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Identification of reactive dyes in spent dyebaths and wastewater by capillary electrophoresis–mass spectrometry

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Abstract

Capillary electrophoresis with diode array detection and mass spectrometry combined with solid-phase extraction were employed for the identification of reactive vinylsulfone and chlorotriazine dyes and their hydrolysis products in spent dyebaths and raw and treated wastewater. Recoveries of dyes from treated wastewater as their tetrabutylammonium ion-pairs using C_{18} reversed-phase cartridges ranged from 81 to 121%. Detection limits in sewage effluent of the different dyes and hydrolysis products ranged from 23 to 42 μg/l. The method was successfully applied to the detection of the hydrolysis products of five reactive dyes in influents and effluents of a municipal wastewater treatment plant receiving dyehouse effluents.

Keywords: Water analysis; Environmental analysis; Dyes; Vinylsulfones; Chlorotriazines; Triazines

1. Introduction

Dyes are a large group of industrial chemicals for which production in 1994 was estimated at $1 \cdot 10^9$ kg/year [1] Most of this quantity is used by the textile industry and includes many hundreds of compounds in various application classes (e.g. acid, direct, reactive, disperse dyes, etc.), of which only very few have a worldwide production of more than $1 \cdot 10^6$ kg/year [2]. Reactive dyes, which will be the focus of this study, contain anionic colorants that form covalent bonds to the substrate (mainly cellulosic fibers). Examples typical of reactive dye structures are given in Fig. 1.

Vinylsulfone reactive dyes may be sold in the vinyl sulfone form or as the sulfato-precursors shown in Fig. 1. In the dyebath under alkaline conditions, the sulfato group is readily released to form the reactive vinylsulfone group (Fig. 2) which then undergoes nucleophillic addition with ionized hydroxyl groups of the cellulose. Chlorotriazine dyes react with nucleophiles by a substitution mechanism. Under alkaline conditions in a dyebath they form a covalent bond with the substrate by nucleophillic substitution with ionized hydroxyl groups of cellulose (Fig. 2).

Important side-reactions of both reactive groups are the nucleophilic addition of water to yield either the alcohol or hydroxytriazine. Since the hydroxy forms are no longer reactive, some manufacturers

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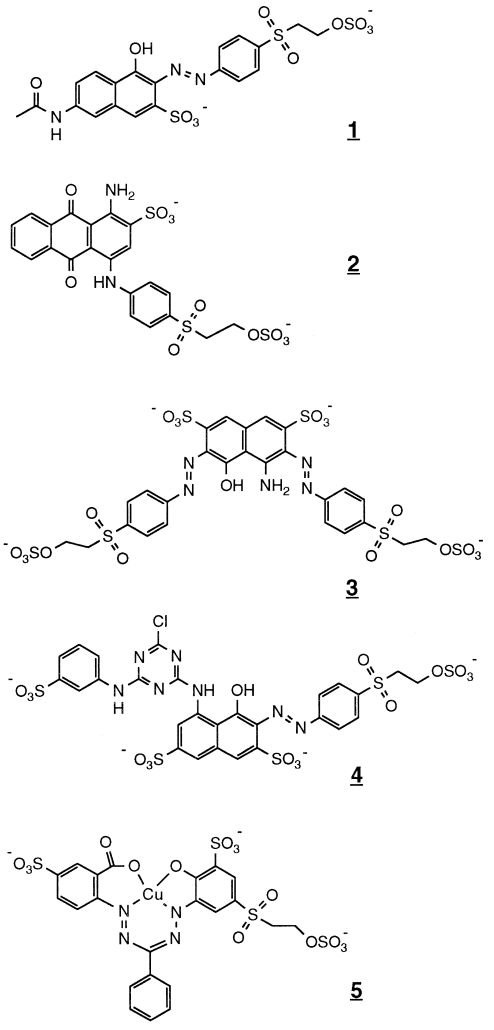

Fig. 1. Reactive dyes investigated in this study.

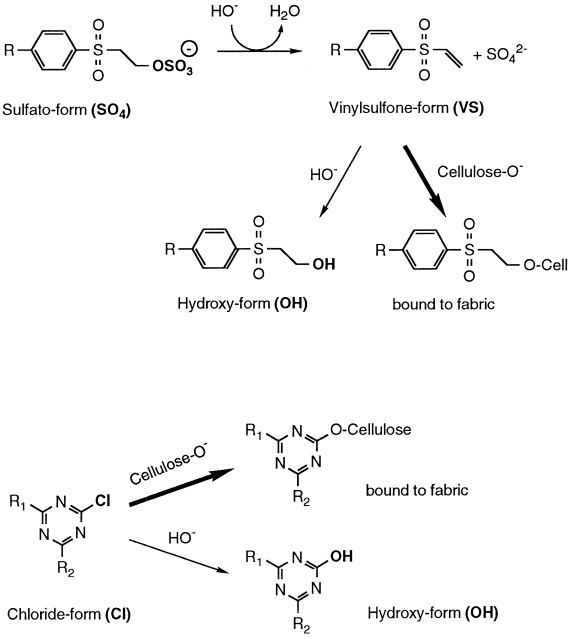

Fig. 2. Reactions of vinylsulfone and chlorotriazine reactive dyes.

have introduced multifunctional dyes (dyes with several reactive groups) to improve the yield of bound versus hydrolyzed dye (for example 3 and 4, Fig. 1). Other side-reactions of vinylsulfone dyes are reported in the literature, such as formation of ethers of the form dye–O–dye [3,4].

