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Analysis of sulphonated dyes and intermediates by electrospray mass spectrometry

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Abstract

Molecular weights of dyes and intermediates containing one to four sulphonic acid groups were determined by negative-ion electrospray mass spectrometry. Mass spectra of monosulphonated dyes exhibit an important ion that arises from the loss of a cation from a sulphonic group. Sulphonated dyes form molecular ions with different charges either by subsequent loss of protons, i.e. $[M–xH]^{x-}$, or from adducts with sodium ions, i.e. $[M–(x+y)H+y\mathrm{Na}]^{x-}$. The maximum values of x or $(x+y)$ are equal to the total number of sulphonic and carboxylic acid groups, where x is higher than y. This method is applicable to various types of poly-sulphonated dyes, including anthraquinone, azo or metal complex dyes. The HPLC/MS technique can be used for the analysis of mixtures of dyes and intermediates also.

Keywords: Sulphonated dyes; Molecular weight; Electrospray ionization; Mass spectrometry; HPLC/MS

1. Introduction

Sulphonated dyes are important industrial products with widespread applications. Sulphonic groups are incorporated into dye molecules to improve their solubility in water. The production of sulphonated dyes is increasing every year and the environmental aspects of these as well as other dye has become important, because of the toxicity of certain dyes and their degradation products [1,2]. Hence, a simple, sensitive and reliable method of determining sulphonated dyestuffs in environment is required, making possible the identification of precursors, intermediates and by-products during the synthetic process.

A variety of spectroscopic methods are employed for structure elucidation or confirmation of the identity of dyes. In addition to UV/VIS and IR spectroscopy, mass spectrometry (MS) is suitable for this purpose. MS with conventional electron ionization (EI) cannot be used for non-volatile compounds such as polysulphonated dyes because of excessive fragmentation and inability to transfer ionic compounds to the gaseous phase. EI mass spectra have been reported only for monosulphonated aromatic acids containing none or one other functional group (e.g. CH_3, OH or

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Cl) using high-performance liquid chromatography–mass spectrometry (HPLC/MS) with a particle beam interface [3]. The resultant mass spectra were noisy and the molecular ion signal was not the base peak. Generally, EI is not suitable for ionic dyes, especially those containing two or more sulphonic acid groups.

Soft ionization techniques (thermospray ionization-TSI, atmospheric pressure chemical ionization-APCI, electrospray ionization-ESI, matrix-assisted laser desorption/ionization-MALDI) make possible the analysis of non-volatile ionic dyes with high molecular weights. The sulphonic group is strongly acidic and therefore completely dissociated in the aqueous solution, so that the formation of $[R\text{-}(SO_3)_n]^{n-}$ ions is very easy. Hence, negative-ion MS is much more sensitive than positive-ion MS [4]. APCI [4] and TSI [4–6] permit molecular weight (MW) determination for compounds containing up to two sulphonic acid groups, and APCI mass spectra may contain also some fragment ions. Negative-ion ESI mass spectra have been recorded for compounds with one to eight sulphonic groups [7–13], which indicates that the use of negative-ion ESI is probably not restricted by total number of acid groups. MALDI-MS was demonstrated [13] to be a suitable alternative method for the MW determination of dyes containing one to six sulphonic acid groups.

The coupled HPLC/MS technique simplifies the MW determination in complex industrial mixtures. Conventional HPLC methods for separation of ionic species usually employ non-volatile additives such as an ion-pair reagent [14–17] in the mobile phase, or a high concentration of inorganic salts [16–19]. Good resolution of positional isomers of sulphonated aromatics was achieved on a column packed with β-cyclodextrin bonded phase [20]. Non-volatile salts are not compatible with MS detection due to the deposition of salts in the ion source, resulting in the instrument failure. Fortunately, use of the more volatile ammonium acetate at concentrations of 10–30 mM is often possible, without a significant decrease in the separation selectivity [4,6,8,20].

Capillary zone electrophoresis (CZE) can effectively complement HPLC techniques in the separation of sulphonic acids [17,21–24], the coupled CZE/MS technique being particularly useful in this respect [25–27]. The coupling of planar chromatography with fast atom bombardment (FAB) MS was utilised for the identification of food dyes containing up to two sulphonic acid groups [28]. Gas chromatography is not effective for the analysis of sulphonated compounds, because time-consuming derivatization has to be done prior to the analysis to increase the volatility [29,30]. Analytical methods suitable for the analysis of sulphonated compounds have been reviewed by Reemtsma [31].

The objective of the present work was to investigate the utility of ESI/HPLC in the characterization of polysulphonated dyes and intermediates. Special attention was paid to the determination of MW and of the total number of acid groups in polysulphonated compounds.

2. Experimental

Methanol for HPLC was used as received from Baker (Deventer, The Netherlands), water was redistilled in glass in the presence of potassium permanganate. The solvents were filtered through a 0.45 μm Millipore filter prior use and degassed by continuous stripping by a stream of helium. Ammonium acetate was purchased from Lachema (Brno, Czech Republic). Samples of sulphonated dyes and intermediates were obtained from Synthesia (Pardubice, Czech Republic) and are listed in Tables 1 and 2.

