RAPID COMMUNICATIONS IN MASS SPECTROMETRY
*Rapid Commun. Mass Spectrom.* 2006; **20**: 2807–2815

# Analysis of electrochemical degradation products of sulphonated azo dyes using high-performance liquid chromatography/tandem mass spectrometry

**Dana Vaněrková[1], Anastasios Sakalis[2], Michal Holčapek[1]*, Pavel Jandera[1] and Anastasios Voulgaropoulos[2]**

[1]Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Nám. Čs. Legií 565, CZ-532 10 Pardubice, Czech Republic
[2]Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, University Campus, GR-541 24 Thessaloniki, Greece

Received 25 May 2006; Revised 7 July 2006; Accepted 11 July 2006

**Electrochemical treatment of wastewaters containing azo dyes in the textile industry is a promising approach for their degradation. The monitoring of the course of the decomposition of azo dyes in wastewaters is essential due to the environmental impact of their degradation products. In this work, aqueous solutions of a simple azo dye with a low molecular weight (C.I. Acid Yellow 9) and more complex commercial dye (C.I. Reactive Black 5) were electrochemically treated in a laboratory-scale electrolytic cell in sodium chloride or ammonium acetate as supporting electrolytes. Ion-pairing reversed-phase high-performance liquid chromatography coupled with negative-ion electrospray ionization mass spectrometry is applied for the identification of electrochemical degradation products. In addition to simple inorganic salts, the formation of aromatic degradation products obtained due to the cleavage of azo bonds and further degradation reactions is shown, as well as chlorination where sodium chloride is the supporting electrolyte. Degradation mechanisms are suggested for the treatment with sodium chloride as the supporting electrolyte. Copyright © 2006 John Wiley & Sons, Ltd.**

The azo functional group can be found in all groups of textile dyes (e.g., disperse, acid, basic and reactive dyes) and it plays an important role in dyestuffs chemistry in general. The presence of such dyes in industrial wastewaters is of great interest, because they can produce potentially hazardous products, either by photochemical degradation or via metabolic processes in plants and animals.[1] These compounds are highly persistent in the environment. The colour removal processes typically include physical (nanofiltration, electroflotation), photocatalytic (UV/$H_2O_2$, UV/$O_3$, UV/$TiO_2$), biological (anaerobic, aerobic), chemical (ozonation, chlorination, ion exchange, etc.) and electrochemical methods. Each process has its own constraints in terms of costs, reliability, stability, environmental impact, sludge production and potential toxic by-products.[2] Electrochemical degradation is a relatively new method for wastewater treatment, giving promising results.[3–8]

A pilot-plant electrochemical process for the treatment of synthetic dye solutions and real wastewaters was applied for batch operation in one cell or continuous experiments in a cascade system.[3] Almost complete decolourization, neutral final pH value, no sludge formation, decrease of biological and chemical oxygen demand and better biodegradability of treated wastewaters were observed. The treatment of real wastewaters was successful without the addition of other chemicals, because sodium chloride is already present in wastewaters containing textile dyestuffs. Optimized electrode materials, electrolyte composition, pH value and potential in the laboratory electrochemical cell may improve the course of the electrochemical process, as demonstrated by the calculation of the process efficiency and the monitoring of several parameters, such as colour removal, temperature, pH value, biological oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), $ClO^-$ and $Cl^-$ concentrations.[8] This technique is applicable in a pH range from 2 to 9, which is broader than for other established treatment processes, such as biological or ozonation reactions.

The quality of treated wastewaters is usually evaluated using environmental parameters, such as pH value, temperature, BOD, COD, TOC and total suspended solids (TSS),[2] but it does not provide information on the composition of the wastewaters. The identification of

---

**Correspondence to*: M. Holčapek, Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Nám. Čs. Legií 565, CZ-532 10 Pardubice, Czech Republic.
E-mail: Michal.Holcapek@upce.cz
Contract/grant sponsor: Ministry of Education of the Czech Republic; contract/grant number: MSM0021627502.
Contract/grant sponsor: Grant Agency of the Czech Republic; contract/grant numbers: 203/04/P126; 203/04/0917.
Contract/grant sponsor: COM-CHROM of the Commission of European Communities 5th framework programme 'Improving the Human Research Potential and Socio-Economics Knowledge Base'; contract/grant number: HPRN-CT-2001-00180.

degradation products is discussed in a few papers only, where the structures of some products were proposed using gas chromatography/mass spectrometry,[6,9,10] high-performance liquid chromatography/mass spectrometry (HPLC/MS),[10–12] or HPLC with UV-Vis detection.[13,14] For the separation of anionic (poly)sulphonated dyes, intermediates and degradation products, salting-out HPLC with high concentration of inorganic salts[15–17] or ion-pairing HPLC with tetraalkylammonium salts[15] can be applied. However, these methods are not compatible with mass spectrometric detection, because they seriously impair the performance of the mass spectrometer causing increased baseline noise, ion suppression and memory effects.[18] In HPLC/MS of sulphonic acids, more volatile di- and trialkylammonium acetates or formates at low concentrations should be used instead of non-volatile reagents as a compromise between the chromatographic and mass spectrometric demands. Tributylammonium acetate,[19,20] dihexylammonium acetate[21] and triethylammonium acetate (TEAA)[19,22] additives have been successfully applied for the HPLC/MS analysis of various sulphonated dyes. TEAA was chosen for the present work, because it offers sufficient chromatographic separation and causes relatively low signal suppression in HPLC/MS.[18] Negative-ion electrospray ionization mass spectrometry (ESI-MS) is the most suitable technique for the analysis of anionic dyes,[19–25] enabling the determination of the molecular weight and the number of sulphonic acid groups. Before the advent of ESI, fast-atom bombardment (FAB) was successfully applied for the analysis of non-volatile sulphonated, sulphated and phosphonated dyes.[26–29] Studying the fragmentation behaviour in tandem mass spectrometric (MS/MS) experiments extends the possibilities of the structural identification.[20,30–32] The characteristic neutral losses and observed ions for particular functional groups are the following:[26] losses of $H_2SO_4$ and $SO_3$ and ions $[HSO_4]^-$ for sulphates, losses of $SO_3$ and $SO_2$ and ions $[SO_3]^{-\bullet}$ for sulphonic acids, rearrangement losses of $N_2$ and cleavages of or next to the azo bond. The rearrangement loss of $N_2$ from the azo group is dependent on collision conditions, such as the collision energy.[26]

The objective of this study is to develop a suitable HPLC/MS/MS method for the monitoring of the electrochemical degradation process and to identify products formed during the electrochemical treatment using two model compounds, a relatively simple azo dye, C.I. Acid Yellow 9 (AY), and a more complex commercial dye, C.I. Reactive Black 5 (RB). Laboratory-scale electrochemical treatment is used for the investigation of the degradation process. The influence of electrolytes used in the electrochemical degradation, the treatment time and the structures of treated dyes are also studied. Sodium chloride is the main salt present in azo dye wastewaters due to common dyeing process conditions. The effect of this salt on the electrochemical degradation is compared with that of ammonium acetate, which is electrochemically inactive. To describe the course of the degradation process, the formation of the decomposition products is studied depending on the time elapsed from the start of the electrochemical treatment. Detailed characterization of the reaction mixture components is allowed by the interpretation of the HPLC/MS/MS spectra.

