doi: 10.1111/j.1472-3581.2006.00001.x

Mass spectrometric analysis of sulphonated dyes based on diaminobiphenyls

Coloration Technology

Society of Dyers and Colourists

Kateřina Volná,[1] Michal Holčapek,[1] Potjanart Suwanruji[2] and Harold S Freeman[2]*

[1]*Departmentof Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Nám. Čs. Legií 565, 53210 Pardubice, Czech Republic*
Email: harold_freeman@ncsu.edu

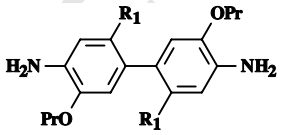
[2]*Polymerand Color Chemistry Programme, College of Textiles, North Carolina State University, Raleigh, NC 27695, USA*

Received: 3 August 2005; Accepted: 13 October 2005

The title dyes, a group of new homo-bireactive dis-azo reactive dyes having molecular masses of 1000–1400 Da, were developed because of their potential use as low salt, easy wash-off colorants for cotton. Following dye synthesis from diaminobiphenyls with and without substituents in the 2,2′-positions, negative-ion electrospray ionisation mass spectrometry (ESI-MS) was used to characterise the dyes. The MS obtained were characterised by signals arising from $[M - xH]^{x-}$ ions plus fragment ions produced by cleavage at C–N bonds adjacent to the azo linkage. In addition, better results were produced when J-acid was the coupler employed rather than H-acid. The characteristic fragmentation behaviour of the studied dyes is discussed and illustrated on selected example.

Introduction

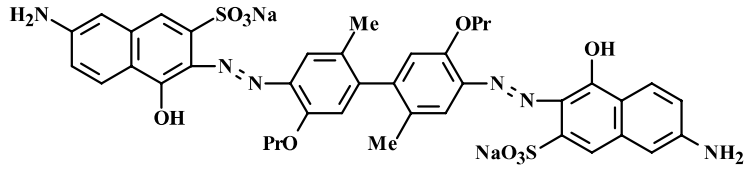
The present investigation is part of a continuing research programme aimed at developing synthetic alternatives to aromatic amines known to be genotoxic. In this regard, the design and synthesis of non-genotoxic benzidine analogues were pursued, leading to diaminobiphenyls of type **1** [1,2]. In addition to determining that these diamines were non-mutagenic, it was shown that azo dyes (**2** and **3**) and organic pigments (**4**) could be obtained and that these colorants were themselves non-mutagenic [3,4]. It was also found that the presence of a non-hydrogen substituent in the 2,2′-positions produced a significant hypsochromic shift in the colours of the dyes and pigments obtained, owing to the large dihedral angle at the biphenyl linkage [5–7].



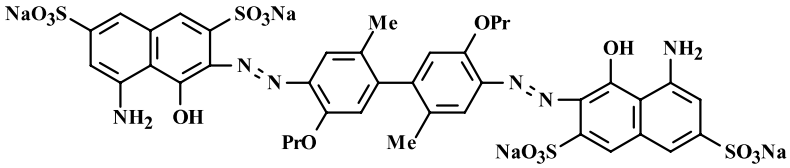
1 (R_1 = Me, Cl)

Bearing in mind that twisted dye structures arise from the use of type **1** diamines, it seemed reasonable to anticipate that the hydrolysed forms of reactive dyes based on such diamines would have lower affinity and better wash-off properties than reactive dyes of similar molecular size. This idea led to the synthesis of dis-azo reactive dyes derived from twisted and non-twisted diaminobiphenyls, such as the homo-bireactive dyes **5** and **6**. Following the synthesis of the target dyes methods for their characterisation were pursued. Special consideration was given to mass spectrometry (MS), as it had been shown in previous studies that organic pigments derived from type **1** diamines were amenable to analysis by this method [8].