A VG Platform quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with electrospray and APCI probes was used for operation in positive or negative-ion mode. The data was acquired in the range $m/z = 35$–1200 at 1.9 s per scan in the negative-ion ESI mode. The temperature in the ion source was held at 100°C. The voltage of 30 V was applied on the cone electrode. Samples of dyes were dissolved in 40% methanol–60% 5 mM ammonium acetate in water and introduced directly into the mass spectrometer in the stream of solvent pumped at the flow rate of 20 μl/min. The injection volumes were 10 μl.

Table 1
Sulphonated dye intermediates studied in this investigation

No.	Compound	Common name or abbreviation	Molecular formula	MW[a]	Acid groups[b]	t_R[c] (min)
Naphthalene sulphonic acids						
1	Naphthalene-1-sulphonic acid	1-NSA	$C_{10}H_8O_3S$	208.0	1	13.4
2	Naphthalene-2-sulphonic acid	2-NSA	$C_{10}H_8O_3S$	208.0	1	15.2
3	1-Aminonaphthalene-6-sulphonic acid	1,6-Cleve's acid	$C_{10}H_9O_3NS$	223.0	1	6.9
4	1-Aminonaphthalene-7-sulphonic acid	1,7-Cleve's acid	$C_{10}H_9O_3NS$	223.0	1	9.3
5	2-Aminonaphthalene-7-sulphonic acid	Amino-F acid	$C_{10}H_9O_3NS$	223.0	1	8.5
6	5-Aminonaphthalene-1-sulphonic acid	Laurent's acid	$C_{10}H_9O_3NS$	223.0	1	3.0
7	8-Aminonaphthalene-1-sulphonic acid	Peri acid	$C_{10}H_9O_3NS$	223.0	1	13.1
8	7-Amino-1-hydroxynaphthalene-3-sulphonic acid	Gamma acid	$C_{10}H_9O_4NS$	239.0	1	4.6
9	6-Amino-1-hydroxynaphthalene-3-sulphonic acid	I acid	$C_{10}H_9O_4NS$	239.0	1	3.4
10	1,6-Dihydroxynaphthalene-3-sulphonic acid	Dioxy-I acid	$C_{10}H_8O_5S$	240.0	1	5.1
11	6-Aminonaphthalene-1,3-disulphonic acid	Amino-I acid	$C_{10}H_9O_6NS_2$	303.0	2	1.8
12	8-Amino-3-hydroxynaphthalene-1,6-disulphonic acid	W acid	$C_{10}H_9O_7NS_2$	319.0	2	1.8
13	1-Amino-8-hydroxynaphthalene-3,6-disulphonic acid	H acid	$C_{10}H_9O_7NS_2$	319.0	2	2.5
14	1,8-Dihydroxynaphthalene-3,6-disulphonic acid	Chromotropic acid	$C_{10}H_8O_8S_2$	320.0	2	1.6
15	5,5′-Dihydroxy-2,2′-dinaphthylamine-7,7′-disulphonic acid	Di-I acid	$C_{20}H_{15}O_8NS_2$	461.0	2	3.8
16	7-Aminonaphthalene-1,3,6-trisulphonic acid	7-NH_2-1,3,6-NTSA	$C_{10}H_9O_9NS_3$	383.0	3	1.5
17	8-Aminonaphthalene-1,3,6-trisulphonic acid	Koch's acid	$C_{10}H_9O_9NS_3$	383.0	3	1.7
Other sulphonic acids						
18	2,5-Dichloroaniline-4-sulphonic acid	DCASA	$C_6H_5O_3NSCl_2$	241.0	1	4.7
19	1-(2′,5′-Dichloro-4′-sulpho)-phenyl-3-methylpyrazolone	DCSPMP	$C_{10}H_9O_4N_3SCl_2$	337.0	1	5.2
20	4,4′-Dinitrostilbene-2,2′-disulphonic acid	DNSA	$C_{14}H_{10}O_{10}N_2S_2$	430.0	2	9.0
Anthraquinone sulphonic acids						
21	Anthraquinone-1-sulphonic acid	1-ASA	$C_{14}H_8O_5S$	288.0	1	9.0
22	Anthraquinone-2-sulphonic acid	2-ASA (Silver salt)	$C_{14}H_8O_5S$	288.0	1	13.4
23	1-Aminoanthraquinone-2-sulphonic acid	1-NH_2-2-ASA	$C_{14}H_9O_5NS$	303.0	1	8.1[d]
24	1-Amino-4-hydroxyanthraquinone-2-sulphonic acid	1-NH_2-4-OH-2-ASA	$C_{14}H_9O_6NS$	319.0	1	11.6[d]
25	1-Chloroanthraquinone-2-sulphonic acid	1-Cl-2-ASA	$C_{14}H_7O_5SCl$	322.0	1	16.8[d]
26	1-Amino-4-bromoanthraquinone-2-sulphonic acid	Bromamine acid	$C_{14}H_8O_5NSBr$	381.0	1	13.0[d]
27	Anthraquinone-1,5-disulphonic acid	1,5-ADSA	$C_{14}H_8O_8S_2$	368.0	2	1.7
28	Anthraquinone-1,8-disulphonic acid	1,8-ADSA	$C_{14}H_8O_8S_2$	368.0	2	3.9
29	Anthraquinone-2,6-disulphonic acid	2,6-ADSA	$C_{14}H_8O_8S_2$	368.0	2	2.7

[a] Molecular weight.
[b] Total number of sulphonic and carboxylic acid groups.
[c] Retention time, HPLC conditions: 10% MeOH–90% 5 mM aq. NH_4OAc, flow rate 1 ml/min, others in Experimental.
[d] Mobile phase 40% MeOH–60% 5 mM aq. NH_4OAc, flow rate 1 ml/min.