## EXPERIMENTAL

### Reagents

C.I. Acid Yellow 9 (AY, structure shown in Table 1) of chemical purity grade (95%) was obtained from Sigma (St. Louis, MO, USA), while C.I. Reactive Black 5 (RB, structure shown in Table 2), obtained from DyStar Textilfarben GmbH & Co. (Brunsbuettel, Germany), was of commercial purity grade. All salts used as electrolytes were of analytical grade and methanol was of HPLC grade (Merck, Darmstadt, Germany). Water was doubly distilled in glass in the presence of potassium permanganate. The solvents were filtered through a 0.45 μm Millipore filter prior to use and degassed in the ultrasonic bath before HPLC analyses. Triethylamine of the best available purity (>99%) was obtained from Aldrich (Milwaukee, WI, USA) and acetic acid (99%) from Sigma. Triethylammonium acetate (TEAA), the ion-pairing reagent, was prepared by mixing equimolar amounts of triethylamine and acetic acid.

### Electrochemical process

A laboratory-scale Plexiglass electrolytic cell[33] with a volume capacity of 0.5 L was obtained from the Institute of Physical Chemistry, University of Erlangen-Nurenberg (Germany). Batch operation was supported by compact air pumps for aeration to enhance the migration of dye molecules towards electrodes. The carbon fleece used as a cathode was Sigatherm model PR201-16 obtained from SGL Carbon Group (Germany). The anode made of platinated titanium (Pt/Ti, 50g Pt/m$^2$) was obtained from Metakem GmbH (Germany). Aqueous solutions containing 0.5 mmol/L of the azo dye and 0.2 mol/L of the electrolyte (sodium chloride or ammonium acetate) were treated in the electrolytic cell at a potential ranging from 10 to 20 V applied between anode and cathode. The potential was optimized in each process to obtain adequate current. The treated solution was sampled from the cell every 5 or 10 min depending on the course of the process. A UV-Vis spectrum was measured for each sample. HPLC/MS/MS analysis was applied for selected samples taken at the beginning, during and at the end of the process.

### Instrumentation

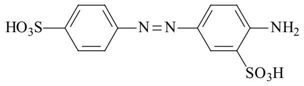
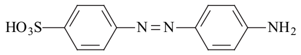
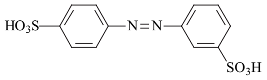
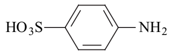
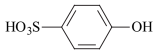
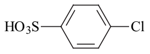
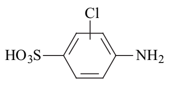
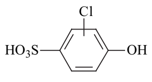
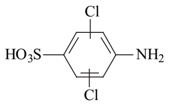
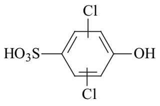
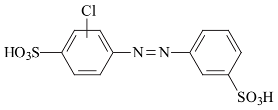
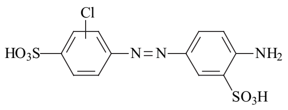
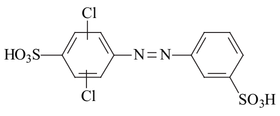
The HPLC/MS system consisted of a Waters (Milford, MA, USA) liquid chromatograph assembled from a model 616 pump, a model 996 diode-array UV detector, a model 717+ autosampler and an Esquire 3000 mass spectrometer with an ion trap analyzer (Bruker Daltonics, Bremen, Germany). A pH meter from Hanna Instruments was used for pH measurements. UV-Vis spectra were measured with a Jasco V-530 spectrophotometer (Tokyo, Japan).

### HPLC/MS/MS conditions

A LiChrospher C18 column (125 × 4 mm i.d., 5 μm particle size; Merck) was used for the HPLC separation. The gradient elution from 15% to 50% B in A in 20 min for RB and from 0% to 20% B in A in 20 min for AY was used. Solvent A was 2.5 mM TEAA in water and solvent B was 2.5 mM TEAA in methanol. The column temperature was 40°C, the flow rate 0.8 mL/min and the injection volume 20 μL in the case of the initial dyes and 80 μL after the treatment. The mass

DOI: 10.1002/rcm

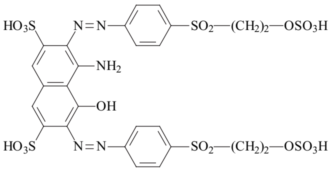
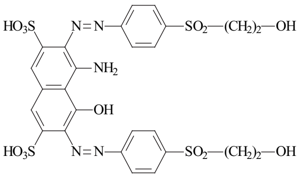
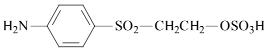
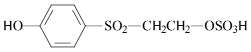
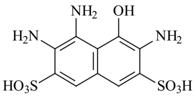
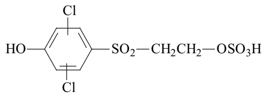
**Table 1.** Molecular weights (MW), retention times ($t_R$), product ions and proposed structures of impurities and some electrochemical degradation products of C.I. Acid Yellow 9 dye