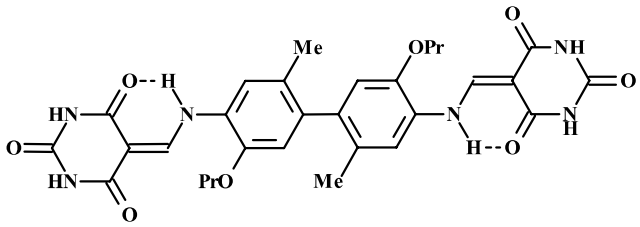
Among the available ionisation techniques, negative-ion electrospray ionisation (ESI) has proved to be the most suitable for the MS analysis of sulphonated dyes [9–13]. Negative-ion ESI-MS containing only the peaks of deprotonated molecules $[M - H]^-$ for monosulphonic acids, and a series of $[M - xH]^{x-}$ ions and sodiated adducts $[M - (x + y)H + yNa]^{x-}$ for polysulphonic acids afforded easy molecular weight (MW) determination for sulphonated dyes (acid, direct and metal complexed) and dye intermediates (benzene, naphthalene and anthraquinone derivatives) [9,10]. Based on the knowledge of MWs and of the detailed interpretation of fragment ions observed in tandem spectra, probable structures of trace impurities in commercial acid, direct, mordant and reactive dye samples were proposed [11]. For the identification of trace impurities and by-products in the samples of commercial dyes and reaction mixtures, the coupling of high-performance liquid chromatography (HPLC) and MS is preferred, but conventional non-volatile ion-pairing agents, such as tetraalkylammonium salts, must be replaced by more volatile dialkylammonium acetates and trialkylammonium acetates [10,11]. The latter compounds offer a reasonable compromise between separation selectivity and MS performance in the analysis of anionic dyes. Unfortunately, dialkyl-
ammonium acetates and trialkylammonium acetates also suppress the ionisation of target analytes, but to a lower degree than the tetraalkylammonium analogues [11]. For metal-complex azo dyes without sulphonic acid groups, 5 mM ammonium acetate can be used instead of alkylammonium acetates to give acceptable separation selectivity [12]. Based on results from the



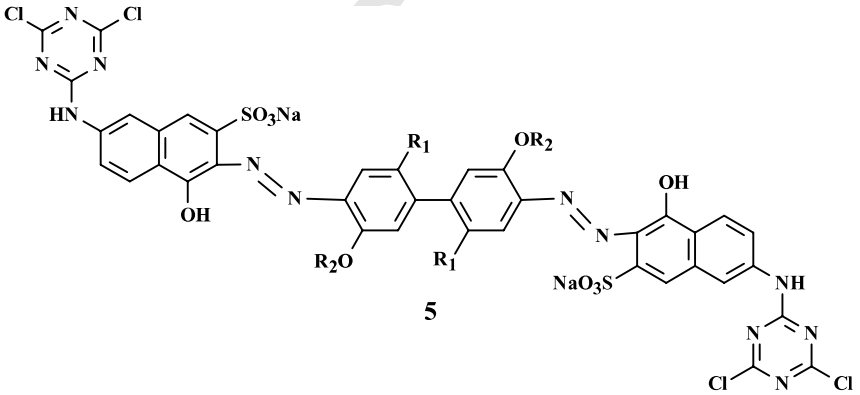
2



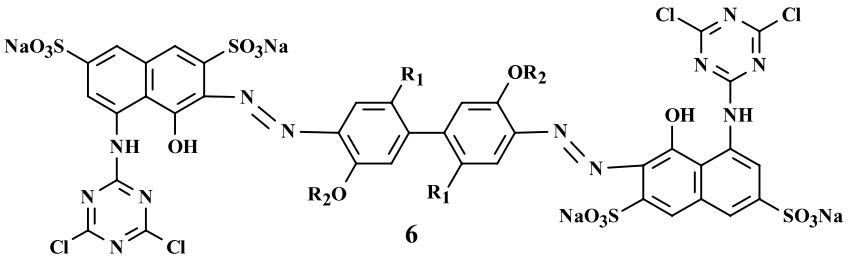
3



4



5



6

R_1 = H, Me; R_2 = C_1-C_3 alkyl

above studies, ESI-MS was chosen for the analysis of type **5–6** dyes.