For HPLC/MS experiments, the chromatographic apparatus consisted of a Model 616 pump, a Model 996 diode-array detector, a Model 717+ autosampler (all from Waters, Milford, MA, USA). An octadecyl silica glass cartridge column, Separon® SGX C18 (150×3 mm I.D., 7 μm particle size) purchased from Tessek Ltd. (Prague, Czech Republic) was used for the separation. The mobile phase contained 10% MeOH and 90% 5 mM aq. NH_4OAc in water. For substituted anthraquinones, 40% MeOH–60% 5 mM aq. NH_4OAc as the mobile phase was used, because in 10% MeOH–90% 5 mM aq. NH_4OAc these compounds were strongly retained on the chromatographic column. The flow rate for HPLC with UV detection was 1 ml/min (see retention times in Table 1). When the MS detection was applied, the flow rate was reduced to 0.6 ml/min to enhance the electrospray response with optimum flow rate after splitting (Fig. 3). The post-column splitting 1:20 was used, so that 30 μl/min of effluent was introduced into the electrospray ion

Table 2
Sulphonated commercial dyes studied in this investigation

No.	Colour index name	Trade name	Molecular formula	MW[a]	Acid groups[b]
30	Acid Yellow 36	Egacid Yellow M	$C_{18}H_{15}O_3N_3S$	353.1	1
31	Acid Blue 40	Egacid Blue A2G	$C_{22}H_{17}O_6N_3S$	451.0	1
32	Acid Violet 7	Egacid Red 6B	$C_{20}H_{18}O_9N_4S_2$	522.0	2
33	Direct Yellow 28	Saturn Yellow LFF	$C_{28}H_{20}O_6N_4S_4$	636.0	2
34	Direct Blue 106	Saturn Blue LB	$C_{30}H_{18}O_8N_4S_2Cl_2$	696.0	2
35	Acid Yellow 23	Egacid Yellow T	$C_{16}H_{12}O_9N_4S_2$	468.0	3
36	Direct Green 28	Saturn Green L5G	$C_{42}H_{30}O_{11}N_{10}S_2$	914.1	3
37	Direct Red 79	Saturn Red L4B	$C_{37}H_{32}O_{17}N_6S_4$	960.0	4
38	Direct Blue 78	Saturn Blue L4G	$C_{42}H_{29}O_{13}N_7S_4$	967.0	4
Metal complex dyes					
39	Acid Orange 142	Rylan Orange R	$C_{32}H_{23}O_{14}N_{10}S_2Cr$	887.0	2
40	Acid Red 357	Rylan Red 3G	$C_{32}H_{23}O_{14}N_{10}S_2Cr$	887.0	2
41	Acid Violet 90	Rylan Bordeaux B	$C_{40}H_{29}O_{10}N_8S_2Cr$	897.0	2
42	Acid Yellow 194	Rylan Yellow 3R	$C_{32}H_{25}O_{16}N_8S_2Co$	900.0	2
43	Acid Brown 355	Rylan Brown B	$C_{36}H_{23}O_{14}N_8S_2Cr$	907.0	2

[a] Molecular weight.
[b] Total number of sulphonic and carboxylic acid groups.

source. Samples were dissolved in the mobile phase and the injection volumes were 10 μl in all cases.

3. Results and discussion

In agreement with previous works [4,9,12,13], our preliminary experiments with ESI and APCI techniques proved that the best sensitivity and signal stability was achieved using the negative-ion ESI. The negative-ion APCI was applicable only for mono- and disulphonated dyes, but the sensitivity was worse. Using positive-ion APCI or ESI, the signal was obtained only for certain compounds with proton-acceptor groups such as hydroxyl, amino or carbonyl. Therefore, the negative-ion ESI mode was selected as the universal ionization technique for MW determination of compounds employed in this study.