| Compound, MW | $t_R$ [min] | Ions observed in first-order MS (*m*/*z* - structure) | Product ions in MS/MS (*m*/*z* - structure) | Proposed structures |
|---|---|---|---|---|
| Initial dye $M_W = 357$ | 11.9 | 356 - $[M{-}H]^-$<br>378 - $[M{-}2H{+}Na]^-$, 394 - $[M{-}2H{+}K]^-$<br>713 - $[2M{-}H]^-$, 735 - $[2M{-}2H{+}Na]^-$ | 292 - $[M{-}H{-}SO_2]^-$, 276 - $[M{-}H{-}SO_3]^-$<br>248 - $[M{-}H{-}SO_3{-}N_2]^-$<br>184 - $[M{-}H{-}SO_2{-}SO_3{-}N_2]^-$<br>171 - $[M{-}H{-}N_2{-}C_6H_5{-}SO_3]^{-\bullet}$ |  |
| Impurity 1 $MW = 277$ | 14.8 | 276 - $[M{-}H]^-$ | 212 - $[M{-}H{-}SO_2]^-$<br>196 - $[M{-}H{-}SO_3]^-$ |  |
| Impurity 2 $MW = 342$ | 13.9 | 341 - $[M{-}H]^-$ | 277 - $[M{-}H{-}SO_2]^-$<br>233 - $[M{-}H{-}SO_3{-}N_2]^-$<br>169 - $[M{-}H{-}SO_2{-}SO_3{-}N_2]^-$ |  |
| Product A[a] $MW = 173$ | 2.9 | 172 - $[M{-}H]^-$ | 155 - $[M{-}H{-}NH_3]^-$<br>108 - $[M{-}H{-}SO_2]^-$ |  |
| Product B[a] $MW = 174$ | 3.6 | 173 - $[M{-}H]^-$ | 155 - $[M{-}H{-}H_2O]^-$<br>109 - $[M{-}H{-}SO_2]^-$ |  |
| Product C[b] $MW = 192$ | 12.9 | 191 - $[M{-}H]^-$ | 127 - $[M{-}H{-}SO_2]^-$<br>80 - $[SO_3]^-$, 64 - $[SO_2]^{-\bullet}$ |  |
| Product D[b] $MW = 207$ | 11.7 | 206 - $[M{-}H]^-$ | 189 - $[M{-}H{-}NH_3]^-$<br>142 - $[M{-}H{-}SO_2]^-$<br>126 - $[M{-}H{-}SO_3]^-$, 80 - $[SO_3]^{-\bullet}$ |  |
| Product E[b] $MW = 208$ | 13.2 | 207 - $[M{-}H]^-$ | 189 - $[M{-}H{-}H_2O]^-$<br>143 - $[M{-}H{-}SO_2]^-$<br>127 - $[M{-}H{-}SO_3]^-$, 80 - $[SO_3]^{-\bullet}$ |  |
| Product F[b] $MW = 241$ | 14.3 | 240 - $[M{-}H]^-$ | 223 - $[M{-}H{-}NH_3]^-$<br>176 - $[M{-}H{-}SO_2]^-$, 160 - $[M{-}H{-}SO_3]^-$<br>140 - $[M{-}H{-}SO_2{-}HCl]^-$<br>80 - $[SO_3]^{-\bullet}$ |  |
| Product G[b] $MW = 242$ | 17.6 | 241 - $[M{-}H]^-$ | 223 - $[M{-}H{-}H_2O]^-$<br>177 - $[M{-}H{-}SO_2]^-$<br>161 - $[M{-}H{-}SO_3]^-$<br>141 - $[M{-}H{-}SO_2{-}HCl]^-$ |  |
| Product H[b] $MW = 376$ | 16.4 | 375 - $[M{-}H]^-$ | 347 - $[M{-}H{-}N_2]^-$ , 311 - $[M{-}H{-}SO_2]^-$<br>295 - $[M{-}H{-}SO_3]^-$, 267 - $[M{-}H{-}SO_3{-}N_2]^-$<br>231 - $[M{-}H{-}SO_3{-}N_2{-}HCl]^-$<br>203 - $[M{-}H{-}SO_2{-}SO_3{-}N_2]^-$<br>190 - $[M{-}H{-}N_2{-}C_6H_5{-}SO_3]^{-\bullet}$ |  |
| Product I[b] $MW = 391$ | 17.2 | 390 - $[M{-}H]^-$ | 373 - $[M{-}H{-}NH_3]^-$, 362 - $[M{-}H{-}N_2]^-$<br>310 - $[M{-}H{-}SO_3]^-$, 282 - $[M{-}H{-}SO_3{-}N_2]^-$<br>218 - $[M{-}H{-}SO_2{-}SO_3{-}N_2]^-$<br>205 - $[M{-}H{-}N_2{-}C_6H_5{-}SO_3]^{-\bullet}$ |  |
| Product J[b] $MW = 410$ | 16.8 | 409 - $[M{-}H]^-$ | 381 - $[M{-}H{-}N_2]^-$, 345 - $[M{-}H{-}SO_2]^-$<br>329 - $[M{-}H{-}SO_3]^-$, 301 - $[M{-}H{-}SO_3{-}N_2]^-$<br>237 - $[M{-}H{-}SO_2{-}SO_3{-}N_2]^-$<br>224 - $[M{-}H{-}N_2{-}C_6H_5{-}SO_3]^{-\bullet}$<br>201 - $[M{-}H{-}SO_2{-}SO_3{-}N_2{-}HCl]^-$<br>189 - $[M{-}H{-}N_2{-}C_6H_5{-}SO_3{-}Cl]^{-\bullet}$ |  |

[a] Formed during the treatment in acetate electrolyte.
[b] Formed during the treatment in chloride electrolyte.

DOI: 10.1002/rcm

**Table 2.** Molecular weights (MW), retention times ($t_R$), product ions and proposed structures of hydrolysed dye and some electrochemical degradation products of C.I. Reactive Black 5 dye

