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Determination of sulfonated azo dyes in river water samples by capillary zone electrophoresis

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Abstract

A method based on capillary zone electrophoresis coupled with photodiode-array detection has been developed to determine several sulfonated dyes, including a sulfonated dye (acid yellow 1), and the sulfonated azo dyes acid orange 7, acid orange 12, acid orange 52, acid red 26, acid red 27 and acid red 88. A CElect-FS75 CE column is used. The electrophoresis buffer contains a 1:5 dilution of 10 m*M* phosphoric acid and tetrabutylammonium hydroxide buffer (pH 11.5), and 25 m*M* of triethylamine, the final pH being 11.55. The detection limits for the seven dyes ranged from 0.1 to 4.53 μg/ml. Spiked river water samples (100 ml), containing different concentration levels (0.025–0.150 μg/ml) of the dyes were analyzed after acidification (pH 3) and pre-concentration in disposable SPE Oasis HLB, 1 ml cartridges.

Keywords: Water analysis; Azo dyes

1. Introduction

Azo dyes are a class of dyes, which are widely used in a variety of products, such as textiles, paper, foodstuffs or leather. However, these compounds and their degradation products can be hazardous because of their toxicity and carcinogenicity [1]. An estimation shows that 12% of the amount of dyes used in textile processes is lost to waste streams and, of this percentage, 20% is released into the environment. In addition, these compounds are difficult to remove from water and can be transported from municipal sewers to rivers. Therefore, it is necessary to optimize the analytical procedures for the determination of these compounds at low levels.

In recent years, several papers have described the separation of aromatic sulfonic acids and related dyes by using high-performance liquid chromatography (HPLC) methods [2–4], capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) [5–15]. Different methods and conditions have been used to separate the analytes. The extraction and clean-up from spiked water and soil samples have been studied [5] at μg/g levels of seven compounds including sulfonic azo dyes. These compounds were separated by CZE (buffer borate, pH 8.3) and MEKC (100 m*M* sodium cholate). By using a buffer system containing 0.5% polyethylene glycol and 0.01% polyvinylpyrrolidone, nine synthetic organic dyes have been identified [6], including seven azo compounds used as textile dyes. A

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buffer containing sodium dodecyl sulfate (SDS) was used to separate dyes and dyes intermediates [7,8]. A CZE method was described [9], for the determination of seven synthetic dyes used as food additives, the electrophoretic buffer was a mixture of 25 m*M* sodium phosphate buffer–25 m*M* sodium borate buffer (1:1) containing 10 m*M* SDS, showing that the use of β-cyclodextrin instead of SDS improved separation. A CZE separation of 11 synthetic food colorants via host–guest complexation has been reported [10] with hydrodynamically closed separation compartment. The influences of buffer composition and the effects of α-, β-, and γ-cyclodextrins have been described on the separation of synthetic food colorants [11]. The influence of cyclodextrins has also been studied on the separation of naphthalene sulfonic acids (used as intermediates in production of synthetic dyes) [12]. Methods to quantify synthetic food dyes have used borate buffer adjusted to pH 7–9 [13], and 10.5 [14]. A linear relationship between 2 and 50 mg/l in the first case and detection limits from 0.35 to 2.12 mg/l in the second have been reported.

In wastewater, the concentration levels of the sulfonated dyes could be in the μg/l level; therefore, a pre-concentration step will be necessary for CZE analysis.

The aim of this work is to combine a pre-concentration step by using solid-phase extraction (SPE) with CZE separation in order to obtain better detection and quantification limits than those reported before. The influences of buffer pH and addition of tetrabutylammonium hydroxide and triethylamine on the separation are also investigated.

The presented method allows the quantification of the analytes (after the pre-concentration step) in river water samples spiked at 0.037–0.150 μg/ml levels.

Structures, numbers and names of the dyes used are shown in Fig. 1.