Mass spectra of monosulphonated dyes **1–10**, **18**, **19**, **21–26**, **30**, **31** (Tables 1 and 2) were very simple. The sulphonate anions $[M–H]^-$ were the only observed ions in negative-ion ESI mass spectra of each compound. Mass spectra of disulphonated (**11–15**, **20**, **27–29**, **32–36**, **39–43**), trisulphonated (**16**, **17**) and tetrasulphonated (**37**, **38**) dyes were recorded, compounds **35** and **36** also containing one carboxylic acid group. The spectra of compounds with two and more sulphonic carboxylic acid groups showed decationised molecular ions with different charges, e.g. $[M–H]^-$, $[M–2H]^{2-}$, $[M–3H]^{3-}$, or generally $[M\text{-}xH]^{x-}$. The adducts with various number of sodium cations were also observed. Hence, a series of ions were observed, including monocharged ions, $[M–H]^-$, $[M–2H+Na]^-$, $[M–3H+2Na]^-$, dicharged ions, $[M–2H]^{2-}$, $[M–3H+Na]^{2-}$, $[M–4H+2Na]^{2-}$. The general expression is $[M–(x+y)H+y\text{Na}]^{x-}$, where x and y are integers, x is higher than y, and the maximum value of x or $(x+y)$ equals the total number of acid groups. Fig. 1A shows the mass spectrum of disulphonated dye Egacid Red 6B (**32**), where ions $[M–H]^-$ ($m/z=521$), $[M–2H+Na]^-$ ($m/z=543$) and $[M–2H]^{2-}$ ($m/z=260$) are apparent. Ions $m/z=97$ and 179 correspond to $[HSO_4]^-$ and $[M–2H–2HSO_3]^{2-}$, respectively. Similar ions were observed in the spectrum of H-acid (**13**) in Fig. 1B: $[M–H]^-$ ($m/z=318$), $[M–2H+Na]^-$ ($m/z=340$), $[M–2H]^{2-}$ ($m/z=158.5$) and includes the loss of a sulphonic group $[M–H–HSO_3]^-$ ($m/z=237$). Other typical ions of these sulphonated compounds were the odd-electron ion $[SO_3]^{-\bullet}$ ($m/z=80$) and ion $[HSO_4]^-$ ($m/z=97$), whose relative intensity increased with increasing voltage applied on the cone electrode. The ion

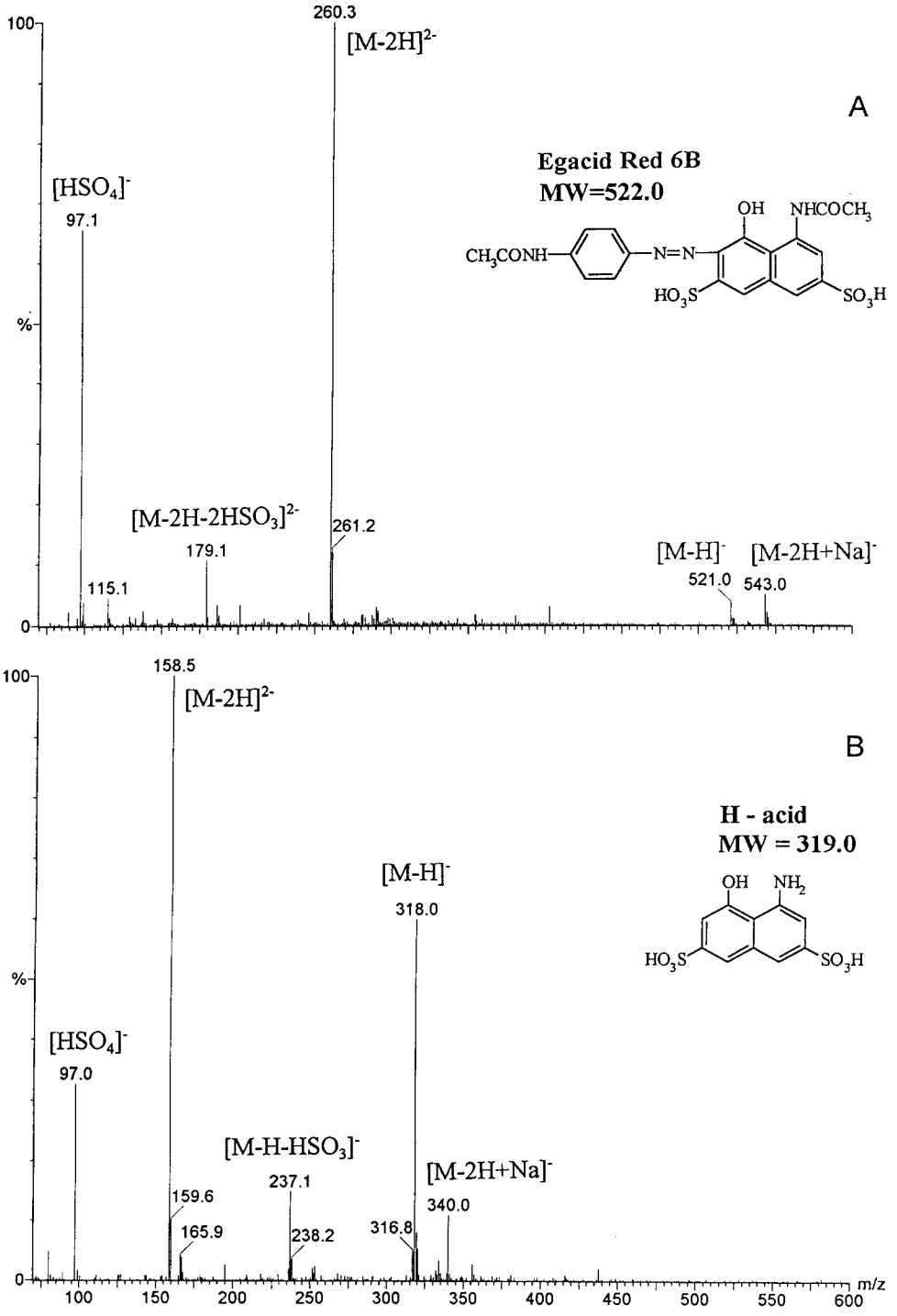

Fig. 1. Negative-ion ESI mass spectra of disulphonated dyes Egacid Red 6B (A) and H-acid (B).