| Compound, MW | $t_R$ [min] | Ions observed in first-order MS (*m*/*z* - structure) | Product ions in MS/MS (*m*/*z* - structure) | Proposed structures |
|---|---|---|---|---|
| Initial dye<br>MW = 903 | 13.8 | 924 - $[M-2H+Na]^-$ ,<br>902 - $[M-H]^-$<br>844 - $[M-2H-SO_3+Na]^-$<br>822 - $[M-H-SO_3]^-$,<br>742 - $[M-H-2SO_3]^-$<br>724 - $[M-H-SO_3-H_2SO_4]^-$<br>706 - $[M-H-2H_2SO_4]^-$<br>660 - $[M-H-SO_2-SO_3-H_2SO_4]^-$<br>421.5 - $[M-3H-SO_3+Na]^{2-}$<br>410.5 - $[M-2H-SO_3]^{2-}$<br>370.5 - $[M-2H-2SO_3]^{2-}$,<br>97 - $[HSO_4]^-$ | 696 - $[724-N_2]^-$, 660 - $[724-SO_2]^-$<br>644 - $[724-SO_3]^-$, 632 - $[724-SO_2-N_2]^-$<br>568 - $[724-2SO_2-N_2]^-$<br>552 - $[724-SO_2-SO_3-N_2]^-$<br>524 - $[724- HNC_6H_4SO_2C_2H_4OH]^{-\bullet}$<br>511 - $[724- N_2C_6H_4SO_2C_2H_4OH]^{-\bullet}$<br>465 - $[511-N_2-H_2O]^{-\bullet}$, 447 - $[511-SO_2]^{-\bullet}$<br>419 - $[511-N_2-SO_2]^{-\bullet}$ |  |
| Impurity 1<br>MW = 885 | 17.1 | 906 - $[M-2H+Na]^-$,<br>884 - $[M-H]^-$<br>826 - $[M-2H-SO_3+Na]^-$<br>804 - $[M-H-SO_3]^-$,<br>724 - $[M-H-2SO_3]^-$<br>706 - $[M-H-SO_3-H_2SO_4]^-$<br>642 - $[M-H-SO_2-SO_3-H_2SO_4]^-$<br>412.5 - $[M-3H-SO_3+Na]^{2-}$<br>401.5- $[M-2H-SO_3]^{2-}$<br>361.5 - $[M-2H-2SO_3]^{2-}$,<br>97 - $[HSO_4]^-$ | 678 - $[706-N_2]^-$, 642 - $[706-SO_2]^-$<br>626 - $[706-SO_3]^-$, 614 - $[706-SO_2-N_2]^-$<br>550 - $[706-2SO_2-N_2]^-$<br>534 - $[706-SO_2-SO_3-N_2]^-$<br>524 - $[706- HNC_6H_4SO_2C_2H_3]^{-\bullet}$<br>511 - $[706- N_2C_6H_4SO_2C_2H_3]^{-\bullet}$<br>483 - $[511-N_2]^{-\bullet}$,460 - $[524-SO_2]^-$<br>447 - $[511-SO_2]^{-\bullet}$, 419 - $[511-N_2-SO_2]^{-\bullet}$ | Initial dye minus $H_2O$ |
| Impurity 2<br>MW = 867 | 21.2 | 866 - $[M-H]^-$,<br>728 - $[M-2H-2SO_3+Na]^-$<br>706 - $[M-H-2SO_3]^-$,<br>352.5 - $[M-2H-2SO_3]^{2-}$ | 642 - $[706-SO_2]^-$<br>524 - $[706- HNC_6H_4SO_2C_2H_3]^{-\bullet}$ | Initial dye minus $2H_2O$ |
| Hydrolysed dye<br>MW = 743 | 15.0 | 764 - $[M-2H+Na]^-$<br>742 - $[M-H]^-$<br>370.5 - $[M-2H]^{2-}$ | 714 - $[M-H-N_2]^-$, 678 - $[M-H-SO_2]^-$<br>662 - $[M-H-SO_3]^-$, 650 - $[M-H-SO_2-N_2]^-$<br>586 - $[M-H-2SO_2-N_2]^-$<br>570 - $[M-H-SO_2-SO_3-N_2]^-$<br>542 - $[M-H- HNC_6H_4SO_2C_2H_4OH]^{-\bullet}$<br>529 - $[M-H- N_2C_6H_4SO_2C_2H_4OH]^{-\bullet}$<br>501 - $[529-N_2]^{-\bullet}$, 478 - $[542-SO_2]^{-\bullet}$<br>465 - $[529-SO_2]^{-\bullet}$, 449 - $[529-SO_3]^{-\bullet}$<br>437 - $[529-N_2-SO_2]^{-\bullet}$, 393 - $[529-2N_2-SO_3]^{-\bullet}$<br>329 - $[529-2N_2-SO_2-SO_3]^{-\bullet}$ |  |
| Product K[a,b]<br>MW = 281 | 5.7 | 302 - $[M-2H+Na]^-$,<br>280 - $[M-H]^-$<br>238 - $[M-2H-SO_2+Na]^-$,<br>182 - $[M-H-H_2SO_4]^-$<br>97 - $[HSO_4]^-$ | 262 - $[M-H-H_2O]^-$, 253 - $[M-H-C_2H_3]^{-\bullet}$<br>182 - $[M-H-H_2SO_4]^-$<br>97 - $[HSO_4]^-$ |  |
| Product L[a,b]<br>MW = 282 | 8.6 | 303 - $[M-2H+Na]^-$ ,<br>281 - $[M-H]^-$<br>223 - $[M-2H-SO_3+Na]^-$<br>183 - $[M-H-H_2SO_4]^-$<br>141 - $[M-2H-SO_2-H_2SO_4+Na]^-$ | 263 - $[M-H-H_2O]^-$, 217 - $[M-H-SO_2]^-$<br>183 - $[M-H-H_2SO_4]^-$<br>156 - $[M-H-H_2SO_4-C_2H_3]^{-\bullet}$<br>97 - $[HSO_4]^-$, 93 - $[C_6H_5O]^{-\bullet}$ |  |
| Product M[a,b]<br>MW = 349 | 1.8 | 370 - $[M-2H+Na]^-$<br>348 - $[M-H]^-$<br>173.5 - $[M-2H]^{2-}$ | 331 - $[M-H-NH_3]^-$, 330 - $[M-H-H_2O]^-$<br>284 - $[M-H-SO_2]^-$, 268 - $[M-H-SO_3]^-$<br>188 - $[M-H-2SO_3]^-$ |  |
| Product N[b]<br>MW = 350 | 14.5 | 371 - $[M-2H+Na]^-$<br>349 - $[M-H]^-$<br>251 - $[M-H-H_2SO_4]^-$ | 331 - $[M-H-H_2O]^-$, 313 - $[M-H-HCl]^-$<br>251 - $[M-H-H_2SO_4]^-$<br>224 - $[M-H-H_2SO_4-C_2H_3]^{-\bullet}$ |  |

[a] Formed during the treatment in acetate electrolyte.
[b] Formed during the treatment in chloride electrolyte.

spectrometric conditions were as follows: negative-ion ESI mode, $m/z$ range 50–1000, nitrogen flow rate 12 L/min, temperature 365°C, nebulizer pressure 70 psi, capillary voltage 3500 V, automatic measurement of MS/MS spectra with an isolation width of 4 Th and a collision amplitude (i.e., collision energy) of 1 V.

## RESULTS AND DISCUSSION

Two main electrochemical processes take place during the electrolysis of azo dyes: direct oxidation/reduction on the surface of electrodes and indirect reactions, which occur in the bulk solution if there is a sufficient amount of oxidative or reductive intermediates produced by electrolysis of the supporting electrolyte. Chloride anions are the main salts present in azo dye wastewaters and are very reactive and can enhance the indirect oxidation of the dye. On the other hand, ammonium acetate is a relatively inactive electrolyte, therefore not contributing significantly to the degradation of the dyes. To understand the effect of the electrolyte and the extent of the indirect degradation process, the identification of degradation products by HPLC/MS/MS analysis is very useful.