2. Experimental

2.1. Reagents

Ultrapure Milli-Q water from Millipore was used for the preparation of solutions. Dyes were obtained from Aldrich. Stock standard solutions between 68 and 670 μg/ml were prepared in Ultrapure Milli-Q water and diluted as required. A mixed standard solution containing the seven dyes was used to examine the optimum conditions of separation. HPLC-grade methanol was supplied by LiChrosolv. The chemicals used for the preparation of the buffer were of analytical-reagent grade. Other reagents used were tetrabutylammonium hydroxide (Aldrich), 40% (w/w) solution in water, orthophosphoric acid (Fluka), triethylamine and sodium hydroxide (Merck).

Disposable SPE Oasis HLB Waters, 1 ml (30 mg) cartridges, were used in the pre-concentration step.

Before use, the dyes were purified by recrystallization with dichloromethane to separate them from impurities such as salts and surfactants.

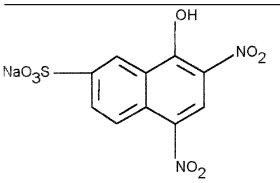
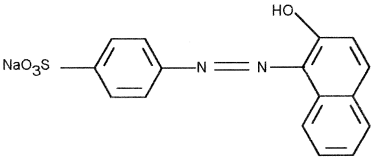
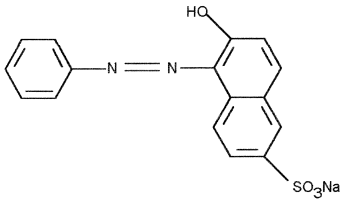
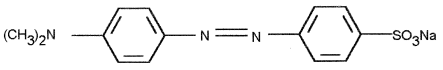
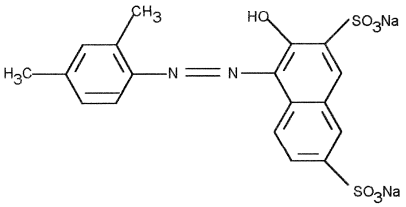
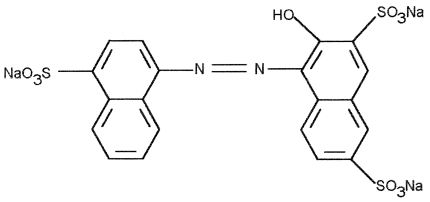
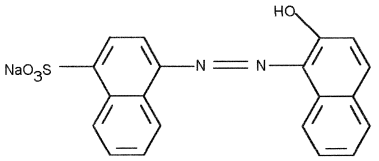
2.2. Apparatus and conditions

CZE was carried out with a P/ACE System 5500 (Beckman, Palo Alto, CA, USA) equipped with a diode array detection (DAD) system. The separation capillary Supelco (Bellefonte, PA, USA) was made from fused-silica of 57 cm (50 cm effective length)× 75 μm I.D. The capillary was activated by pressure injection of 1.0 *M* sodium hydroxide solution for 15 min followed by a 5-min rinse with Ultrapure Milli-Q water and by a 5-min rinse with the run electrolyte. The electrophoresis buffer (prepared daily) contains a 1:5 dilution of a pH 11.5 buffer prepared from 10 m*M* phosphoric acid and tetrabutylammonium hydroxide and 25 m*M* triethylamine. The final pH was 11.55. Separation was performed at 25°C and the applied voltage was 15 kV. Injections were made in hydrodynamic mode for 4 s. The absorbance from 300 to 500 nm was monitored with an on-column DAD system.

Sample pre-concentration was done using a Watson Marlow peristaltic pump.