$m/z = 80$ can be used as specific marker for selective monitoring of sulphonic acids.

Fig. 2A shows an example of the mass spectrum of a high molecular weight dye with a complex structure-Saturn Blue L4G (**38**). The calculated masses of dicharged ions are $[M–2H]^{2-}$ ($m/z = 482.5$), $[M–3H + Na]^{2-}$ ($m/z = 493.5$), $[M–4H + 2Na]^{2-}$ ($m/z = 504.5$), tricharged ions $[M–3H]^{3-}$ ($m/z = 321.3$), $[M–4H + Na]^{3-}$ ($m/z = 328.7$) and tetracharged ion $[M–4H]^{4-}$ ($m/z = 240.8$). The maximum charge of four is equal to the total number of the acid groups in the molecule. The total number of the acid groups was further confirmed by the maximum number of exchangeable protons. For example, four protons are dissociated and partly replaced, Fig. 2A, corresponding to (ions $[M–4H + 2Na]^{2-}$ and $[M–4H + Na]^{3-}$).

When analysing an unknown sulphonated compound, the MW can be determined in a manner that is applicable to polycharged peptides. This approach can be illustrated by the following example. Let us suppose that the structure of the compound in Fig. 2A is unknown. First, a series of ions with having the same charge, but differing in the number of sodium ions can be identified. In this case, they are dicharged ions $[M–2H]^{2-}$, $[M–3H + Na]^{2-}$ and $[M–4H + 2Na]^{2-}$, with $m/z = 482.5$, 493.6 and 504.8, or tricharged ions $[M–3H]^{3-}$, $[M–4H + Na]^{3-}$ with $m/z = 321.8$ and 328.9. The mass differences between adjacent peaks are characteristic for each group of ions with the same charge. For monocharged ions $\Delta m/z = Na–H = 22$, for dicharged ions $\Delta m/z = (Na–H)/2 = 11$, for tricharged ions $\Delta m/z = (Na–H)/3 = 7.33$, and so on. In our example, the differences among dicharged ions $m/z = 504.8$, 493.6 and 482.5 are 11.1 and 11.2, the difference between tricharged ions $m/z = 328.9$ and 321.8 is 7.1, which is in a good agreement with the theoretical values. If we choose two deprotonated molecular ions differing in charge by one, then two simple equations apply, where MW and n are unknowns:

$$(m/z)_1 = \frac{MW - n}{n}, \quad (m/z)_2 = \frac{MW - (n+1)}{n+1}.$$

By solving these equations, the correctness of the charge determination can be checked and then MW can be readily calculated from the m/z values of each ion. It is recommended to calculate unknown MW from all identified ions and take into account an average value, to improve the precision. The precision of MW determination depends also on the accuracy of the calibration of the mass spectrometer. The number of acidic groups is equal to the maximum charge ($[M–4H]^{4-}$) of the ion with all acidic protons removed, but this value can be easily determined also from the ions with all acid protons removed and replaced in part by sodium ions (e.g. $[M–4H + Na]^{3-}$ and $[M–4H + 2Na]^{2-}$ ions).

Negative-ion ESI is the only ionization technique, that was successfully utilised for recording the mass spectra of azo dye metal complexes [10,12,26]. The mass spectrum of a 1:2 symmetrical complex of Cr^{3+} with two molecules of monosulphonated azo dye (Rylan Red 3G-**40**) is shown in Fig. 2B. Azo dye metal complexes (**39–43**) did not undergo fragmentation. The molecular weight was calculated the same way as outlined for the analysis of the dye in Fig. 2A. Important ions were $[M–3H + Na]^{2-}$ ($m/z = 453.6$), $[M–2H]^{2-}$ ($m/z = 442.6$) and $[M–3H]^{3-}$ ($m/z = 295.0$). The mass spectra of azo dye metal complexes show lower background noise than the other sulphonated dyes were studied. Chromium (III)-azo dye complexes carry a net negative charge, because of four coordination bonds to the central chromium ion (Cr^{3+}). Monocharged ions were not observed for all azo dye metal complexes and for dyes with more than two sulphonic acid groups. Generally, the relative abundances of low-charged ions decreased with increasing total number of acidic groups. The structures of similar azo dye metal complexes were previously confirmed based on their NMR spectra [32,33]. We have found that NMR and negative-ion ESI–MS are complementary methods for structure elucidation or confirmation of (poly-)sulphonated dyes.

