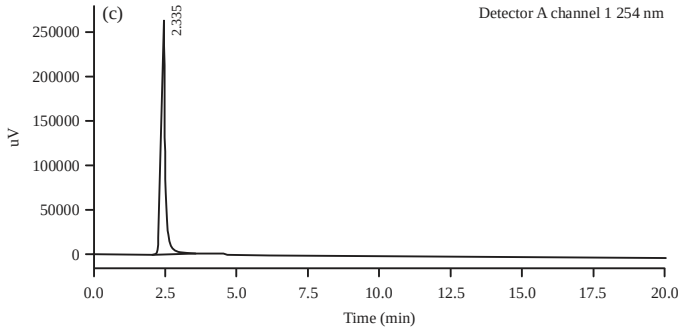
Detector A channel 1 254 nm Peak Table

Peak#	Ret. time	Area	Height	Concentration	Unit	Makr	Name
1	2.337	2170856	209645	0.000	mg L^{-1}	M	PPD
Total		2170856	209645				



Detector A channel 1 254 nm Peak Table

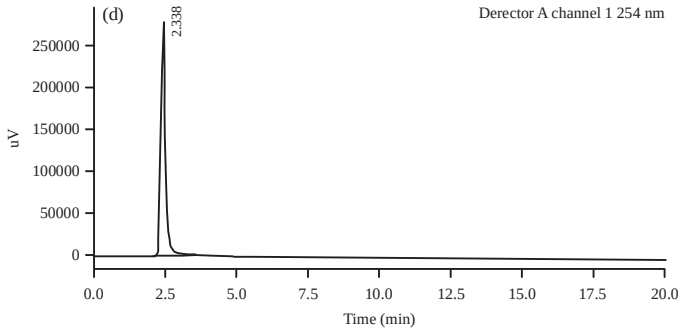
Peak#	Ret. time	Area	Height	Concentration	Unit	Makr	Name
1	2.333	1609888	152578	0.000	mg L^{-1}	M	PPD
Total		1609888	152578				



Detector A channel 1 254 nm Peak Table

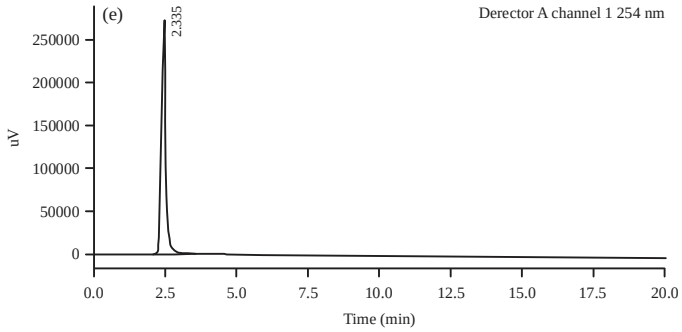
Peak#	Ret. time	Area	Height	Concentration	Unit	Makr	Name
1	2.335	2773899	262094	0.000	mg L^{-1}	M	PPD
Total		2773899	262094				

Fig. 7(a-f): Continue



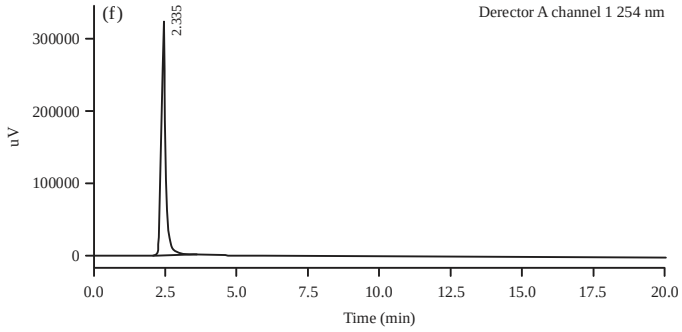
Detector A channel 1 254 nm Peak Table

Peak#	Ret. time	Area	Height	Concentration	Unit	Makr	Name
1	2.338	2932758	279022	0.000	mg L^{-1}	M	PPD
Total		2932758					



Detector A channel 1 254 nm Peak Table

Peak#	Ret. time	Area	Height	Concentration	Unit	Makr	Name
1	2.335	2883042	273263	0.000	mg L^{-1}	M	PPD
Total		2883042	273263				