2.3. Sample preparation and pre-concentration

The river water was filtered using Whatman glass microfiber filters, 4.7 cm. The river water samples were spiked with a dye mixture to obtain levels equal to 25, 37.5, 50, 100 and 150 μg/l of each dye and acidified with 0.1 *M* hydrochloric acid to give pH 3. The dyes were extracted from these samples using

Structure		pKa	CI[a] number and name
	(NYS)		10316 Acid Yellow 1
	(OII)	10.62	15510 Acid Orange 7
	(CO)	10.43	15970 Acid Orange 12
	(MO)	3.20	13025 Acid orange 52
	(P2R)	11.59	16150 Acid Red 26
	(AMR)	10.36	16185 Acid Red 27 Food Red 9
	(R88)	11.06	15620 Acid Red 88

[a]CI = Colour Index

Fig. 1. Structures, numbers and names of the dyes used.

SPE cartridges. They were preconditioned with 1 ml of methanol followed by 2 ml of water. The sample (100 ml) was passed through the cartridge using a peristaltic pump at 2 ml/min. After this the dyes were eluted with 2 ml of methanol, evaporated to dryness and reconstituted with 250 μl of electrophoresis buffer. The recoveries were determined as the ratio of the peak areas obtained for processed samples over the peak areas of matrix matched standards.

3. Results and discussion

Separations by capillary electrophoresis (CE) are strongly influenced by pH and composition of the electrophoretic buffer and the concentration of additives. The additives used were tetrabutylammonium hydroxide and triethylamine. The first is capable of forming reversible ion pairs, alters the net average molecular mass of the analyte and the net average charge of the ionized analyte. Triethylamine was added as a pH stabilizer. The use of triethylamine does not augment the ionic strength of the electrolyte and does not introduce new counter ions into the medium since it is available in the form of free base [16].

The ideal pH for electrophoretic separation of a mixture is in a range near the pK_a values of the target analytes [16]. Since the analytes have pK_a values from 3.2 to 11.56 [4], the pH range studied was between 3 and 12. Several tests were performed.