Structures and m/z ratios of the important ions of sulphonated dyes employed in this study are listed in Table 3. Monosulphonic acids are not included, because their spectra contained only $[M–H]^-$ ions. (Poly-)sulphonic acids are completely ionised in aqueous solutions, hence the nature of the cation in the original dye (proton,

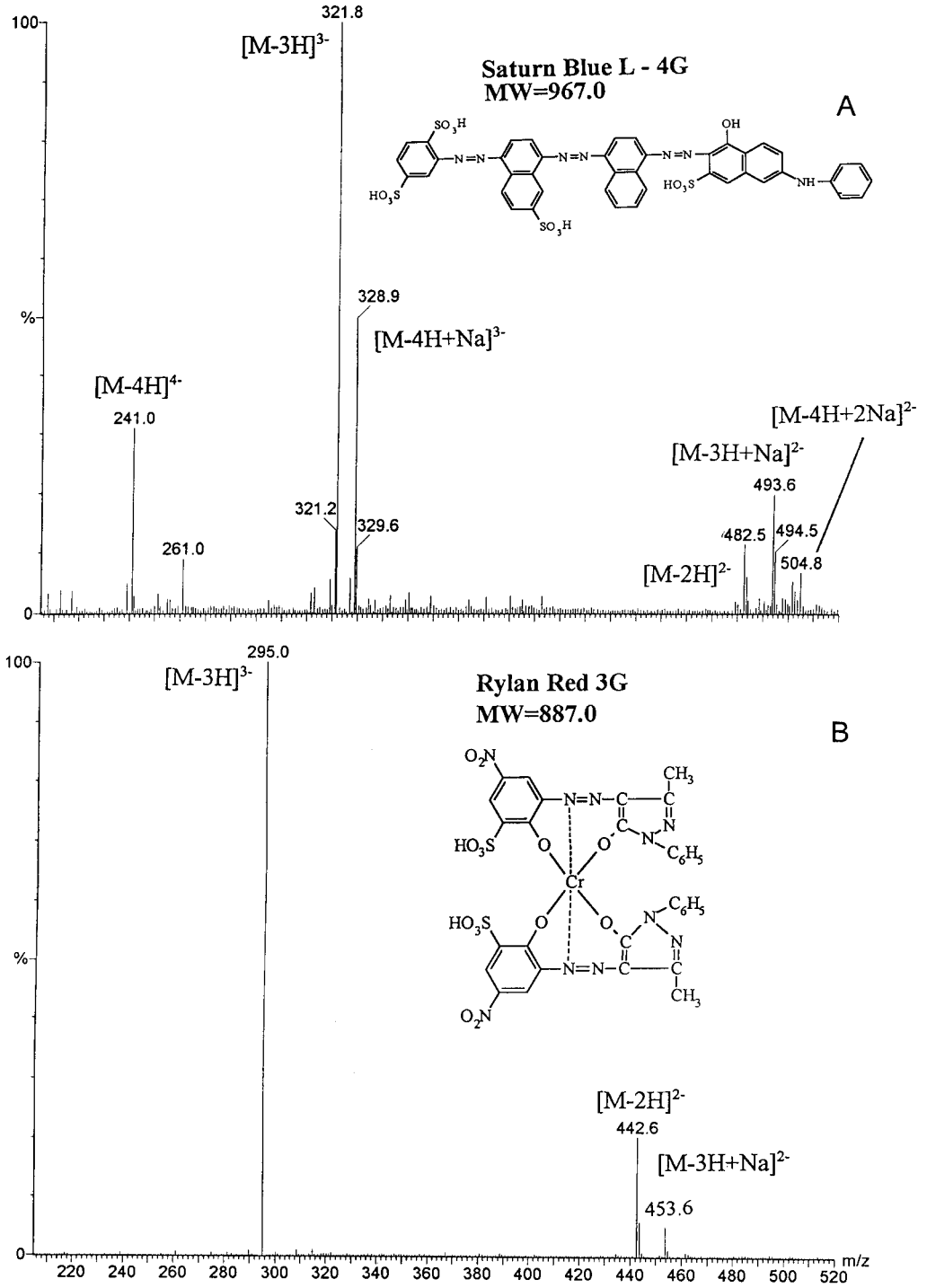

Fig. 2. Negative-ion ESI mass spectra of tetrasulphonated dye Saturn Blue L4G (A) and disulphonated azo dye metal complex Rylan Red 3G (B).

Table 3
Structures and m/z ratios of observed ions of sulphonated dyes and intermediates[a]