HPLC/ESI-MS/MS (see Experimental section for conditions) was used for the separation and identification of the degradation products of azo dyes on the basis of their retention behaviour, molecular weights (MWs) and product ions observed in MS/MS spectra.[32] The MWs, retention times ($t_R$), ions observed in first-order mass spectra, product ions obtained in MS/MS of important precursor ions and proposed structures of impurities and identified products of electrochemical degradation are listed in Table 1 for AY and in Table 2 for RB. The MWs were determined according to $[M–xH]^{x-}$ ions in the negative-ion ESI mass spectra,[21,23] and information on the structures was obtained from the product ions observed in the MS/MS spectra of the $[M–H]^-$ and $[M–H–SO_3–H_2SO_4]^-$ ions in the case of the RB initial dye ($m/z$ 724) and its impurity 1 ($m/z$ 706). These ions are the most abundant in the first-order mass spectra of both compounds. Deprotonated molecules are the typical base peaks in negative-ion ESI mass spectra of acidic compounds and the neutral losses of $H_2SO_4$ and $SO_3$ are characteristic for the presence of the sulphate functionality.[34]

### Degradation of AY

Two impurities with a higher retention than the original AY dye were identified (Table 1). Impurity 1 was identified as the monosulphonated aminoazobenzene and impurity 2 as the disulphonated azobenzene. Figure 1 shows HPLC/UV chromatograms at 254 nm of AY subjected to the electrolysis in a solution of ammonium acetate. During the treatment, the concentration of the azo dye (retention time 11.9 min) decreases and it finally disappears completely after 60 min, while several reaction products are formed. The peak with the retention time 2.9 min corresponds to the major product with MW 173 (product A). This compound was identified as *p*-aminobenzenesulphonic acid, on the basis of the aromatic absorption band in the UV-Vis spectrum, the main ions observed in the first-order mass spectrum ($[M–H]^-$ ion at $m/z$ of 172) and neutral losses of $NH_3$ (17 Da) and $SO_2$ (64 Da) observed in the MS/MS spectrum (see Table 1). The structure was confirmed by the agreement of retention times, UV-Vis spectra and mass spectrometric behaviour with the data for the authentic standard. The formation of this product unambiguously proves the reductive cleavage of the azo bond on the cathode surface. In a similar way, *p*-hydroxybenzenesulphonic acid reaction product B was identified in the final solution after the electrochemical treatment. The second product of the azo bond cleavage, the diaminobenzenesulphonic acid, eluted at a retention time of 2.1 min, hence coeluting with high concentrations of inorganic salts formed during the electrochemical process. Further electrochemical processes on the electrode surface or in the bulk of the ammonium acetate solution are slow. Hence the degradation leads to the formation of only a few products, simple sulphonated aromatic amines or phenols, without any further reactions.

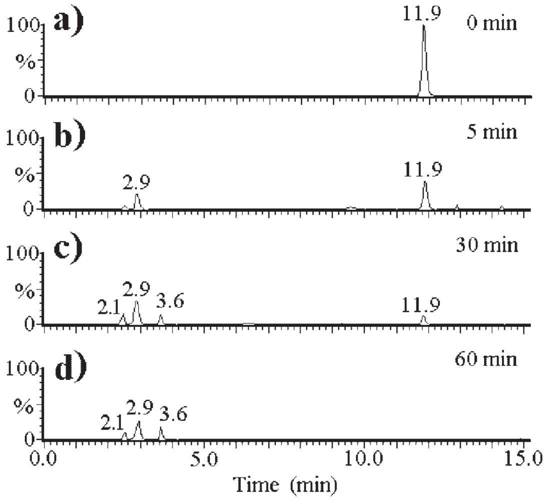


**Figure 1.** HPLC/UV chromatograms of C.I. Acid Yellow 9 treated in ammonium acetate electrolyte at a potential of 20 V. LiChrospher C18 column (125 × 4 mm, 5 μm), gradient elution from 0% to 20% B in A in 20 min, with 2.5 mM TEAA in water as solvent A and in methanol as solvent B, column temperature 40°C, flow rate 0.8 mL/min, injection volume 80 μL, detection at 254 nm: (a) at 0 min (original dye), (b) after 5 min, (c) after 30 min, and (d) after 60 min of treatment. Original dye (AY) – retention time of 11.9 min, the main degradation product (A, i.e. *p*-aminobenzenesulphonic acid) – retention time of 2.9 min. The scale of the Y axis is normalized with respect to the chromatogram of the original dye.

In the sodium chloride electrolyte, however, the degradation process is accelerated and becomes more effective. Chlorinated aromatic compounds are formed, such as products C–J (Table 1). The MS and MS/MS spectra of singly and doubly chlorinated AY products H and J are depicted in Fig. 2. The MWs, product ions and the presence of the expected isotopic distributions for compounds with one and two chlorine atoms support the suggested structures. In the first-order mass spectrum of the singly chlorinated product H (Fig. 2(a)), the $[M–H]^-$ ion at $m/z$ 375 and the $[M–2H+Na]^-$ ion at $m/z$ 397 were observed, both with the typical 3:1 isotopic distribution. The mass spectrum of product J (Fig. 2(b)) shows the $[M–H]^-$ ion at $m/z$ 409, the $[M–2H+Na]^-$ ion at $m/z$ 431, both with the typical 9:6:1 isotopic distribution for the presence of two chlorine atoms in