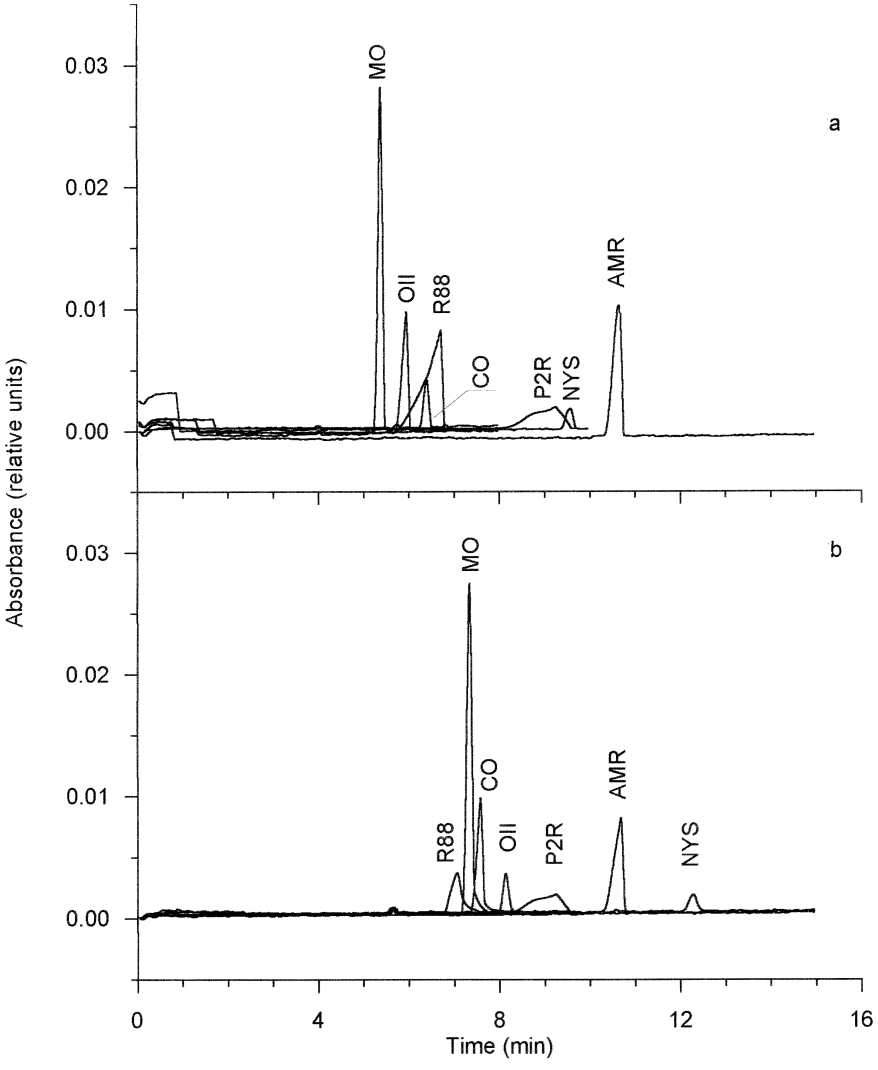


Fig. 2. Electropherogram of the dyes using the following conditions: CElect-FS75 CE column, 57 cm (50 cm effective length)×75 μm I.D.; electrophoretic buffer: (a) buffer phosphates, pH 11.05, (b) 10 m*M* phosphoric acid–tetrabutylammonium hydroxide, pH 11.05. Applied voltage 15 kV and λ=460 nm. Injections were made in hydrodynamic mode.

In the first group of assays, only the pH was varied by the addition of different buffer solution [prepared from succinic acid, sodium dihydrogenphosphate, tris(hydroxymethyl)aminomethane, boric acid, hydrochloric acid and sodium hydroxide]. Under these conditions, as is shown in Fig. 2a and Table 1, the sulfonated-azo dyes have an electrophoretic migration times according with the order of its molecular mass, but the sulfonic dye (NYS) has a different behavior, it is also shown that it was not possible to obtain good separation of the analytes. In the second group of assays the buffers were prepared using formic, orthophosphoric, or boric acid at 10 m*M* concentration, and the desired pH values (3, 5, 7, 9, 11 and 11.5) were obtained by the addition of tetrabutylammonium hydroxide (instead of sodium hydroxide). Under these conditions the better results were obtained for pH 11 and 11.5. All migration times compared to the same pH of the first group of assay were increased but in higher proportion for the analytes having lower molecular mass, this could indicate the formation of ion-pairs with tetrabutylammonium which decrease the mobility of the analytes. Although the results improved, the resolution obtained was low as shown in Fig. 2b and Table 1. In the third group of assays we used a 1:5 dilution of the electrophoretic buffer (10 m*M* phosphoric acid and tetrabutylammonium hydroxide at pH 11.5), to which 25 m*M* triethylamine was added to give a final pH of 11.55. The addition of triethylamine (pK_b = 3.3) at this concentration contributes to maintain the buffer pH. The electropherogram obtained under these conditions is shown in Fig. 3.

Table 1
Migration time of the dyes under the different conditions studied[a]

Dye	Migration time (min)		
	A	B	C
Acid yellow 1 (NYS)	9.56	12.07	7.73
Acid orange 7 (OII)	5.72	7.39	5.40
Acid orange 12 (CO)	6.23	7.98	5.65
Acid orange 52 (MO)	5.22	7.16	4.74
Acid red 26 (P2R)	9.33	9.30	5.89
Acid red 27 (AMR)	10.33	10.29	6.86
Acid red 88 (R88)	6.74	6.79	5.01

[a] A, Buffer phosphates, pH 11.05. Same conditions as in Fig. 2a. B, 10 m*M* phosphoric acid–tetrabutylammonium hydroxide, pH 11.05. Same conditions as in Fig. 2b. C, Dilution (1:5) of 10 m*M* phosphoric acid and tetrabutylammonium hydroxide buffer (pH 11.5), and 25 m*M* of triethylamine, final pH 11.55. Same conditions as in Fig. 3.