No.	m/z	Ion structure	No.	m/z	Ion structure
11	302	$[M–H]^-$		260	$[M–2H]^{2-}$
	221	$[M–H–SO_3H]^-$		179	$[M–2H–2SO_3H]^{2-}$
	150.5	$[M–2H]^{2-}$	**33**	657	$[M–2H+Na]^-$
12	318	$[M–H]^-$		635	$[M–H]^-$
	252	$[M–H–SO_2H_2]^-$		317	$[M–2H]^{2-}$
	237	$[M–H–SO_3H]^-$	**34**	717	$[M–2H+Na]^-$
	158.5	$[M–2H]^{2-}$		695	$[M–H]^-$
13	318	$[M–H]^-$		347	$[M–2H]^{2-}$
	237	$[M–H–SO_3H]^-$	**35**	533	$[M–4H+3Na]^-$
	158.5	$[M–2H]^{2-}$		511	$[M–3H+2Na]^-$
14	341	$[M–2H+Na]^-$		489	$[M–2H+Na]^-$
	319	$[M–H]^-$		467	$[M–H]^-$
	238	$[M–H–SO_3H]^-$		244	$[M–3H+Na]^{2-}$
	159	$[M–2H]^{2-}$		233	$[M–2H]^{2-}$
15	482	$[M–2H+Na]^-$		155	$[M–3H]^{3-}$
	460	$[M–H]^-$	**36**	467	$[M–3H+Na]^{2-}$
	229.5	$[M–2H]^{2-}$		456	$[M–2H]^{2-}$
16	382	$[M–H]^-$		303.7	$[M–3H]^{3-}$
	201.5	$[M–3H+Na]^{2-}$	**37**	501	$[M–4H+2Na]^{2-}$
	190.5	$[M–2H]^{2-}$		490	$[M–3H+Na]^{2-}$
	150	$[M–2H–SO_3H]^{2-}$		479	$[M–2H]^{2-}$
	126.7	$[M–3H]^{3-}$		326.3	$[M–4H+Na]^{3-}$
17	382	$[M–H]^-$		319	$[M–3H]^{3-}$
	190.5	$[M–2H]^{2-}$		239	$[M–4H]^{4-}$
	150	$[M–2H–SO_3H]^{2-}$	**38**	504.5	$[M–4H+2Na]^{2-}$
	126.7	$[M–3H]^{3-}$		493.5	$[M–3H+Na]^{2-}$
20	451	$[M–2H+Na]^-$		482.5	$[M–2H]^{2-}$
	429	$[M–H]^-$		328.7	$[M–4H+Na]^{3-}$
	214	$[M–2H]^{2-}$		321.3	$[M–3H]^{3-}$
	199	$[M–2H–NO]^{2-}$		240.8	$[M–4H]^{4-}$
	191	$[M–2H–NO_2]^{2-}$	**39**	453.5	$[M–3H+Na]^{2-}$
27	389	$[M–2H+Na]^-$		442.5	$[M–2H]^{2-}$
	367	$[M–H]^-$		294.7	$[M–3H]^{3-}$
	286	$[M–H–SO_3H]^-$	**40**	453.5	$[M–3H+Na]^{2-}$
	183	$[M–2H]^{2-}$		442.5	$[M–2H]^{2-}$
	151	$[M–2H–SO_2]^{2-}$		294.7	$[M–3H]^{3-}$
28	389	$[M–2H+Na]^-$	**41**	458.5	$[M–3H+Na]^{2-}$
	367	$[M–H]^-$		447.5	$[M–2H]^{2-}$
	183	$[M–2H]^{2-}$		298	$[M–3H]^{3-}$
29	367	$[M–H]^-$	**42**	460	$[M–3H+Na]^{2-}$
	286	$[M–H–SO_3H]^-$		449	$[M–2H]^{2-}$
	183	$[M–2H]^{2-}$		299	$[M–3H]^{3-}$
	151	$[M–2H–SO_2]^{2-}$	**43**	463.5	$[M–3H+Na]^{2-}$
32	543	$[M–2H+Na]^-$		452.5	$[M–2H]^{2-}$
	521	$[M–H]^-$		301.3	$[M–3H]^{3-}$

[a] Ions with $m/z = 80$ and 97 ($[SO_3]^{-\bullet}$ and $[HSO_4]^-$) are typical for most sulphonated compounds and are not included in this table.

sodium or potassium ion) do not influence mass spectra and the type of the cation cannot be determined this way. Therefore, regardless of the cation present in the sulphonic groups of the dyes, they are written in the free acid form $–SO_3H$ (Tables 1 and 2).

To record the reported mass spectra, dye concentrations were in the range of 50–300 mg/l. No

