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Detection and Determination of Aromatic Amines as Products of Reductive Splitting from Selected Azo Dyes

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The current environment-friendly regulations concerning textile products ban the marketing of textiles dyed with azo dyes capable of reductively splitting carcinogenic aromatic amines. The study analyzes seven azo dyes whose chemical structure determines various quantities of splitting aromatic amines, such as benzidine. For tests, seven commercially available azo dyes with aromatic amines in their structure were selected. These included two hazardous dyes: Acid Red 85 and Direct Blue 6, both capable of reductively splitting carcinogenic benzidine. Of the remaining five azo dyes, three—Ponceau SS, Sudan II, and Disperse Yellow 7—are capable of splitting *p*-phenylenediamine and aniline, while Mordant Orange 1 and Disperse Orange 3 can split only *p*-phenylenediamine. For Acid Red 85 and Direct Blue 6, the quantity of benzidine split from them was analyzed, depending on the conditions of the reduction process (e.g., in the HPLC method, 104 g/kg of dye for reduction in NaOH, and 41 g/kg of dye for reduction in acetate buffer). The spectrophotometric method proved useful for preliminary analysis of amine content in examined samples. Spectrophotometric analysis may be used to determine the total content of amines counted as aniline. A full qualitative and quantitative analysis of amines released from azo dyes is possible using high-performance liquid chromatography (HPLC). © 2002 Elsevier Science (USA)

Key Words: **azo dye; aromatic amines; spectrophotometric method; HPLC.**

INTRODUCTION

Publications in the literature [1, 3, 4, 11] have set forth evidence that azo dyes can pose threats to public health. It is claimed that textile chemicals not only act as allergens but also exhibit toxic and even mutagenic or carcinogenic properties [2]. The sources of mutagenic activity are the dyestuffs and the amines contained in their chemical structures. The literature [3, 4] quotes opinions that the dyestuffs themselves provide only a minor part of the mutagenic potential, and for many years work has been conducted to draw up lists of dangerous azo dyes and amines [3, 22, 23]. The result is a systematic removal from the market of those dyes that are suspected to have carcinogenic or mutagenic influence on humans. The main hazard criterion for a dye is its ability to split reductively aromatic amines in contact with sweat, saliva, or gastric juice [12]. The process of the reduction of azo dyes with the cleavage of aromatic $R–NH_2$ amines is one of the means of degradation of those dyes.

Figure 1 illustrates how amines are split from an azo dye. Other methods of splitting amines from dyestuffs are photodegradation and biodegradation by means of hydroxylation, oxidation, or hydrolysis. However, in humans, the biological reduction of an azo dye is responsible for the possible presence of toxic amines in organism. Aromatic amines are specified [12, 23, 20] in groups III A1 and III A2 of the Maximale Arbeintsplatz Konzentration (MAK) list as well as in International Agency for Research on Cancer (IARC) and Ecological and Toxicological Association of the Dyestuffs Manufacturing (ETAD) lists (e.g., benzidine, *o*-toluidine, 4-aminodiphenyl). The ability to form any of the listed carcinogenic amines through the cleavage of one or more azo groups is the reason for classification as a banned azo dye.

Legally, the issue of toxic chemicals (including aromatic amines that split from dyestuffs or dyed textiles) has been regulated by the European Union directive [6]. However, according to the European Union Law, each member state may define its own regulations to protect people's health. Thus, first Germany [5] and then other European Union countries approved bans on importing and marketing textiles dyed with dyes capable of reductively splitting carcinogenic amines. In Poland also [5], efforts are being made to pass a relevant act complying with the European Union directives. The danger to the user of a dyed product is caused not only by the hazardous dye itself, but also by the aromatic amine that splits off.

Regarding textile products, European Union law provides a list of hazardous substances and also specifies their maximum acceptable quantities and obligatory detection

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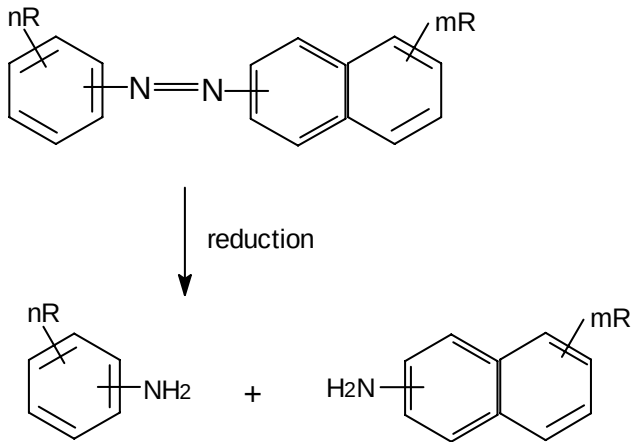