Estimates indicate that approximately 12% of dyes are discharged with textile wastewater [5]. Despite their widespread use and the associated potential hazards of dyes, dye impurities, and metabolites to human and environmental health, little is known about the fate of dyes in sewage treatment or in the aquatic environment. This is partly because, until recently, suitable analytical techniques for the trace analysis of ionic compounds in complex mixtures, such as wastewater, were unavailable.

Because most dyes, and particularly ionic sulfonated dyes are non-volatile compounds, they are not amenable to analysis by gas chromatography–mass spectrometry. Instead, methods based on paper and thin-layer chromatography and, more recently, high-performance liquid chromatography (HPLC) [6–10] and capillary electrophoresis (CE) [11–15] were developed. Mass spectrometry (MS) with different inlet systems including thermospray [16], fast atom bombardment (FAB) [17–19], liquid secondary ion mass spectrometry (LSI-MS) [20–22] and various atmospheric pressure ionization techniques were used as such or coupled to HPLC [23–26] or CE [27–29]. For the analysis of sulfonated dyes, the coupling of CE and negative-ion electrospray ionisation (ESI)-MS currently appears to be the most selective approach with acceptable sensitivity [30].

Investigations on the fate of dyes in the environment are complicated by a number of factors, one of the most important being the great number of dyes in use, many of which are mixtures of different colorants. Another important factor is that the structures

of modern dyes are typically treated as trade secrets, a fact that normally prevents development of suitable analytical methods or interpretation of data in a meaningful way. On the other hand, dyes for which structures are known may not have much commercial relevance. Further, dyes are typically used in batch processes, and day-to-day usage varies greatly, depending on market demand, thus leading to release of very complex mixtures with frequently changing composition.

Considering the above-mentioned obstacles for studies of dyes in real samples it is not surprising that most analytical methods for dyes are performed with spiked sewage and drinking water, and only very few analyses of real samples are reported in the literature [7,8,31–33]. The aim of the project reported here was to develop a sensitive and selective analytical method based on CE–MS for currently used vinylsulfone and chlorotriazine reactive dyes and to identify these dyes and some of their transformation products in spent dyebaths, dyehouse effluents, and sewage effluents.

2. Experimental

2.1. Dye samples

Dyes were supplied by textile dyeing facilities located in Washington (GA, USA). In all cases, dye structures were provided by the manufacturers on condition that structures could not be used with commercial names. Dye structures that are not in the public domain are thus given without names (Fig. 1).

Vinylsulfones were prepared from their sulfato precursors by the following procedure. Samples of 15 mg dye were dissolved in 2 ml of 0.01 *M* sodium hydroxide for 2 h at room temperature. Then 1 ml of 1 *M* sodium phosphate buffer (pH 6.5) and 100 μl of 0.5 *M* tetrabutylammonium phosphate (TBA) were added. The product was isolated by solid-phase extraction (SPE, see below).

The hydroxy forms were prepared in the same way as the vinylsulfones except that 0.1 *M* sodium hydroxide was used and the mixture was kept at 70°C in a water bath for 2 h. Then, the solution was neutralized with 2 ml of 0.1 *M* hydrochloric acid and 1 ml of 1 *M* phosphate buffer (pH 6.5) and subjected to SPE after addition of 100 μl of 0.5 *M* TBA. The resulting methanol–water (70:30, v/v) solutions of isolated product were stable for months when stored at 4°C. Standards for CE were made up by dilution of stock solutions in acetonitrile–water (1:1, v/v).

2.2. Dye bath samples

Samples (40 ml) of liquor containing reactive dyes were taken directly from the dyebaths before and after dyeing. Samples were immediately neutralized by adding 1 ml of 1 *M* sodium phosphate buffer (pH 6.5) and titration to pH 6.5 with 1 *M* hydrochloric acid. Samples of 10 ml were mixed with 100 μl 0.5 *M* TBA and immediately subjected to SPE to prevent any transformation of reactive dyes during transportation to the laboratory. The extracts were stored on ice during transportation and kept at 4°C until analyzed by CE.

2.3. Wastewater samples

Special care was taken when dealing with wastewater samples. To avoid skin contact, particularly with raw wastewater, surgical gloves were worn at all times during sampling and sample preparation. Effluent samples were taken by automatic sampling devices as 24 h flow-proportional composites at the sewage outfall of the municipal sewage treatment plant in Washington. Grab samples of raw wastewater were obtained from the same facility. A major portion of influent to this plant is from two textile mills both of which have dyeing operations. Wastewater samples were stored at 4°C and analyzed within 1–2 days of sampling to avoid anaerobic degradation of azo dyes to the corresponding amines [34].

2.4. Solid-phase extraction

Reversed-phase C_{18} bonded silica SPE cartidges (Supelco, Bellefonte, PA, USA) were attached to a 12-port vacuum manifold (Baker, Philippsburg, NJ, USA) and conditioned with two bed volumes of methanol, four bed volumes of water and two bed volumes of 5 m*M* TBA. Dyebath samples (10–30 ml) and preparations of vinylsulfone and hydroxy-forms of dyes (3–5 ml) were extracted using 3-ml

cartridges containing 500 mg packing material. After extraction, the cartridges were rinsed with 2 ml of water and eluted with 2 ml of methanol–water (70:30). For CE, extracts were diluted at least 1:1 with acetonitrile–water (1:1).