The capillary length was initially 57 cm in total length, but it was reduced to 47 cm. This change reduces the migration time for all the compounds but it has not influence on the quality of separation of the peaks.

3.1. Quantification

Calibration plots with correlation coefficient $r^2 \geq 0.995$ were obtained by reporting peak heights as a function of analyte concentrations, at values ranging between one and 10-times the quantitation limit. Detection and quantitation limits were calculated as the concentration corresponding to three- and 10-times the variation in the blank response [17]. Table 2 reports the limits of detection (LODs) and quantitation (LOQs) for each dye.

Water from the Llobregat River (Sant Joan Despi, Barcelona, Spain) was analyzed under the optimized conditions and none of the analyzed dyes was found. Spiked river water samples, prepared as described, were analyzed under the same conditions. Table 3 presents the concentration range for the analyzed spiked river water, the mean values of recoveries after pre-concentration and the corresponding relative standard deviations. The recoveries obtained decrease with the increase of sulfonic groups in the compounds because of its solubility in water increases thus reducing the retention in the SPE cartridge. The recoveries obtained were between 80 and 100% for the monosulfonated dyes, 82% for the disulfonated dyes and 65% for the trisulfonated dyes.

4. Conclusions

A CE method for the determination of one sulfonated dye and six sulfonated azo dyes in water samples has been developed. CZE has been shown to offer higher resolution separation compared to HPLC for analytes like acid orange 7 (OII) and acid orange 12 (CO). The time of analysis is short and it is not necessary to use solvents for separation. The method, in conjunction with SPE, allows analysis of spiked river water samples containing between 0.037 and 0.150 μg/ml of the studied compounds. The con-

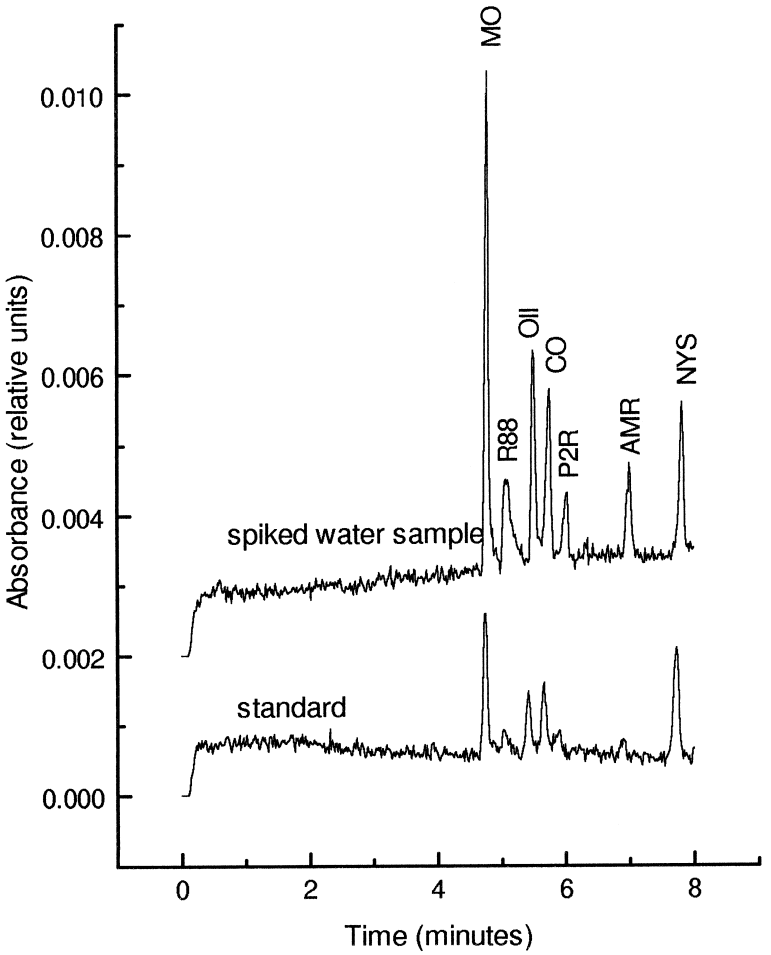


Fig. 3. Electropherogram of a dye mixture obtained under the optimized conditions; voltage 15 kV and λ=460 nm. Injection was made in hydrodynamic mode by 4 s. The dye concentrations in the standard were 15 μg/ml except methyl orange (30 μg/ml); in the spiked river water they were 20 μg/ml except methyl orange (40 μg/ml).