Detector A channel 1 254 nm Peak Table

Peak#	Ret. time	Area	Height	Concentration	Unit	Makr	Name
1	2.335	3471120	323257	0.000	mg L^{-1}	M	PPD
Total		3471120	323257				

Fig. 7(a-f): Identification of Bandrowski's Base formation-after 48 h in henna based hair colour dark brown shade, (a) Henna based hair colour-dark brown+40 mL water. Sample A at pH 7.5, (b) Henna hair colour-dark brown+6 mL of H_2O_2 (3%)+34 mL water. Sample B at pH 7.7, (c) Henna hair colour-dark brown+6 mL of H_2O_2 (3%)+30 mL water and adjusted with NH_3 solution. Sample C at pH 10.0, (d) Henna hair colour-dark brown+6 mL of H_2O_2 (3%)+30 mL water and adjusted with NH_3 solution. Sample D at pH 12.0, (e) Henna hair colour-dark brown+12 mL of H_2O_2 (3%)+30 mL water and adjusted with NH_3 solution. Sample E at pH 10.0 and (f) Henna hair colour-dark brown+12 mL of H_2O_2 (3%)+30 mL water and adjusted with NH_3 solution. Sample F at pH 12.0

No traces of Bandrowski's Base has been identified after 48 h in dark brown sample treated with different pH condition

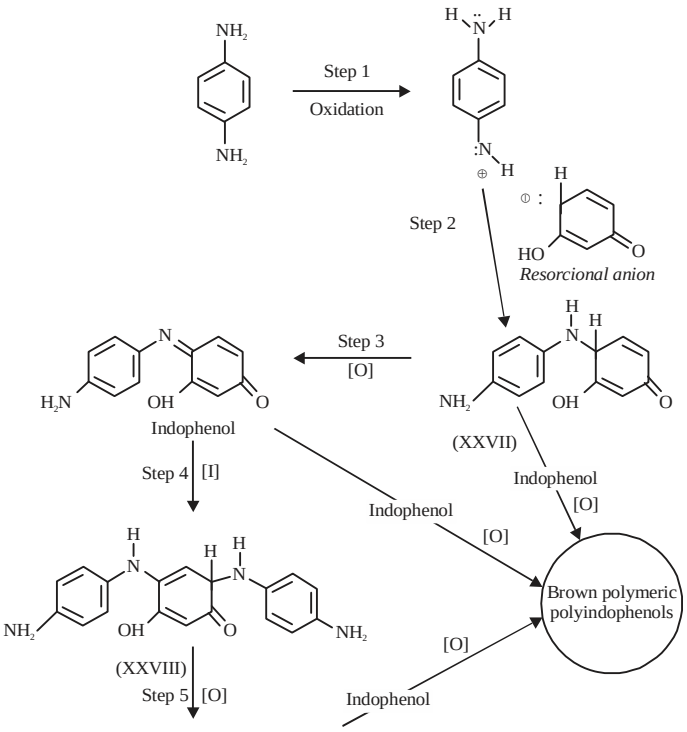
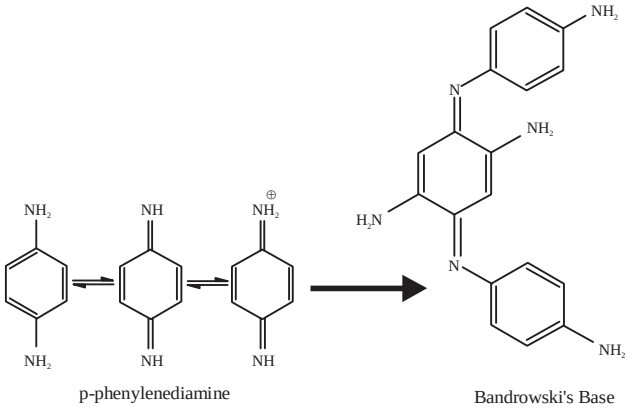


Fig. 8: Schematic diagram of paraphenylenediamine reaction resorcinol

the time and suitable pH condition. Henna based hair colour delivers the colour in 30 min and the study confirms that natural black and dark brown shades are safer to use with that exposure time.