FIG. 1. Reductive splitting of an aromatic amine from an azo dye.

methods. It is assumed that the legally allowed concentrations of aromatic amines in textiles should be determined by means of the TLC, GC/MS, or HPLC methods [23, 7, 13, 14], and these are currently the routine procedures. It should be stressed, though, that those regulations are binding only for the European Union countries, and the limits do not have to be observed in Asian countries [19] or in East European markets.

It is therefore necessary to seek alternative, inexpensive methods of chemical analysis of carcinogenic amines in dyestuffs or dyed textiles. The aim is to be able to quickly detect those products that do not conform to the Chart of Substance Safety Characteristics. The Chart is an attachment that producers supply to the buyers of their dyestuffs. It contains key product information (including trade name, physical and chemical properties, toxicological data) describing the product as a nonhazardous substance.

Regardless of the method of determining the content of aromatic amines in samples, an area that needs to be addressed is that of structure–activity relations. These relationships reveal how the reduction conditions affect the quantity of amines splitting off a dyestuff. It is worthwhile to determine whether this quantity is somehow dependent on the dye structure, i.e., the presence and position of specific functional groups in the dye. Usually [11, 8, 15, 16, 9], only genotoxic, mutagenic, or carcinogenic properties of azo dyes are examined, which result from the presence of certain functional groups within the chemical structure of the dye. Here, a different approach is suggested: combining the analytical objective, i.e., determining the amine content in a sample, with a structural interpretation, i.e., explaining how the chemical structure of a dyestuff and the reduction conditions affect the quantity of a released aromatic amine.

EXPERIMENTAL

In Fig. 2 the structural formulas of the dyes selected for analysis are found. Acid Red 85 and Direct Blue 6 are industrial dyes no longer produced; they were supplied by Instytut Barwników i Produktów Organicznych from Zgierz, Poland. Ponceau SS, Sudan III, Disperse Yellow 7, Mordant Orange 1, and Disperse Orange 3 were supplied by Sigma–Aldrich, Poland. The following reagents and solutions were used for analyses: 2% solution of α-naphthol (p.a. Fluka, Sigma–Aldrich) in ethanol; 1 M sodium nitrate $NaNO_2$ (p.a. Fluka, Sigma-Aldrich); sodium hydroxide NaOH (p.a. Fluka, Sigma-Aldrich); solutions 1 M and 30%; 1 M HCl (p.a. Fluka, Sigma-Aldrich); sodium dithionite $Na_2S_2O_4$ (p.a. Fluka, Sigma-Aldrich); methylene chloride absolute (Merck, Poland).

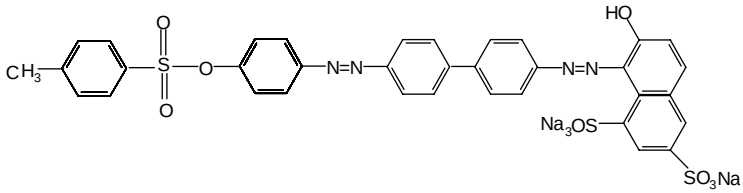
Two methods of chemical reduction were adopted. More drastic conditions of reduction [21] involved 0.05 g of dye (i.e., the quantity fitting to standard of 3% dyeing or 4 g of dyed cotton fabric with 3% dyeing). The sample was boiled in 1 M NaOH (30 ml) for 1 h in a round-bottom flask under a reflux condenser. After 30 min 0.8 g of sodium dithionite was added, and reduction was continued. The entire process lasted 1 h. For less drastic conditions [1], the reduction was carried out a 70°C at pH 6, using 17 ml of citrate buffer with 0.8 g of sodium dithionite per 1 g of fiber. Other conditions were identical to those of the previous method.

In each case, the extract obtained was transferred to Extrelut 20 columns (Merck) to which 17 ml of methylene chloride was subsequently poured. Next, the samples were evaporated to dryness, and the remains were dissolved in volumetric flasks in 100 ml of 0.1 M HCl. For evaporating the samples, a 350P vacuum evaporator (EQUIMED, Poland) was used. The temperature was set at 40°C.