Raw sewage (200 ml) and sewage effluent (1 l) samples were filtered through glass fiber filters (Type A/E, Gelman, Ann Arbor, MI, USA), 5 mMol/l TBA were added, and the samples were passed through preconditioned SPE cartridges (6 ml/1000 mg). The cartridges were then rinsed with 2 ml of water and sequentially eluted with 7 ml each of methanol–water (40:60), (50:50) and (70:30). All extracts were evaporated separately to dryness under a stream of air with mild heating (40°C). The dry residues were redissolved in 2 ml acetonitrile–water (1:1), transfered to polypropylene microcentrifuge tubes (Elkay, Shrewsbury, MA, USA) and centrifuged for 10 min at 14 000 rpm. For CE–MS, in some cases, excess TBA was removed by percolating the sample through a 3-ml cartridge containing 500 mg of strong cation-exchange resin (Baker) previously rinsed with 2 ml of acetonitrile–water (1:1).

2.5. Capillary electrophoresis

All analyses were performed on a P/ACE 5500 capillary electrophoresis system with diode-array detection (DAD) system (Beckman, Fullerton, CA, USA). Untreated fused-silica capillaries were obtained from Supelco. Capillaries of 110 cm (20 cm to the DAD system)×50 μm I.D. were used for CE–MS experiments, whereas 57 cm (50 cm to detector)×50 μm I.D. capillaries were used for CE alone. New capillaries were rinsed with buffer for 20 min. Between runs, capillaries were rinsed with buffer for 5 min. Acid and base rinses usually employed in CE were not used in CE–MS experiments, because it took in excess of 20 min to completely remove any residues of the rinses. A buffer stock solution was prepared at a concentration of 0.1 *M* by titrating ammonium acetate solution with ammonium hydroxide to pH 9. The run buffer was prepared by dilution with water and acetonitrile to yield a buffer with 30% acetonitrile and 30 m*M* ammonium acetate (CE–DAD) or 40% acetonitrile and 5 m*M* ammonium acetate (CE–MS). Samples were introduced by pressure injection (0.5 p.s.i.; 1 p.s.i.=6894.76 Pa) for 2 s (CE alone) and 15 s (CE–MS). The separation voltage was 30 kV. DAD was at 500 nm with a wide bandwidth of ±100 nm.

2.6. Capillary electrophoresis–mass spectrometry

A Fisons Platform II (Micromass, Manchester, UK) quadrupole mass spectrometer, fitted with an electrospray source and a CE probe was connected to a P/ACE® 5500 CE system (Beckman, Fullerton, CA, USA). The CE probe consisted of a triaxial arrangement with the CE capillary surrounded by a stainless steel capillary supplying the sheath liquid, and another stainless steel capillary supplying the sheath gas. The sheath liquid (isopropanol–water, 80:20) was supplied by an Isco 100D syringe pump (Lincoln, NE, USA) at a flow-rate of 2 μl/min. Sheath and drying gas were both industrial grade nitrogen at flow-rates of 20 and 50 l/h, respectively. The source temperature was maintained at 80°C.

The electrospray voltage optimized between −3 and −3.5 kV, resulting in an overall separation voltage of −30 to −33.5 kV, the high voltage lens was set to 0.1 kV, the skimmer lens offset was 5 V, and the multiplier was set to 650 V. High and low mass resolution were set to 14.5, the ion energy was set to 1, which provided approximately unit mass resolution. The cone voltage was set to 20 V to maximize sensitivity. Under these conditions, little or no fragmentation occurred in the ion source. During injection, the electrospray voltage was set to 0 kV to avoid discrimination of ions entering the capillary. The mass spectrometer was calibrated in the negative ion mode using sodium iodide in 80% isopropanol. Optimization of the operating conditions of the interface followed by suggestions by Kirby et al. [35] and was the same as for the analysis of anionic metallized dyes and is described in more detail elsewhere [42].

3. Results and discussion

3.1. Capillary electrophoresis

Optimization of the CE separation conditions for the reactive dyes involved variation of buffer substances and concentration, pH, and organic modifier

content. Addition of other buffer components, such as surfactants and cyclodextrin, as proposed by other researchers [14,36], did not improve the separation of the dyes selected for this study. The buffer pH optimized in the range of 8–10. Therefore, a pH of 9 was used for subsequent experiments. In view of the further application of the method in CE–MS, volatile buffer substances were given preference. Ammonium acetate buffer at concentations between 5 and 30 m*M* was found to yield more symmetric peaks and better resolution than ammonium formate. Ammonium acetate also was found to compare favorably with non-volatile buffers such as sodium citrate, potassium phosphate or sodium borate (data not shown). Peak shapes of dyes were not sensitive to the buffer concentrations (range 5–30 m*M*) when injecting dilute standard solutions. However, when injecting sewage extracts, a pronounced broadening of the dye peaks in the electropherograms was observed, most likely because of co-extraction of a large amount of ionic material. To minimize peak broadening for dyes in sewage extracts, due to electrodispersion, a buffer concentration of 30 m*M* was used.