The molecular formula of Bandrowski's Base is $C_{18}H_{18}N_6$ and CAS number is 20048-27-5 and reaction is as below:



CONCLUSION

Henna based hair colour with higher pH 10-12.0 and 30 min developmental time does not contribute the formation of Bandrowski's Base. However, henna based hair colour products pH fall between 7.0-8.0, it further confirms that there is no BB formation and safe to use. No evidence for the formation of Bandrowski Base after 24 h with the treatment of higher ammonia and hydrogen peroxide. However, in black shade we have noticed the Bandrowski Base formation after keeping the solution for 48 h with higher pH (acidic buffer>8.0). Overall study confirms that the development time play a crucial role in the Bandrowski's Base formation.

SIGNIFICANCE STATEMENTS

This study covers the gray area of PPD toxicity in henna based powder hair colour segments. This study confirms that the henna based powder hair colour does not contribute any adverse or toxic effects to the consumer and safe to use. The HPLC estimation of Bandrowski's Base is immense useful to extrapolate the toxic potential of PPD in powder based henna hair colour. The HPLC estimation confirms that there is no formation of BB base in henna based powder hair colour with 30 min developmental time. Study confirms that the samples prepared with coupler i.e., electron donating group prevent the self coupling of PPD with the presence of higher concentration of alkali in 30 min developmental time. Overall

study clearly indicates that favorable pH, alkali condition and development time plays a crucial role in PPD polymerization and toxic Bandrowski's Base formation. This article acts a guidelines to formulate the henna based powder colour in safer manner.

ACKNOWLEDGMENTS

Authors thank to Mr. Mohit Malhotra, CEO, Mr. Vivek Dhir, Head of Marketing and Mr. Jude Linhares, head of operation for their constant encouragement and support for the study. Authors are also thankful to M/S. The PSN Life Sciences Intentional Lab, Dubai for support the instrument analysis part.