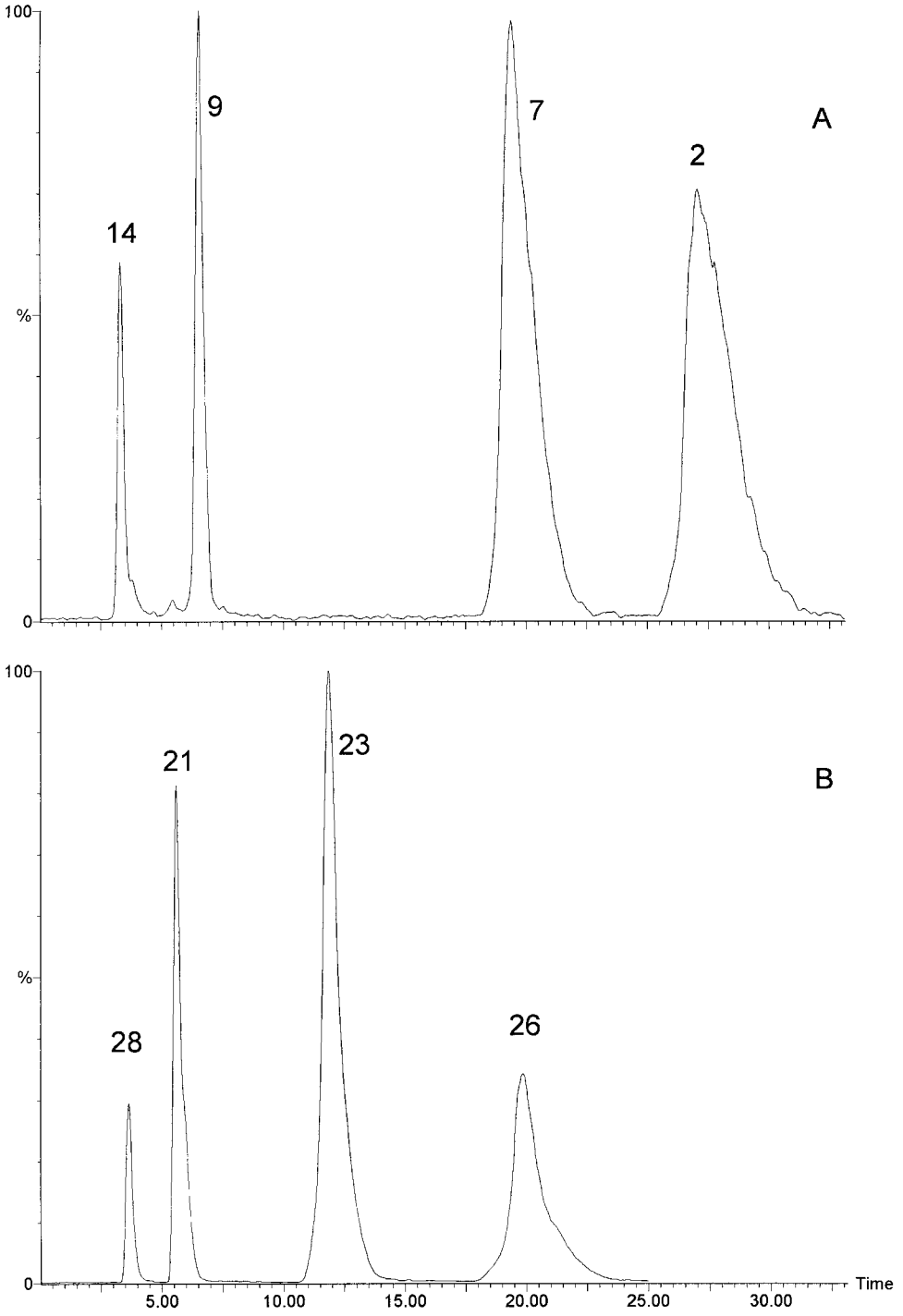

Fig. 3. Reconstructed ion current chromatograms of $[M–H]^-$ ions of naphthalene sulphonic acids **2**, **7**, **9**, **14** (A) and anthraquinone sulphonic acids **21**, **23**, **26**, **28** (B). HPLC conditions: the mobile phase 10% MeOH–90% 5 mM aq. NH_4OAc (A) or 40% MeOH–60% 5 mM aq. NH_4OAc (B), the column Separon® SGX C18 (150×3 mm) and the flow rate 0.6 ml/min.

significant differences in sensitivity were observed for dyes of various structural types. The limits of detection of the analysed dyes was more than one order of magnitude lower than the concentrations used. Further improvement in the sensitivity can be achieved using the selected ion monitoring (SIM) mode. There were no differences between the appearance of mass spectra measured by flow injection analysis versus HPLC/MS, using eluents consisting of 10–50% MeOH in 5 mM aqueous NH_4AOc.

HPLC/MS makes possible the separation and MW determination of individual compounds in complex mixtures. This method is not compatible with mobile phases containing non-volatile additives. Consequently, salting-out or ion-pairing agents are not recommended. By using NH_4OAc at low concentrations, the retention of ionic compounds is increased. Separations involving mobile phases containing MeOH 5 mM aq. NH_4OAc can be achieved for mono- and disulphonated dyes. The reconstructed ion current chromatograms of $[M–H]^-$ ions for model mixtures of naphthalene sulphonic acids **2**, **7**, **9** and **14** and of anthraquinone sulphonic acids **21**, **23**, **26** and **28** are shown in Fig. 3. The mobile phase consisting of 10% MeOH was suitable for the analysis of dye intermediates listed in Table 1, except for substituted monosulphonated anthraquinones. Retention of the anthraquinones was too great under these conditions. A mobile phase consisting of 40% methanol–60% 5 mM aq. NH_4OAc gave better results for the separation of monosulphonated anthraquinones, as shown on Fig. 3B.

The retention times of dyes with more than two sulphonic acid groups were close to the dead volume of the chromatographic system, so that their resolution was only partial. For many commercial dyes listed in Table 2, the retention behaviour proved difficult to reproduce, as tailing peaks highly retained were observed.

Bas on preliminary experiences with (poly-)sulphonated intermediates [17], we anticipate that CZE will be suitable for routine analysis of complex polysulphonated compounds. CZE/MS analysis of polysulphonated dyes will be subjected to our further work in this area.

Conclusions

Negative ion electrospray mass spectrometry is currently the most suitable ionization technique for determining the molecular weight and the total number of sulphonic and carboxylic acid groups in (poly-)sulphonated dyes. Spectra produced via this method are easy to interpret, because they contain only decationised molecular ions with different number of charges. In many cases, the determination of the molecular weight and the total number of acid groups was sufficient for the structure elucidation of unknown impurities or by-products in the reaction mixture. The combination of high-performance liquid chromatography-electrospray mass spectrometry was employed for the separation and molecular weight determination of individual components in certain mixtures of mono- and disulphonated compounds. For dyes with quite complex structures and/or more than two sulphonic acid groups, the HPLC separation often failed. It is possible that capillary zone electrophoresis will be a more effective method for the separation stage.

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