Addition of an organic modifier to the run buffer was by far the most important factor affecting separation efficiency for the dyes. Among the organic solvents tested (acetonitrile, methanol, acetone and isopropanol), acetonitrile gave the best results. Fig. 3 illustrates the effect of various acetonitrile concentrations on the separation of some reactive dyes. Addition of acetonitrile (as most other organic solvents) reduces the electroosmotic flow, thus, increasing the migration times of the dyes and improving peak resolution. This is in agreement with the theoretical prediction that resolution is highest when electrophoretic mobilities of the analytes are approximately equal to the electroosmotic flow, but in the opposite direction [37]. Increased resolution is obtained, however, at the cost of lengthier separations.

The influence of acetonitrile on the peak shape of some reactive dyes is illustrated for the hydroxy forms of dyes **1** and **2** (enlarged section in Fig. 3). At low acetonitrile content the peaks are broad and triangular shaped, typical of electrodispersion. However, at increased acetonitrile content, the peaks become sharper and higher while the dye concentration remains the same. Therefore, the poor peak

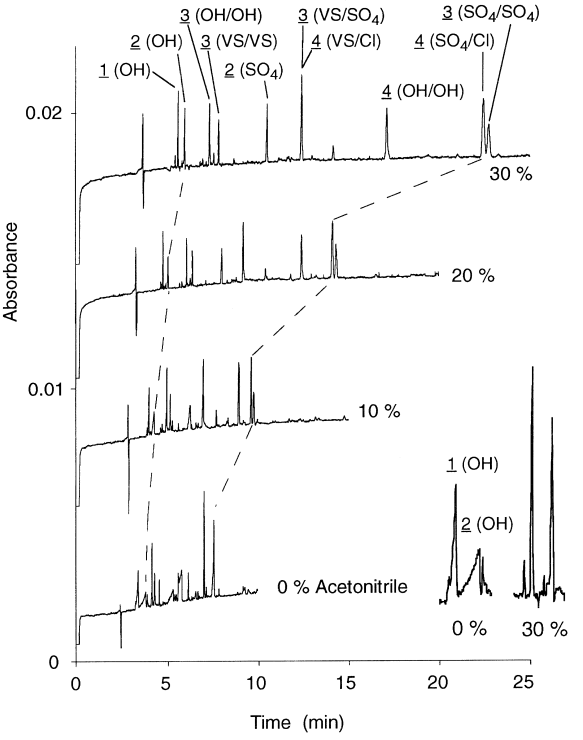

Fig. 3. Influence of acetonitrile on the separation of selected reactive dyes by CE–DAD. Concentration of the dyes: 50 mg/l. Peak annotation: dye number as in Fig. 1 with the dye form in parentheses as in Table 1.

shapes most likely are not caused by overloading. Another possible explanation is the tendency of many dyes to aggregate in solution [38]. Aggregates would have slightly different migration behavior and could cause the broadening of peaks observed at low acetonitrile content. Higher acetonitrile content reduces the tendency of dyes to aggregate and would thus improve peak shapes [39,40].

The difference in migration behavior of hydrolyzed and unhydrolyzed reactive dyes is best illustrated in Fig. 3 by the example of dyes **3** and **4**. Loss of the sulfato groups of **3** and **4** leads to the formation of less negatively charged species that migrate more slowly. Because the negatively charged dyes migrate against the electroosmotic flow, the slower migrating vinylsulfone forms of the dyes actually ‘elute’ earlier than their more negatively charged, faster migrating sulfato forms. The dihydroxy form of **3** migrates even slower than the

corresponding vinylsulfone. This may be explained by increased hydration of the hydroxyl groups and thus, a smaller charge-to-size ratio as compared to the vinylsulfone. The hydroxytriazine form of **4,** on the other hand, migrates faster than the corresponding chlorotriazine, indicating that the hydroxytriazine is at least partially deprotonated at the pH of the CE buffer (pH 9) [41].

3.2. Capillary electrophoresis–mass spectrometry

The CE separation conditions were slightly adapted for CE–MS coupling, to maximize sensitivity. A lower buffer concentration (5 vs. 30 m*M*) was used, which resulted in speedier analyses and, most importantly, in a 4-fold increase in MS signal intensities for the dyes. The lower buffer concentration, however, lead to some loss in separation efficiencies for dyes due to elecrodispersion, when analyzing sewage extracts. In Fig. 4, electropherograms of a sewage extract (a–d) and a reference mixture of dyes (reconstructed ion chromatogram, RIC, e) are shown. As shown in Fig. 4, the peaks for the dyes in sewage extract are broader than those in the reference mixture. Particularly the early eluting dye **2** (OH) showed peak broadening in the sewage extract.

Electrospray is a soft ionization technique and thus typically yields mass spectra that do not provide much structural information, as demonstrated in earlier work [25]. The most abundant ions in the mass spectra of the dyes studied here are listed in Table 1. Typically, the mass spectra contained only singly or multiply charged ions corresponding to the intact dye as in aqueous solution and, in some cases, sodium ion and proton adducts. The charge of the ions corresponded to the number of sulfonate groups in the molecule, less the number of protons and sodium ions in the adducts. Adduct formation increased with increasing number of sulfonate groups in the dye. The sulfato forms of all dyes fragmented to some extent to their respective vinylsulfone forms, which is analogous to the reaction in alkaline solution.