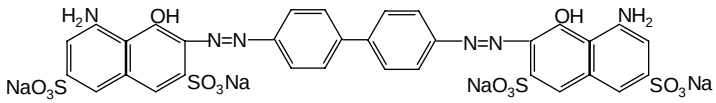
Spectrophotometric analysis was carried out in a Specol absorption spectrophotometer (Carl-Zeiss Jena Germany), using the method of aromatic amine determination developed by Maslowska and Swat [10], with slight modification. In the original method, aromatic amines are isolated using distillation with steam. In the current study, the chemical reduction was carried out as described above. The spectrophotometric method of determining trace amounts of aromatic amines [10] utilizes the color reaction of diazotization of amines with α-naphthol. It allows the determination of total aromatic amine content as aniline.

The working solution of α-naphthol was prepared immediately before carrying out the diazotization reaction. A 100-ml flask was filled with 5 ml of 2% α-naphthol solution in ethanol and 50 ml of 1 M NaOH solution. Stock solutions were prepared, using in each case the amine that split off the dye analyzed, e.g., benzidine for Acid Red 85 and *p*-phenylenediamine for Disperse Orange 3. Each solution was made in the following way: 0.1000 g of amine was

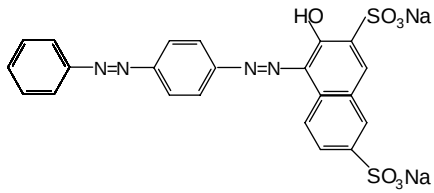
Acid Red 85



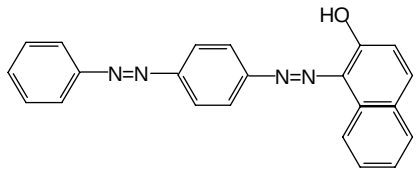
Direct Blue 6



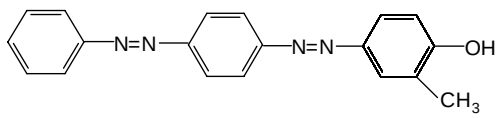
Ponceau SS



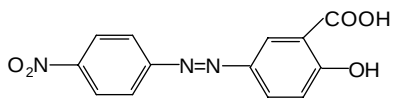
Sudan III



Disperse Yellow 7



Mordant Orange 1



Disperse Orange 3

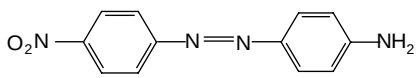


FIG. 2. Structural formulas of the dyes selected for analysis.

weighed and transferred to a 100-ml volumetric flask, to which 10 ml of 1 M HCl was added together with distilled water. If an amine did not dissolve in the acid, hot water was added.

For further examination, a working solution was prepared with a concentration of 100 μg/ml (by diluting 10 ml of stock solution in a 100-ml volumetric flask). Next, 0.1, 0.2, 0.3, 0.4, …, 1 ml of the amine solution were poured in

TABLE 1
Content of Aromatic Amines Determined by Spectrophotometric and HPLC Methods

No.	Dye[a]	Splitting amine	Content of aromatic amines (g/kg dye)	
			Spectrophotometry	HPLC
1.	Acid Red 85	Benzidine	128 ± 0.11	104
2.	Direct Blue 6	Benzidine	23.2 ± 0.07	24.6
3.	Ponceau SS	*p*-Phenylenediamine, aniline	160 ± 0.07	129.1; 31.5
4.	Sudan III	*p*-Phenylenediamine, aniline	110.8 ± 0.10	73.7; 15.1
5.	Disperse Yellow 7	*p*-Phenylenediamine, aniline	256 ± 0.07	165.9; 89.2
6.	Mordant Orange 1	*p*-PD	94.7 ± 0.10	132.8
7.	Disperse Orange 3	*p*-PD	143.6 ± 0.10	0.088

[a]Dyes were analyzed under more drastic conditions of reduction process.

a series of test tubes with glass stoppers. Each sample was supplemented with 1 ml of 1 M HCl and 0.2 ml of 1 M $NaNO_2$. After 3 min of shaking, the test tubes were filled with 5 ml of freshly prepared working solution of α-naphthol and filled to 10 ml with distilled water, taking into consideration the amount of amine solution they already contained. A zero sample was prepared in the same way, without the amine stock solution and filled with 9 ml of distilled water.

Absorbance was measured with the spectrophotometer at wavelength $\lambda = 520$ nm. For study samples, 0.5 or 1 ml of each solution (product of dyestuff reduction) in 0.1 M HCl was used, with the remaining components added as described above.