DOI: 10.1002/rcm

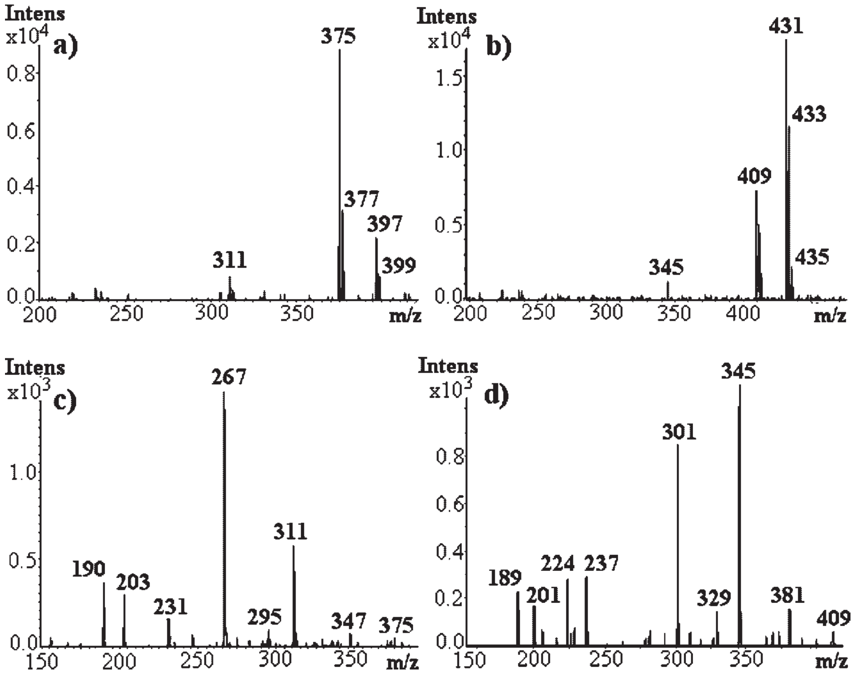


**Figure 2.** HPLC/MS/MS analysis of C.I. Acid Yellow 9 electrochemically treated in sodium chloride solution. Negative-ion ESI mass spectra: (a) MS spectrum of singly chlorinated product H with MW 376, (b) MS spectrum of doubly chlorinated product J with MW 410, (c) MS/MS spectrum of deprotonated molecule (*m/z* 375) of product H, and (d) MS/MS spectrum of deprotonated molecule (*m/z* 409) of product J.

the molecule. Both products exhibit similar fragmentation behaviour in the MS/MS spectra of deprotonated molecules (Figs. 2(c) and 2(d)). The spectra confirm a cleavage of the C–N bond next to the azo bond and the aromatic ring, in addition to the neutral losses of $N_2$ (28 Da, the rearrangement loss typical for an azo bond), $SO_2$ and $SO_3$ (64 and 80 Da, typical for a sulphonic acid group) and HCl (36 Da, typical for a chlorine substituent).[34]

The presence of chlorinated products confirms that chlorination takes place during the electrolysis in the chloride solution, and this has an important environmental impact regardless of the low concentrations of these compounds. The evidence of such compounds in the final reaction mixture proved the necessity of detailed analytical control of treated wastewaters during the treatment process. For the safety evaluation of treated wastewaters, the conventional monitoring of established environmental parameters, such as COD, BOD, TOC, total organic halogen and extent of decolourization, should be complemented with HPLC/MS/MS data that provides more information on the structures of the obtained products.

The electrochemical degradation mechanism is proposed on the basis of the results shown in Table 1 (Fig. 3). It includes the reductive cleavage of the azo bond on the cathode surface and chlorination in the bulk solution. These reactions occur as initial steps at the beginning of the process and are followed by further indirect oxidation, reduction, degradation and partial mineralization, resulting in water, carbon dioxide and inorganic salts (sulphates, chlorates and perchlorates). These final products are identified in the treated samples, when the treatment time is adequately long, and this is confirmed by the decrease of BOD, COD, TOC and chloride concentrations. A similar mechanism can be expected in the electrochemical treatment of real wastewaters, because chloride anions are the main salts present in the dye bath. The degree of degradation can be monitored by HPLC/MS/MS and controlled by the optimization of several parameters, such as the type and concentration of salts, the potential applied between the electrodes, the materials of the anode and cathode, and the treatment time.

## Degradation of RB

The same approach is used for the identification of RB degradation products (Table 2). The electrochemical method with sodium chloride as electrolyte and with a longer treatment time results in effective dye degradation, although the dye structure is more complicated and the number and concentrations of impurities are much higher than with AY. HPLC/UV chromatograms at 254 nm of the RB dye monitored at different stages of the electrochemical treatment with sodium chloride are shown in Fig. 4. Before the start of the process (Fig. 4(a)), the peaks of the original dye with retention time of 13.8 min and some impurities in this dye of commercial purity are observed. The impurities with retention times of 17.1 and 21.2 min were identified as the original dye after the elimination of one (impurity 1) and two molecules of water (impurity 2). The absence of the $[M–H–2H_2SO_4]^-$ ion in the first-order mass spectrum of impurity 1 (in comparison with the mass spectrum of original dye) confirms the elimination of one molecule of water. The typical ESI mass spectra of sulphated dyes are rather complex with many fragment ions being present in the first-order spectra and unfavourable signal-to-noise ratio.[32–34]

DOI: 10.1002/rcm

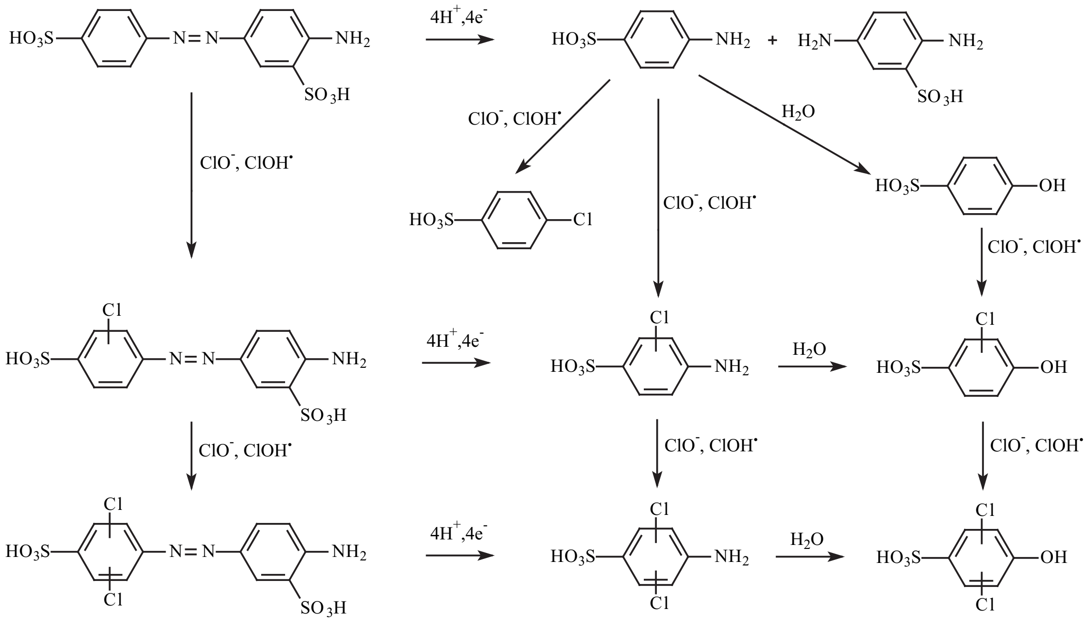


Figure 3. Scheme of the electrochemical degradation of C.I. Acid Yellow 9 in the presence of sodium chloride as the supporting electrolyte based on products identified by HPLC/MS/MS.