The simplicity of the mass spectra is an asset for the detection of the dyes in sewage for two reasons. The first is that a set of compounds with widely differing molecular masses (470–900) can be de-

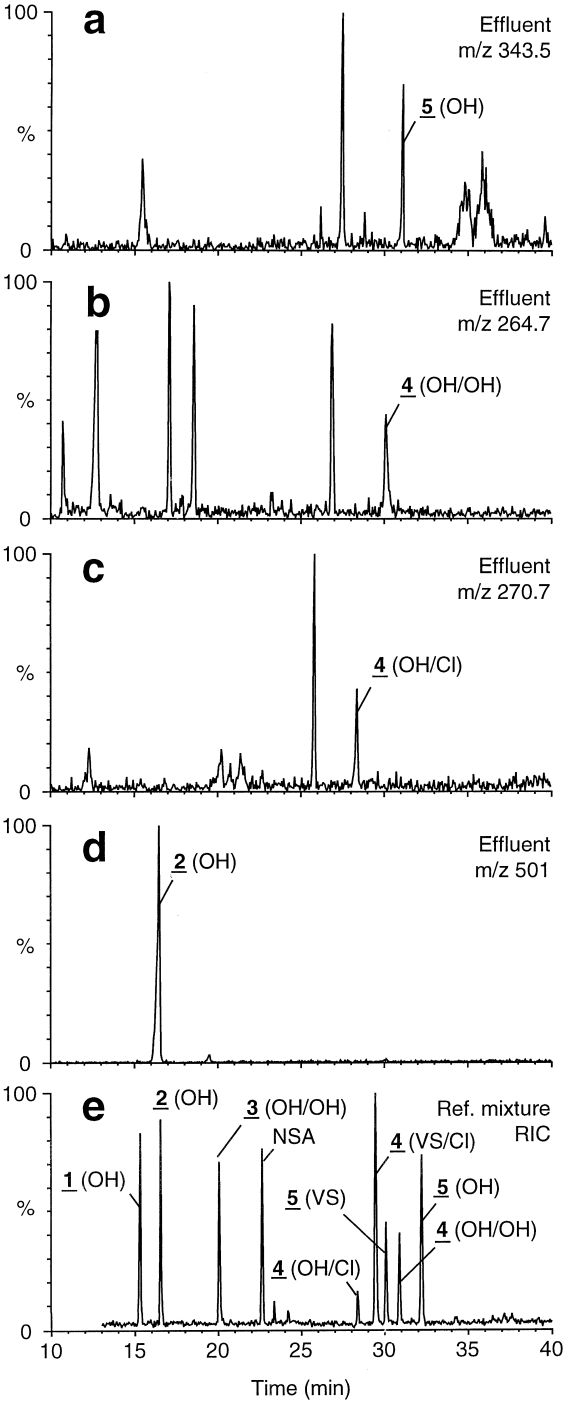

Fig. 4. Separation of an extract of sewage effluent (mass traces, a–d) and a reference mixture of reactive dyes (reconstructed ion chromatogram, RIC, e) by CE–MS. Concentration of the dyes in the reference mixture: 50 mg/l; peak annotation as in Fig. 3; NSA, naphthalene sulfonic acid.

Table 1
Fragment ions and their relative abundance (RA) in the mass spectra of some reactive dyes in CE–MS[a]

Dye (form)[b]	M_r[c]	m/z	Fragment ions	RA (%)
1 (OH)	492.1	492.1	M^-	100
1 (VS)	474.0	474.0	M^-	100
1 (SO_4)	571.0	285.5	M^{2-}	100
		474.0	$[M-HSO_4]^-$	9
2 (OH)	501.0	501.0	M^-	100
2 (VS)	483.0	483.0	M^-	100
2 (SO_4)	580.0	290.0	M^{2-}	100
		483.0	$[M-HSO_4]^-$	10
3 (OH/OH)	741.0	370.5	M^{2-}	100
3 (VS/VS)	705.0	352.5	M^{2-}	100
3 (VS/SO_4)	802.0	267.3	M^{3-}	100
		401.5	$[M+H]^{2-}$	7
		412.5	$[M+Na]^{2-}$	2
		352.5	$[M-HSO_4]^{2-}$	10
3 (SO_4/SO_4)	898.9	224.7	M^{4-}	100
		300.0	$[M+H]^{3-}$	26
		307.3	$[M+Na]^{3-}$	9
		267.3	$[M-HSO_4]^{3-}$	28
		352.5	$[M-2HSO_4]^{2-}$	2
4 (OH/OH) and (VS/Cl)	794.0	264.7	M^{3-}	100
		397.5	$[M+H]^{2-}$	20
		408.5	$[M+Na]^{2-}$	3
4 (OH/Cl)	812.0	270.7	M^{3-}	100
		406.5	$[M+H]^{2-}$	22
		417.5	$[M+Na]^{2-}$	4
5 (OH)	686.9	228.6	M^{3-}	100
		343.5	$[M+H]^{2-}$	80
		354.5	$[M+Na]^{2-}$	12
5 (VS)	667.9	222.6	M^{3-}	100
		334.5	$[M+H]^{2-}$	70
		345.5	$[M+Na]^{2-}$	10

[a] Cone voltage = 20 V (low collision energy).
[b] Number refers to dye number in Fig. 1. Dye forms: OH, hydroxy; VS, vinylsulfone; SO_4, sulfato; Cl, chlorotriazine.
[c] Molecular mass of the dye anion.

tected by scanning a relatively narrow molecular mass range (220–500). The second advantage is that most of the ions formed in the electrospray source can be observed at one or two masses. For this study, scanning the molecular ion plus, in some cases, a proton adduct, was adequate to identify the dyes in sewage extracts. However, for structural information, in-source fragmentation [26,42] and MS–MS techniques can be applied [24,26,27].