Experimental

Materials

Acetonitrile for HPLC was purchased from Merck (Darmstadt, Germany). Water was deionised using Demiwa 5-roi purifying system (Watek, Ledeč nad Sázavou, Czech Republic). The solvents were filtered 2 through 0.45 μm Millipore filter prior to use. The dyes used in this study were synthesised at North Carolina State University [14].

Mass spectrometry

Negative-ion ESI-MS were recorded using an Esquire 3000 mass spectrometer with an ion trap analyzer (Bruker

Daltonics, Bremen, Germany) in the mass range (m/z) of 50–1500. The samples were dissolved in 50% aqueous acetonitrile and analysed by direct infusion at a flow rate of 5 μl/min using a syringe pump (COLE Palmer Instrument Company, Vernon Hills, IL, USA). The ion trap analyzer was tuned to obtain an optimal response according to the expected m/z values of doubly charged ions, i.e. the tuning parameter 'target mass' was set to m/z 400–600. The source parameters were as follows: drying gas flow rate = 4 l/min, nebulising gas pressure = 10 psi and drying gas temperature = 300 °C. The selected precursor ions were further analysed by MS/MS analyses under the following conditions: the isolation width m/z = 4 and the collision amplitude = 0.6–0.9 V. The key observed ions are summarised below.

Dye **5a**: R_1 = Me, R_2 = Pr (MW = 1122 Da)
Negative-ion ESI-MS: m/z 860; 725; 709 (100%); 681; 560 $[M - 2H]^{2-}$; 491; 463.5; 399; 385; 349.

Dye **5b**: R_1 = Me, R_2 = Et (MW = 1094 Da)
Negative-ion ESI-MS: m/z 681 (100%); 645; 667; 631; 496; 385; 349; 306; 263.

Dye **5c**: R_1 = Me, R_2 = Me (MW = 1066 Da)
Negative-ion ESI-MS: m/z 1065 $[M - H]^-$; 910; 653; 532 $[M - 2H]^{2-}$ (100%); 472; 385; 349.

Dye **5d**: R_1 = H, R_2 = Me (MW = 1038 Da)
Negative-ion ESI-MS: m/z 737; 625; 589; 518 $[M - 2H]^{2-}$; 445; 385; 349 (100%); 306; 288; 263.

Dye **6a**: R_1 = Me, R_2 = Pr (MW = 1372 Da)
Negative-ion ESI-MS: m/z 466.5.

Table 1 Key ions observed in negative-ion ESI mass spectra of reactive dyes **5**[a]

Dye	R_1	R_2	$[M - 2H]^{2-}$	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
5a	Me	Pr	560	385	349	–	–	**709**	681	–	399
5b	Me	Et	–	385	349	306	–	**681**	–	645	–
5c	Me	Me	**532**	385	349	–	–	653	–	–	–
5d	H	Me	518	385	**349**	306	288	625	–	–	–

[a]Ions corresponding to the base peaks in individual spectra are printed in bold (see Figure 1 for notation of fragment ions F_1–F_8)

Dye **6b**: R_1 = Me, R_2 = Et (MW = 1344 Da)
Negative-ion ESI-MS: m/z 763; 723.

Dye **6c**: R_1 = Me, R_2 = Me (MW = 1316 Da)
Negative-ion ESI-MS: m/z 735.

Dye **6d**: R_1 = H, R_2 = Me (MW = 1288 Da)
Negative-ion ESI-MS: m/z 867; 722.

Results and Discussion

Negative-ion ESI-MS of reactive dyes employed in this study generally showed singly charged and doubly charged deprotonated molecules $[M - H]^-$ and $[M - 2H]^{2-}$, which enabled the determination of MWs of individual dyes. Additionally, fragment ions arising from cleavage at the azo bond or adjacent to the azo bond were observed in the first-order MS. The cleavage reactions were followed by neutral losses of HCl, CHN_2, H_2O or N_2 to give other fragment ions observed in the spectra. The typical fragment ions and their m/z values for individual dyes are presented in Table 1. The general fragmentation scheme for dyes **5** and **6** is illustrated in Figure 1. The notation of observed ions in MS was derived from the acid form of sulphonic acids (i.e. RSO_3H) based on the established convention [9–11], but the synthesised dyes were in the form of sodium salt (i.e. RSO_3Na).