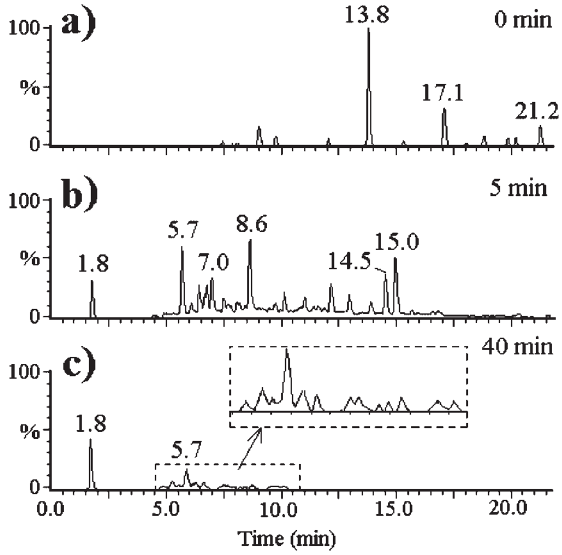


Figure 4. HPLC/UV chromatograms of C.I. Reactive Black 5 electrochemically treated in sodium chloride electrolyte at a potential of 12 V. LiChrospher C18 column (125 × 4 mm, 5 $\mu$m), gradient elution from 15% to 50% B in A in 20 min, with 2.5 mM TEAA in water as solvent A and in methanol as solvent B, column temperature 40°C, flow rate 0.8 mL/min, injection volume 80 $\mu$L, detection at 254 nm: (a) at 0 min (original dye), (b) after 5 min, and (c) after 40 min of treatment. Original dye (RB) – retention time 13.8 min, main product M – retention time of 1.8 min. The scale of the Y axis is normalized with respect to the chromatogram of the original dye.

Many degradation products are already formed after 5 min of the treatment (Fig. 4(b), major ones are listed in Table 2), but only two of them are still present after 40 min (Fig. 4(c) and Table 2, products M and K with retention times of 1.8 and 5.7 min). The RB dye has a more complex structure than the AY dye and the mass spectrum of RB (shown in Fig. 5(a))

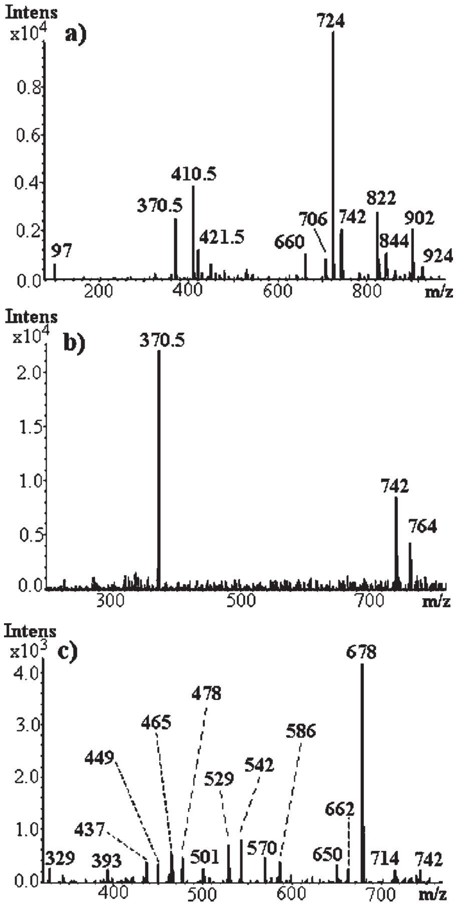


Figure 5. HPLC/MS/MS analysis of C.I. Reactive Black 5 treated electrochemically in sodium chloride solution. Negative-ion ESI mass spectra: (a) MS spectrum of the original dye with MW 903, (b) MS spectrum of hydrolyzed dye with MW 743, and (c) MS/MS spectrum of deprotonated molecule (*m/z* 742) of hydrolyzed dye.

DOI: 10.1002/rcm

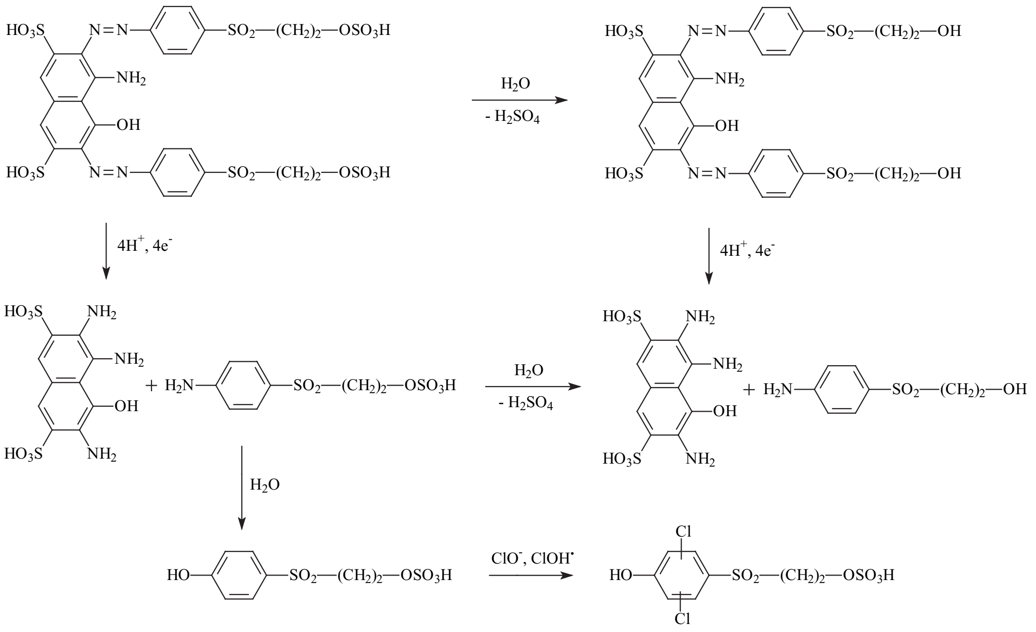


**Figure 6.** Scheme of the electrochemical degradation of C.I. Reactive Black 5 in the presence of sodium chloride as supporting electrolyte based on products identified by HPLC/MS/MS.