Detection limits of the dyes and hydrolysis products ranged from 45 to 150 pg, illustrating the excellent sensitivity of the CE–MS coupling for dyes (Table 2). However, very small volumes of sample are injected (~ 12 nl), so that the few picograms required for detection still correspond to rather high concentrations of 4 to 12 mg/l. Therefore, an effective method for the preconcentration of dyes is needed for their detection in wastewater samples.

3.3. Solid-phase extraction

At first thought, the logical choice for the extraction of anionic dyes from water samples would be an anion-exchange resin. In fact, anion-exchange

Table 2
Detection limits (LODs) of reactive dyes in CE–MS

Dye (form)[a]	m/z[b]	Standard solution LOD (pg)	Wastewater LOD (μg/l)[c]
1 (OH)	492.1	48	24
1 (VS)	474.0	53	27
2 (OH)	501.0	45	23
2 (VS)	483.0	51	26
3 (OH/OH)	370.5	90	30
3 (VS/VS)	352.5	96	32
4 (OH/OH)	264.7	150	42
4 (VS/Cl)	264.7	98	38
4 (OH/Cl)	270.7	94	36
5 (OH)	343.5	147	28
5 (VS)	334.5	153	33

[a] See Table 1.
[b] Detected ion (scan range m/z 200–600).
[c] Extracted from 1 l sewage effluent ($S/N=3$).

resins are excellent at extracting sulfonated dyes from wastewater. However, very harsh elution conditions (strong base) are necessary to recover the dyes from the resin, which likely would also lead to partial hydrolysis. In addition, the eluents needed for elution of the dyes from anion-exchange resins would result in samples with high ionic strength, which would cause peak broadening due to electrodispersion, and thus lead to poor CE separations. Therefore, after some preliminary experiments, we concluded that anion-exchange resins could not be used for preconcentration of reactive dyes, particularly when CE was used for analysis.

Anionic dyes also can be efficiently extracted from aqueous samples as ion-pairs using reversed-phase C_{18} SPE, as was already shown by other researchers [23]. Extraction is also possible without ion-pairing reagents, but may result in losses of the more polar, highly sulfonated dyes [24,26,29]. Using 5 m*M* TBA, up to 1 l of strongly colored wastewater could be extracted onto 1 g C_{18} SPE cartridges without visible breakthrough of dyes. Elution of dyes from C_{18} cartridges under mild conditions yielded samples with low salt content, which is compatible with CE separations. Residual ion-pair reagents in the extracts did not interfere with the CE separation as long as small volumes of extracts were injected (not more than 12 nl or 2 s pressure injection). If the ion-pair reagent was found to interfere with the separation (retention time shift), it was readily removed by percolation of the sample through a small cartridge containing a strong cation-exchange resin. With a final extract volume of 2 ml, concentration factors of 100 (raw wastewater) and 500 (effluent) were achieved. The detection limits, however, did not decrease as much as the concentration increased, because the peaks were broader for wastewater samples than for standard solutions.

Although the study presented here was essentially qualitative and was not aimed at quantitative determination of dyes, we determined recoveries of some reactive dyes from 1 l sewage effluent samples to demonstrate the feasibility of the extraction procedure (Table 3). As shown, recoveries between 81 and 121% were obtained. Recoveries of <100% are, most likely, due to incomplete elution from the SPE material rather than breakthrough, because the dyes formed a visible dark band at the top of the extraction cartridge. This band became wider as the extraction proceeded, but did not reach the bottom of the cartridge before the extraction was completed. However, some color remained on the SPE material even after prolonged rinses of the cartridges with 70% methanol, indicating that some of the dyes were bound more strongly to the packing material. Lower recoveries of the vinylsulsulfones as compared to the respective hydroxy forms also point to formation of covalent bonds with the solid phase.

Reversed-phase SPE was also used for desalting of hydrolysis products of reactive dyes and for isolation of dyes from dyebath samples. Without desalting, only very poor separations were achieved with these samples. Recoveries for reactive dyes and products from 40-ml dyebath samples are listed in Table 3. Recoveries fell within a narrow range of 92–103%, with much lower variability than those for wastewater samples, most likely because the much higher dye concentrations lead to a correspondingly smaller fraction of dye lost due to strong binding to the solid-phase material.