Chromatography was performed using a Merck–Hitachi L4500A HPLC chromatograph equipped with a diode-array detector, an L 7840 fluorescence detector, and column packing of the chemically bonded alkyl stationary phases (Li Chrosorb C_{18} (7μm) (250 × 4 mm)).

From 100 ml of the samples dissolved in 0.1 M HCl, 3 ml of each solution was taken to be vaporized, with the dry remains dissolved in 1 ml of methanol. Twenty microliters of the solution was injected into the column. The methanol:water (55:45 v/v) mixture was used as the mobile phase [14], at a flow rate of 1.2 ml/min.

RESULTS AND DISCUSSION

It is well known [17] that the degree to which a dye azo group is reduced depends on the electron density around the –N = N– bond. Electron-withdrawing groups such as $-NH_2$ and –OH decrease the electron density around the –N = N– bond and facilitate the reduction of the azo group with a simultaneous release of an aromatic amine. Also, placing an electrodonor substituent (ED) in an ortho- position in relation to the azo group causes a reduction through the creation of hydrogen bonds on the nitrogen azo group. A similar effect in a simpler reduction within the –N = N– group is observed for water-soluble dyes [4], i.e., those with groups such as $-SO_3Na$ and –COOH in their structure.

Those criteria were reflected in the analyses of Ponceau SS, Sudan III, and Acid Red 85, which have an –OH group in an ortho- position in relation to the azo bond and thus are easily reduced to one or more amines. The splitting of substantial amounts of amines for Disperse Orange 3 and Mordant Orange 1 is caused by the presence of a nitro group in the dye structure. Nitro groups are reduced [18] much more easily than azo groups. Introducing an –OH group in a para- position in relation to the azo bond makes the dye less alkali-resistant [18], which, indirectly, may also facilitate reduction. This effect was observed for Disperse Yellow 7. Only small amounts of amines split off Direct Blue 6. This weak reactivity was caused by steric hindrances to the reduction process [11].

The structural conditions described above, stemming from the presence of specific functional groups in the azo dyes examined, are quantitatively justified by the data in Table 1. It lists the amounts, obtained by means of two measuring methods, of aromatic amines released by the azo dyes during their reduction, under drastic conditions of a strongly alkalic environment and at boiling point. The table proves that spectrophotometry allows determination of the total amount of amine (recounted as total aniline content) that splits off a dye during its chemical reduction.

For three dyes—Ponceau SS, Sudan III, and Disperse Yellow 7—on the basis of the well-known procedure of dye reduction (see Fig. 1), which involves the breaking off of azo bonds and recreation of amines, it is justified to say that two amines (*p*-phenylenediamine and aniline) split off rather than one. In this case, determining the amount of each amine is possible only by using HPLC. Then comparable amounts of released amines are expected, regardless of the measuring method applied. This is confirmed by Table 1. For example, when Ponceau SS is reduced, 160 g of amine splits off every 1 kg of dye (in the spectrophotometric method). For the same dye analyzed using HPLC, 129.1 g

TABLE 2
Content of Aromatic Amines Determined by Spectrophotometric and HPLC Methods

No.	Dye and method of chemical reduction	Splitting amine	Content of aromatic amines (g/kg dye)	
			Spectrophotometry	HPLC
1.	Acid Red 85 in NaOH	Benzidine	128 ± 0.11	104
2.	Acid Red 85 in pH 6 buffer	Benzidine	39.2 ± 0.10	41
3.	Direct Blue 6 in NaOH	Benzidine	23.2 ± 0.07	24.6
4.	Direct Blue 6 in pH 6 buffer	Benzidine	17.5 ± 0.09	21.9
5.	Direct Blue 6 fabric in NaOH	Benzidine	10.7 ± 0.07	6

of *p*-phenylenediamine and 31.5 g of aniline splits off every 1 kg of dye, which gives about 160 g of amine per 1 kg of dye.

Depending on the method of chemical reduction, different amounts of splitting aromatic amines were observed (Table 2). It seems that a strongly alkaline environment is most conducive to dye degradation. For example, Acid Red 85, when reduced in 1 M NaOH, splits off 128 g of benzidine per 1 kg of dye analyzed spectrophotometrically, compared with 104 g of benzidine for the HPLC method.

Less amine is released during a chemical reduction in acetate buffer. The smallest amount of amine splits off during the reduction of a dye that is bound on a cotton fabric dyed with an azo dye. This indirectly explains the process of dye depletion from a dyeing bath. Normally, the fabric is dyed with 3% dyeing, but only a part of the dye binds to the fabric, the rest being retained in the solution as color reaction waste. Also, it is probably more difficult to reduce a dye bound with a fiber than a dye that is an independent competent in a chemical reaction environment.