REFERENCES

1. Coulter, E.M., C. Jenkinson, Y. Wu, J. Farrell and B. Foster *et al*., 2008. Activation of T-cells from allergic patients and volunteers by p-phenylenediamine and Bandrowski's base. J. Invest. Dermatol., 128: 897-905.
2. Jenkinson, C., R.E. Jenkins, J.L. Maggs, N.R. Kitteringham, M. Aleksic, B.K. Park and D.J. Naisbitt, 2009. A mechanistic investigation into the irreversible protein binding and antigenicity of p-phenylenediamine. Chem. Res. Toxicol., 22: 1172-1180.
3. Nohynek, G.J., D. Duche, A. Garrigues, P.A. Meunier, H. Toutain and J. Leclaire, 2005. Under the skin: Biotransformation of para-aminophenol and para-phenylenediamine in reconstructed human epidermis and human hepatocytes. Toxicol. Lett., 158: 196-212.
4. Krasteva, M., B. Bons, C. Ryan and F.G. Gerberick, 2009. Consumer allergy to oxidative hair coloring products: Epidemiologic data in the literature. Dermatitis, 20: 123-141.
5. Kirkland, D.J., L. Henderson, D. Marzin, L. Muller and J.M. Parry *et al*., 2005. Testing strategies in mutagenicity and genetic toxicology: An appraisal of the guidelines of the European Scientific Committee for Cosmetics and Non-Food Products for the evaluation of hair dyes. Mutat. Res./Genet. Toxicol. Environ. Mutagen., 588: 88-105.
6. Yu, M.C., P.L. Skipper, S.R. Tannenbaum, K.K. Chan and R.K. Ross, 2002. Arylamine exposures and bladder cancer risk. Mutat. Res./Fund. Mol. Mech. Mutagen., 506-507: 21-28.
7. Wu, Y., F. Jiang, L. Chen, J. Zheng and Z. Deng *et al*., 2011. Determination of phenylenediamine isomers in hair dyes by coal cinders micro-column extraction and MEKC. Anal. Bioanal. Chem., 400: 2141-2147.
8. Jerschow, E., J.J. Hostynek and H.I. Maibach, 2001. Allergic contact dermatitis elicitation thresholds of potent allergens in humans. Food Chem. Toxicol., 39: 1095-1108.
9. Koopmans, A.K. and D.P. Bruynzeel, 2003. Is PPD a useful screening agent? Contact Dermatitis, 48: 89-92.
10. Alam, M.M., M.L. Rahman and M.Z. Haque, 2007. Extraction of henna leaf dye and its dyeing effects on textile fibre. Bangladesh J. Scient. Ind. Res., 42: 217-222.
11. Jayaganesh, S., M. Jainendra, B.S. Rawat and A.P. Venkataramana, 2016. Influence of gamma radiation on microbial and viscosity parameters of henna based hair colour. Int. J. New Technol. Res., 2: 11-15.
12. Gediya, S.K., B.R. Mistry, K.U. Patel, M. Blessy and N.H. Jain, 2011. Herbal Plants: Used as a cosmetics. J. Nat. Prod. Plant Resour., 1: 24-32.
13. European Commission, 2009. Opinion on Intermediates and reaction products of oxidative hair dye ingredients formed during hair dyeing. SCCP/1198/08, Scientific Committee on Consumer Products (SCCP), Brussels. https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_162.pdf
14. European Commission, 2012. Opinion on p-phenylenediamine. SCCS/1443/11, Scientific Committee on Consumer Products (SCCP), Brussels. https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_094.pdf
15. Karlberg, A.T., D. Basketter, A. Goossens and J.P. Lepoittevin, 1999. Regulatory classification of substances oxidized to skin sensitizers on exposure to air. Contact Dermatitis, 40: 183-188.
16. Morel, O., R.M. Christie, A. Greaves and K.M. Morgan, 2008. Enhanced model for the diffusivity of a dye molecule into human hair fibre based on molecular modelling techniques. Colorat. Technol., 124: 301-309.
17. Coulter, E.M., J. Farrell, K.L. Mathews, J.L. Maggs and C.K. Pease *et al*., 2007. Activation of human dendritic cells by p-phenylenediamine. J. Pharmacol. Exp. Ther., 320: 885-892.
18. Bolduc, C. and J. Shapiro, 2001. Hair care products: Waving, straightening, conditioning and coloring. Clin. Dermatol., 19: 431-436.
19. Kojima, T., H. Yamada, T. Yamamoto, Y. Matsushita and K. Fukushima, 2013. Dyeing regions of oxidative hair dyes in human hair investigated by nanoscale secondary ion mass spectrometry. Colloids Surfaces B: Biointerfaces, 106: 140-188.
20. Ritter, J.J. and G.H. Schmitz, 1929. The constitution of Bandrowski's base. J. Am. Chem. Soc., 51: 1587-1589.
21. Jahn, S., H. Faber, R. Zazzeroni and U. Karst, 2012. Electrochemistry/mass spectrometry as a tool in the investigation of the potent skin sensitizer p-phenylenediamine and its reactivity toward nucleophiles. Rapid Commun. Mass Spectrom., 26: 1453-1464.

22. Sosted, H., J.D. Johansen, K.E. Andersen and T. Menne, 2006. Severe allergic hair dye reactions in 8 children. Contact Dermatitis, 54: 87-91.
23. Sosted, H., T. Rustemeyer, M. Goncalo, M. Bruze and A. Goossens *et al*., 2013. Contact allergy to common ingredients in hair dyes. Contact Dermatitis, 69: 32-39.
24. Meyer, A. and K. Fischer, 2015. Oxidative transformation processes and products of para-phenylenediamine (PPD) and para-toluenediamine (PTD)-a review. Environ. Sci. Eur., Vol. 27. 10.1186/s12302-015-0044-7.
25. Ahmed, H.A.M., R.M.A. Maaboud, F.F.A. Latif, A.M.K. El-Dean, K.M. El-Shaieb, E. Vilanova and C. Estevan, 2013. Different analytical methods of para-phenylenediamine based hair dye. J. Cosmet. Dermatol. Sci. Applic., 3: 17-25.
26. Burnett, C.L., W.F. Bergfeld, D.V. Belsito, C.D. Klaassen and J.G. Marks *et al*., 2010. Final amended report of the safety assessment of toluene-2,5-diamine, toluene-2,5-diamine sulfate and toluene-3,4-diamine as used in cosmetics. Int. J. Toxicol., 29: 61S-83S.