Type 5 dyes

Dye **5a** has the monoisotopic MW of 1122 Da, based on the formula $C_{46}H_{38}N_{12}O_{10}S_2Cl_4$. The important ion in the negative-ion ESI-MS is a doubly charged ion $[M - 2H]^{2-}$ at m/z = 561, which confirms the expected MW. The charge of this ions is determined by the difference among individual isotopic ions, which is $\Delta m/z = 1$ for singly charged ions, $\Delta m/z = 1/2$ for doubly charged ions, $\Delta m/z = 1/3$ for triply charged ions, etc. In addition to the $[M - 2H]^{2-}$ ion peak, key fragment ions were observed at m/z = 709 (fragment ion F_5) and m/z = 385 (F_1). Structures assigned to these fragment ions are shown in Scheme 1, where it is suggested that they were produced by cleavage at C–N bonds adjacent to the azo linkage. In the case of m/z = 709, it is possible that ion **8** forms via sequential cleavage steps at positions 'a' and 'b'.

Dye **5b** has MW of 1094 Da, based on the formula $C_{44}H_{34}N_{12}O_{10}S_2Cl_4$. In this case, the peak of $[M - 2H]^{2-}$

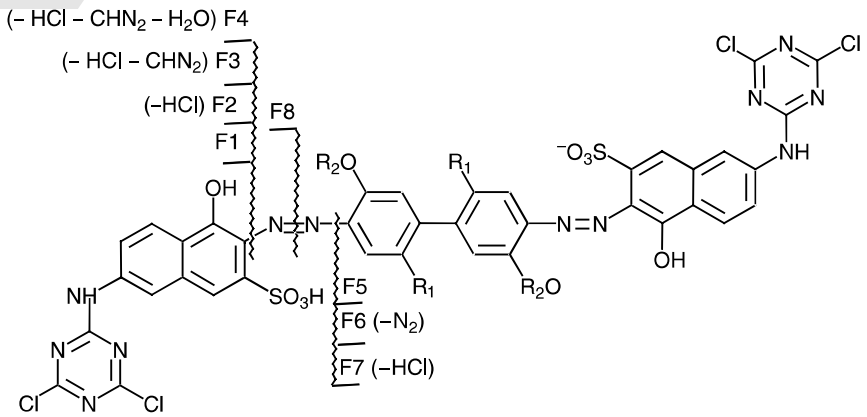
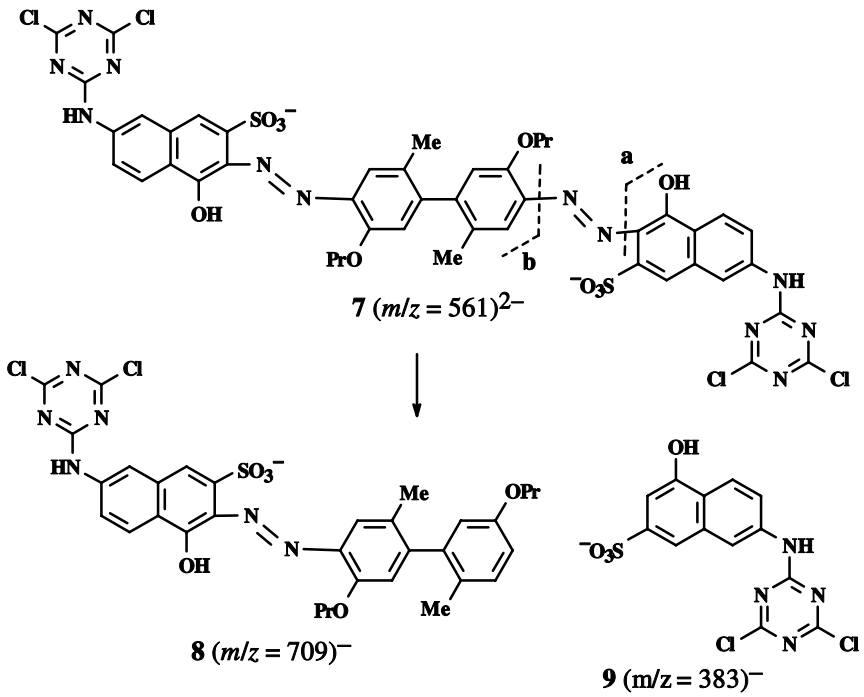