Table 2
Detection and quantitation limits for the CZE separation of studied dyes

CI name	LOD (μg/ml)	LOQ (μg/ml)
Acid yellow 1	0.1	0.34
Acid orange 7	0.90	3.01
Acid orange 12	1.82	6.08
Acid orange 52	0.43	1.42
Acid red 26	4.53	15.09
Acid red 27, food red 9	2.87	9.58
Acid red 88	1.99	6.63

Table 3
Achieved recoveries after pre-concentration of the spiked river water analyzed

CI name	Recovery[a] (%)
Acid yellow 1	89±12
Acid orange 7	92±13
Acid orange 12	99±8
Acid orange 52	92±13
Acid red 26	82±11
Acid red 27, food red 9	65±11
Acid red 88	96±13

[a] Mean of recoveries obtained at 25, 37.5, 50, 100 and 150 μg/l.

centrations of the samples after pre-concentration step were between 0.25 and 13.15 μg/ml. This method gave reliable and reproducible results with a simple sample pre-treatment operation.

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References

[1] E. Rindle, W.J. Troll, Natl. Cancer Inst. 55 (1975) 181.
[2] J.W.M. Wegener, J.C. Klamer, H. Govers, U.A.Th. Brinkman, Chromatographia 24 (1987) 865.
[3] E.M.C. Gennaro, J. Chromatogr. A 767 (1997) 87.
[4] M. Pérez-Urquiza, M.D. Prat, J.L. Beltrán, J. Chromatogr. A 871 (2000) 227.
[5] W.C. Brumley, C.M. Brownrigg, J. Chromatogr. 646 (1993) 377.
[6] Ch.H. Fischer, P. Blatny, A. Rizzi, E. Kenndler, J. Chromatogr. A 717 (1995) 157.
[7] D. Hinks, D.M. Lewis, S.M. Burkinshaw, J. Chromatogr. 640 (1993) 413.
[8] K.P. Evans, G.L. Beaumont, J. Chromatogr. 636 (1993) 153.
[9] M. Shirao, S. Suzuki, M. Aizawa, H. Nakazawa, K. Sasa, H. Sasagawa, J. Chromatogr. A 680 (1994) 541.
[10] M. Masár, D. Kaniansky, V. Madajová, J. Chromatogr. A 724 (1996) 327.
[11] K.L. Kuo, Chromatographia 47 (1998) 249.
[12] J. Fischer, P. Jandera, V. Stanek, J. Chromatogr. A 772 (1997) 385.
[13] T. Zhu, H. Liu, Y. Zhang, S. Qi, A. Huang, Y. Sun, J. Chromatogr. A 718 (1995) 448.
[14] C. Guiberteau Cabanillas, J.J. Berzas Nevado, A.M. Contento Salcedo, Anal. Chim. Acta 378 (1999) 63.
[15] S. Razee, A. Tamura, T. Masujima, J. Chromatogr. A 715 (1995) 327.
[16] H. Parvez, P. Caudy, S. Parvez, P. Roland-Gosselin (Eds), Capillary Electrophoresis in Biotechnology and Environmental Analysis, Progress in HPLC-HPCE, VSP BV, Utrecht, The Netherlands, Vol. 5, 1997.
[17] D.R. Jenke, J. Liq. Chromatogr. Rel. Technol. 19 (1996) 737.