3.4. Application to dyebaths

In view of the number of dyes used even in small dyehouses, it is crucial to know which dyes may be present (and in what form) before analyzing effluent samples. Just due to reaction with water, some bifunctional reactive dyes could be discharged in six

Table 3
Recovery of selected dyes from wastewater and dyebaths

Dye (form)[a]	Recovery from effluent (%)[b]	SD[c]	Recovery from dyebath (%)[d]	SD[c]
1 (OH)	102	5	96	3
1 (VS)	81	5	95	2
1 (SO_4)	–[e]	–	99	4
2 (OH)	121	9	96	3
2 (VS)	112	12	96	3
2 (SO_4)	–	–	100	2
3 (OH/OH)	102	4	95	4
3 (VS/VS)	92	5	97	3
3 (VS/SO_4)	–	–	97	3
3 (SO_4/SO_4)	–	–	103	5
4 (OH/OH)	118	9	98	1
4 (VS/Cl)	117	4	92	4
4 (SO_4/Cl)	–	–	95	3
5 (OH/OH)	107	10	–	–
5 (VS/Cl)	100	6	–	–
5 (SO_4/Cl)	–	–	–	–

[a] Number refers to dye number in Fig. 1. Dye forms: OH, hydroxy; VS, vinylsulfone; SO_4, sulfato; Cl, chlorotriazine.
[b] 1 mg of each dye spiked into 1 l of effluent.
[c] SD, standard deviation ($n=3$).
[d] 10 mg of each dye spiked into 40 ml of 1 *M* sodium chloride solution.
[e] –, not determined.

or more different forms. In addition, other transformation products can result from dye–dye interactions. To obtain an idea of the spectrum of possible products contained in dyehouse effluents, several industrial dyebaths using reactive dyes were analyzed before and after dyeing.

Electropherograms for dyebath samples before and after dyeing (Fig. 5) are very different. None of the parent sulfato form of the vinylsulfone dyes present before dyeing was found in the exhausted dyebath. The vinylsulfone reactive groups of dyes **1**, **2** and **4** were present mostly in the hydroxy form. However, only 50% the triazine reactive group of bifunctional dye **4** was transformed to the corresponding hydroxytriazine, the rest was still present as the (reactive) chlorotriazine.

An estimate of the residual amount of dyes in three different dyebaths is given in Table 4. The estimate does not include the amount of unbound colorants or reaction products that remains in the substrate and is washed out in subsequent rinses. The dyebaths listed in Table 4 vary significantly with respect to the degree of dye exhaustion as well as to the forms of each dye that are present in the residual

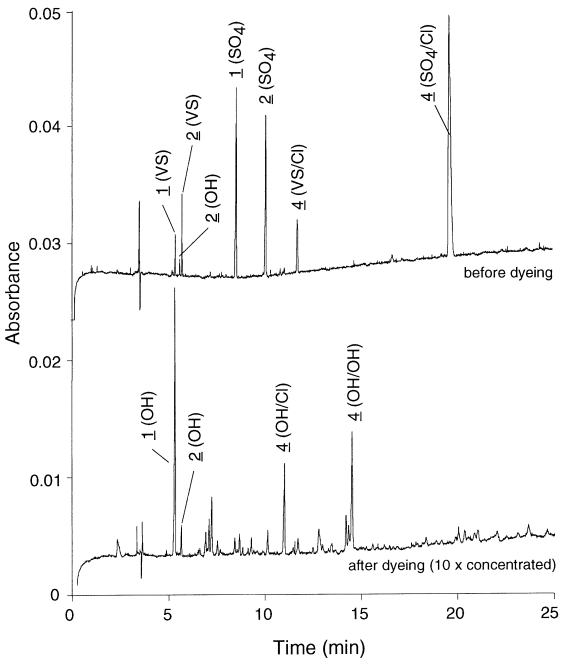

Fig. 5. Analysis of dyebath No. 2 (Table 4) before (top) and after dyeing (bottom) by CE–DAD. Peak annotation as in Fig. 3.

Table 4
Residual dye in exhausted dyebaths in% of initial dye content

Dye	Bath 1	Bath 2	Bath 3
1 (VS)	39	n.d.	
1 (OH)	3	15	
2 (OH)		2.0	
3 (VS/VS)	32		1.6
3 (VS/OH)	7.1		1.6
3 (OH/OH)	<1		0.71
4 (VS/Cl)	41	N.d.	N.d.
4 (OH/Cl)	<1	2.3	N.d.
4 (OH/OH)	<1	2.1	N.d.

liquor. Exhaustion is different for every dye and varies from <60% (dye **4**, bath 1) to 98% (dye **2**, bath 2).

Residual dye was present in the (unreactive) hydroxy form as well as in the reactive vinylsulfone or chlorotriazine form. A situation where massive amounts of the reactive form of the dyes remain in the discarded dyebath is clearly unsatisfactory. A rapid method for the assessment of the condition of a dyebath would certainly improve the efficiency of the dyeing process. When optimized for short separation times instead of maximum resolution, CE is potentially suitable for this purpose.

In addition to the dyes and their hydrolysis products, a number of other components were present in the exhausted dyebaths (Fig. 5). Some of these components were identified using CE–MS coupling. For example, a component with a UV–Vis spectrum identical with that of dye **2** was found. Careful inspection of the mass spectrum of this compound showed an ion at m/z 492 with an ion cluster consisting of signals that were only one half mass unit apart, thus indicating that this ion was doubly charged. The mass of this ion (984) thus corresponds to the mass of the vinylsulfone (483) plus that of the hydroxy form of **2** (501). Thus, this compound most likely is the product of a dye–dye coupling reaction that is known to occur with vinylsulfone dyes [3,4]. Traces of impurities found in the commercial formulation of **2** were also found in dyebaths. These impurities include 1-amino-2-anthraquinonesulfonate, as well as its 4-bromo-, 4-chloro- and 4-hydroxy- analogs. As they exhibit no reactive groups and thus are not incorporated into the dyed goods as much as the dye itself, these impurities are enriched relatively to the dyes and are present in the exhausted dyebaths at concentrations almost equal to those of the hydrolyzed dyes.