Comparing both analysis methods (spectrophotometric and HPLC), one can see that similar amounts of amines are split off during dye reduction, regardless of which technique has been employed. The absolute amount of amine produced during the reduction depends on the structure of the dye analyzed, that is, on the type of functional groups the dye contains, either facilitating or hindering the reduction process.

The spectrophotometric method, being simpler and less expensive, may be used for preliminary analysis and detecting, generally, aromatic amines as impurities or dye reduction products. When interpreting the results of the spectrophotometric analysis, whether the dye is the product of a coupling reaction between an amine and α-naphthol

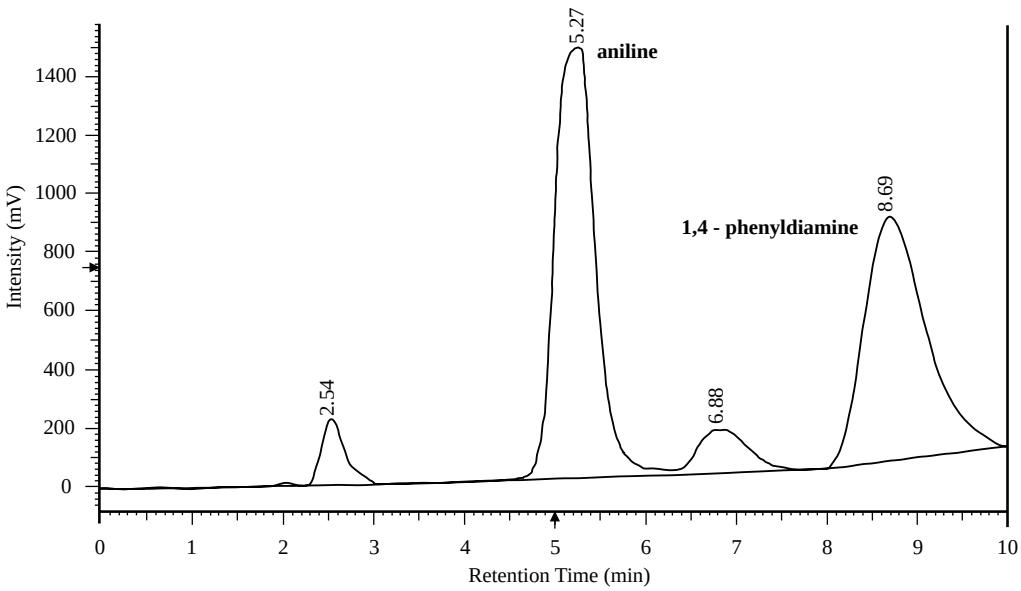


FIG. 3. Chromatogram of separation of aromatic amines: aniline ($t_r = 5.34$ min) and 1,4-phenylenediamine ($t_r = 8.61$ min).

TABLE 3
Statistical Test in the Chromatography of Amines ($n = 3$)

Amine	Added (μg)	$\bar{X}$	SD	Recovery (%)
Benzidine	10	9.73	0.58	97.2
Aniline	10	10.30	0.87	102.9
p-Phenylenediamine	10	9.85	0.70	98.5

should be taken into account. The λ_{max} should be selected individually for each determined aromatic amine, as the dyes produced in the diazotization reaction have diversified coloring. Also, it should be stressed that in the spectrophotometric method the accuracy of amine determination is limited by the adopted procedure of recounting amine as aniline.

The chromatographic method earlier developed by the authors [14] was used to identify and determine benzidine ($t_r = 5.79$ min) in the reduction products of Acid Red 85 and Direct Blue 6, as well as *p*-phenylenediamine ($t_r = 8.61$ min) and aniline ($t_r = 5.34$ min) in the reduction products of other dyes examined. The statistical results for the amine chromatography are found in Table 3.

Figure 3 is a typical chromatogram. Peaks 1 and 3 do not represent amines (this was verified through analyzing standards), but probably some other dye degradation products. The chromatographic method can be used to analyze the reduction products of dyes that release amines of the MAK type.

CONCLUSION

By analyzing the test results provided in Table 1 and 2, it is possible to compare the spectrophotometric and HPLC methods. It is possible to determine the presence or absence of a carcinogenic aromatic amine by employing the less expensive and simpler spectrophotometric method.

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