Figure 1 General fragmentation scheme for dyes studied in this investigation



Scheme 1

ion at $m/z = 546$ was not observed in the spectrum. However, key fragment ions were observed at $m/z = 681$ (F_5), 645 (F_7), 385 (F_1), 349 (F_2) and 306 (F_3). The formation of fragment ions and the notation of fragment ions can be understood from Figure 1. The peak at $m/z = 681$ (F_5) was produced by cleavage at C–N bond adjacent to the biphenyl moiety, followed by the neutral loss of HCl (m/z: 645, F_7). Fragment ions F_1, F_2 and F_3 arise from cleavage at C–N bond adjacent to J-acid group and subsequent neutral losses. All discussed fragment ions are in agreement with the structure.

Dye **5c** has MW of 1066 Da, based on the formula $C_{42}H_{30}N_{12}O_{10}S_2Cl_4$. Doubly charged ion $[M - 2H]^{2-}$ at $m/z = 532$ is the base peak of the spectrum (Figure 2). Moreover, the peak of $[M - H]^-$ ion at $m/z = 1065$ is observed, confirming the expected MW. In addition to $[M - 2H]^{2-}$ and $[M - H]^-$ ions, other diagnostic fragment ions can be found in the spectrum, such as $m/z = 653$ (F_5), 385 (F_1) and 349 (F_2). Figure 2 represents a typical spectrum, which shows a relatively high noise level

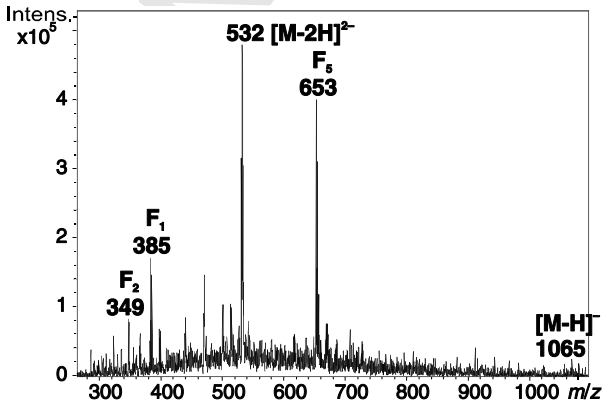


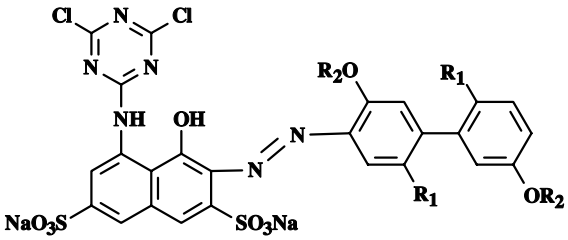
Figure 2 Negative-ion ESI mass spectrum of dye **5c**

probably caused by the salt content level of the dye sample.

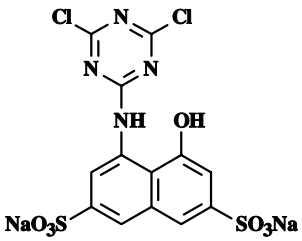
Dye **5d** has MW of 1038 Da, based on the formula $C_{40}H_{26}N_{12}O_{10}S_2Cl_4$. Similarly to **5a** and **5c**, the doubly charged ion at $m/z = 518$ corresponds to the loss of protons (or sodium ions) from the sulphonic acid groups. The base peak of the spectrum is found at $m/z = 349$ (F_2) corresponding to the cleavage at the C–N bond adjacent to the J-acid group followed by the neutral loss of HCl. Other fragment ions were identified in the spectrum: m/z 385 (F_1, the cleavage at C–N bond adjacent to the J-acid group) and further neutral losses from F_1 are peaks at m/z 306 (F_3) and 288 (F_4), and finally the ion corresponding to the cleavage of other C–N bond at m/z 625 (F_5).