Besides the colored components, dyebaths also contain numerous other compounds, such as diluents, surfactants, and complexing agents. One of the compounds that was present in many commercial dyes, dyebaths, and wastewater samples, and was therefore used as a migration time marker, was naphthalene sulfonate. This compound was already identified in wastewater treatment plants receiving water from the textile industry [43].

3.5. Application to sewage effluent

Results of the analysis of a 2-day composite sample of sewage effluent are shown in Fig. 6. Only colored compounds appear in the traces, because the DAD system was set to record only the visible range from 400 to 600 nm. Fractionation of the extract by

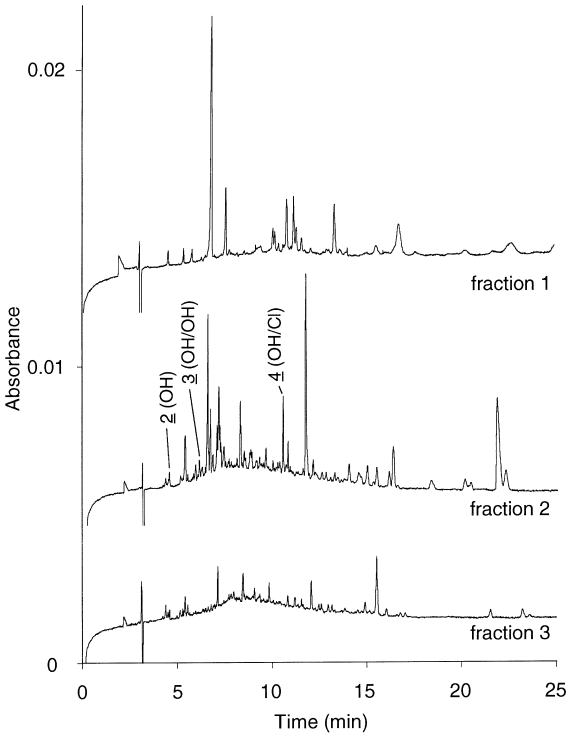

Fig. 6. Analysis of a 48-h composite sample of sewage effluent from the municipal sewage treatment plant at Washington receiving effluent from two textile plants by CE–DAD. Peak annotation as in Fig. 3.

sequential elution with 40, 50 and 70% methanol in water produced three distinct fractions with the bulk of any particular component in only one fraction. Despite successful fractionation, the electropherograms were very complex as a result of the large number of different dyes, dye impurities and reaction products being discharged.

The hydroxy forms of three dyes **2**, **3** and **4** (Fig. 6) could be identified in the second fraction of this extract by matching the UV–Vis spectra and retention times. Other target compounds may have been present but, if so, their peaks overlapped with those of unknown compounds. As the visible spectra of dyes are usually very similar for dyes of the same color, peak overlap, even with compounds other than dyes, makes identification impossible. The only way to overcome this problem is the application of more specific detection, such as mass spectrometry.

Using CE–MS, the presence of the three compounds identified by CE–DAD could be confirmed (Fig. 4). Peak assignment was made by matching the retention times of the dyes in the respective mass traces, relative to the peak of naphthalene sulfonic acid. In addition to the dyes already identified by CE–DAD, the hydroxy forms of **1** and **5** were found. The impurities in dye **2** that were previously identified in exhausted dyebaths were also found in this particular wastewater sample and even in samples that did not contain the dye or its hydrolysis product. This underlines the relative enrichment of these compounds versus the dye itself during the dyeing process and probably also during sewage treatment.

4. Conclusions

The results demonstrate that CE–MS in combination with SPE is suitable for the identification of sulfonated dyes in wastewater samples. Other researchers have shown that quantification is also possible using this technique [26]. In our experience, quantitative studies would require a more rugged interface than that of our system, which allowed continuous analysis typically for less than 2 days.

The successful study of dyes in wastewater depends on several factors besides the availability of an efficient analytical methodology. Our study would not have been possible without the cooperation of both textile plants and dye manufacturers. Cooperation with textile plants is crucial, because information on the actual usage of dyes, including type and quantities must be available; otherwise the chance of identifying any dyes is small. Samples of the dyes used by the plants are necessary because actual structures for some dyes may differ slightly depending on the manufacturer. Small differences in dye structures may not affect the dyeing, but they would prevent identification by CE–MS.

Cooperation with dye manufacturers is also essential, because many structures of commercially important dyes are not publicly available. Given the great number of dyes in use, elucidation of dye structures would not permit cost-effective environmental studies.

Our results also indicate, that in some cases neither the colorants nor their hydrolysis products may be the predominant contaminants in raw wastewater, but other compounds such as dye impurities and combination products. Given the harsh conditions encountered in dyebaths (high or low pH, high temperature), a great number of chemical alterations to colorants should be expected, and their occurrence in dyehouse effluents should be considered.

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