Type 6 dyes

The spectra of tetrasulphonated dyes (series **6**) showed significantly higher level of background noise compared with disulphonated dyes (series **5**), which can probably be explained by higher salt levels in these dyes. For that reason, the spectra interpretation was more complicated and fewer interpretable ions were observed. Attempts to remove salt using various solid-phase extraction procedures with cation- or anion-exchange columns and C18 column did not improve spectral quality. These tetrasulphonated dyes did not give peaks corresponding to the molecular ions. With the exception of dye **6a**; however, peaks consistent with type **10** ions were observed. In the case of dye **6a** (MW = 1282 Da, based on the formula $C_{46}H_{38}N_{12}O_{16}S_4Cl_4$), only a peak at m/z = 466.5 was observed (cf. structure **11**). Dyes **6b** (MW = 1254 Da, based on the formula $C_{44}H_{34}N_{12}O_{16}S_4Cl_4$), **6c** (MW = 1226 Da, based on the formula $C_{42}H_{30}N_{12}O_{16}S_4Cl_4$) and **6d** (MW = 1226 Da, based on the formula $C_{40}H_{26}N_{12}O_{16}S_4Cl_4$) gave a type **10** cleavage product at m/z = 763, 735, and 667, respectively. In



10



11

R_1 = H, Me; R_2 = Me, Et

addition, dyes **6b** and **6d** gave peaks corresponding to the hydrolysed forms of the type **10** structures.

Conclusions

The results of this study indicate that negative-ion ESI-MS can be used to generate useful data for characterising homo-bireactive dyes derived from substituted diaminobiphenyls. It is evident, however, that molecular ion information is more readily obtained in these systems when the molecules contain two rather than four sulphonate groups. Therefore, dyes based on J-acid gave better results than those from H-acid. In the latter case, we believe that rigorous removal of sodium ions and impurities arising from dye synthesis are critical to getting useful spectra.

Acknowledgements

M. Holčapek and K. Volná acknowledge the financial support of the grant project No. 203/04/P126 sponsored by the Grant Agency of the Czech Republic. P. Suwanruji and H. Freeman acknowledge scholarship support provided by the Royal Thai government during the doctoral studies of P. Suwanruji.

References

1. D Hinks and H S Freeman, *Dyes Pigm.*, **44** (2000) 199.
2. J-S Bae and H S Freeman, *AATCC Rev.*, **1** (2001) 67.
3. D Hinks, H S Freeman, Y Arai and H Ando, *Dyes Pigm.*, **48** (2001) 7.
4. J-S Bae and H S Freeman, *Fibers Polym.*, **3** (2002) 140.
5. J Sokolowska-Gajda, D Hinks and H S Freeman, *Dyes Pigm.*, **48** (2001) 15.
6. M Nakpathom, D Hinks and H S Freeman, *Dyes Pigm.*, **48** (2001) 93.
7. A El-Shafei, D Hinks, P D Boyle and H S Freeman, *Acta Crystallogr.*, C**60** (2004) o569.
8. J Sokolowska-Gajda, D Hinks, C A Haney, H S Freeman and M Tanaka, *Dyes Pigm.*, **39** (1998) 159.
9. M Holčapek, P Jandera and J Přikryl, *Dyes Pigm.*, **43** (1999) 127.
10. M Holčapek, P Jandera and P Zderadička, *J. Chromatogr. A*, **926** (2001) 175.
11. D Ansorgová, M Holčapek and P Jandera, *J. Sep. Sci.*, **26** (2003) 1017.
12. K Lemr, M Holčapek, P Jandera and A Lyčka, *Rapid Commun. Mass Spectrom.*, **14** (2000) 1881.
13. A Lyčka, D Luštinec, J Holeček, M Nádvorník and M Holčapek, *Dyes Pigm.*, **50** (2001) 203.
14. P Suwanruji, *Fiber and Polymer Science Program*, PhD thesis, North Carolina State University, NC, USA, 2004.