is more complex than that of the AY dye, mainly because of the presence of two reactive sulphate functionalities, -O-$SO_3H$, which usually shows an extensive fragmentation with typical neutral losses of $H_2SO_4$, $SO_2$ and $SO_3$ and reduced relative abundance of the $[M–H]^-$ ion.[33,34] Because of the four acidic groups in the dye molecule, the solution has a high salt content, so the sodium adducts appear in the spectrum. Both sulphate functionalities hydrolyze during the treatment[12,14] yielding the product with the retention time of 15.0 min (Table 2). The mass spectrum of the hydrolyzed dye (Fig. 5(b)) contains only singly (*m*/*z* 742) and doubly (*m*/*z* 370.5) charged deprotonated molecules and the sodium adduct (*m*/*z* 764) and is much simpler than that of the original dye, because two -O-$SO_3H$ groups are replaced by less reactive hydroxyl groups. The MS/MS spectrum of the $[M–H]^-$ ion (*m*/*z* 742) of the hydrolyzed RB dye (Fig. 5(c)) shows many characteristic product ions (listed in Table 2), with logical neutral losses of $N_2$, $SO_2$ and $SO_3$, cleavage of the C–N bond adjacent to the azo bond and aromatic ring as well as cleavage of the azo bond. The degradation mechanism of RB is more complex than that of AY, but it follows a similar pattern except for the hydrolysis step (Fig. 6). The hydrolyzed dye is slightly more toxic than RB,[35] but it is electrochemically decomposed in a similar way to the parent dye.

## Non-polar products

For the identification of non-polar (i.e. non-sulphonated) products, HPLC separation on a C18 column in aqueous/methanolic mobile phases coupled to positive ion atmospheric pressure chemical ionization (APCI)-MS was applied. No products were found, which means that cleavage of the sulphonic acid group from the aromatic ring does not occur. The sulphonic groups are probably cleaved at the last step of the electrochemical degradation, which could explain the absence of non-sulphonated aromatic products.

## CONCLUSIONS

Electrochemical degradation with a carbon fleece cathode and Pt/Ti anode was applied for the treatment of wastewaters containing azo dyes and HPLC/MS/MS was employed for the identification of products formed during this process. The detailed interpretation of fragmentation behaviour observed in first-order and tandem mass spectra together with their retention times were used for the structural elucidation of unknown degradation products. The concentration and the type of electrolyte are the essential factors influencing the rate and extension of decomposition of azo dyes as well as for the products formed during this process. The azo bond undergoes reductive cleavage on the cathode surface for both dyes regardless of the electrolyte. Further degradation and mineralization were proved as well as the chlorination of products during treatment with sodium chloride as the supporting electrolyte. Therefore, the process of azo dye degradation is faster and more effective with sodium chloride than in the ammonium acetate solution (as confirmed by the identification of organic degradation products, significant concentrations of inorganic products, fast decolourization, etc.), probably because of the formation of the strong oxidative agent $ClO^-$. The formation of different degradation products has to be monitored during the optimization of the treatment process due to the potential environmental hazards. HPLC/UV/MS/MS is well suited for this purpose, as demonstrated in this paper on two examples of model azo dyes.

## Acknowledgements

The authors thank the Ministry of Education of the Czech Republic (research project No. MSM0021627502), the Grant Agency of the Czech Republic (project Nos. 203/04/P126 and 203/04/0917), and the COM-CHROM project No. HPRN-CT-2001-00180 of the Commission of European Communities 5th framework programme 'Improving the Human Research Potential and Socio-Economics Knowledge Base' for financial support of this work.

## REFERENCES

1. Brown MA, Devito SC. *Crit. Rev. Environ. Sci. Technol.* 1993; **23**: 249.
2. Hao OJ, Kim H, Chiang PC. *Crit. Rev. Environ. Sci. Technol.* 2000; **30**: 449.
3. Sakalis A, Mpoulmpasakos K, Nickel U, Fytianos K, Voulgaropoulos A. *Chem. Eng. J.* 2005; **111**: 63.
4. Gutierrez MC, Crespi M. *J. Soc. Dyers Colour.* 1999; **115**: 342.
5. Vlyssides AG, Loizidou M, Karlis PK, Zorbas AA, Pappaioannou D. *J. Hazard. Mater. B* 1999; **70**: 41.
6. Naumczyk J, Spyrkowicz L, Zilio-Grandi F. *Water Sci. Technol.* 1996; **34**: 17.
7. Karunaratne De Fazio SA, Lemley AT. *J. Environ. Sci. Health A* 1999; **34**: 217.
8. Sakalis A, Mpoulmpasakos K, Nickel U, Fytianos K, Voulgaropoulos A. *Chem. Eng. J.* 2006,
9. Feng W, Nansheng D, Helin H. *Chemosphere* 2000; **41**: 1233.
10. Bhaskar M, Gnanamani A, Ganeshjeevan RJ, Chandrasekar R, Sadulla S, Radhakrishnan G. *J. Chromatogr. A* 2003; **1018**: 117.
11. Gosetti F, Gianotti V, Polati S, Gennaro MC. *J. Chromatogr. A* 2005; **1090**: 107.
12. Plum A, Rehorek A. *J. Chromatogr. A* 2005; **1084**: 119.
13. Tanaka K, Padermpole K, Hisanaga T. *Water Res.* 2000; **34**: 327.
14. Plum A, Braun G, Rehorek A. *J. Chromatogr. A* 2003; **987**: 395.
15. Jandera P, Churáček J, Taraba B. *J. Cromatogr.* 1983; **262**: 121.
16. Jandera P, Churáček J. *J. Chromatogr.* 1980; **197**: 181.
17. Rao RN, Alvi SN, Husain S. *HRC - J. High Resol. Chromatogr.* 2000; **23**: 329.
18. Holčapek M, Volná K, Jandera P, Kolářová L, Lemr K, Exner M, Církva A. *J. Mass Spectrom.* 2004; **39**: 43.
19. Storm T, Reemtsma T, Jekel M. *J. Chromatogr. A* 1999; **854**: 175.
20. Reemtsma T. *J. Chromatogr. A* 2001; **919**: 289.
21. Holčapek M, Jandera P, Zderadička P. *J. Chromatogr. A* 2001; **926**: 175.
22. Alonso MC, Castillo M, Barceló D. *Anal. Chem.* 1999; **71**: 2586.
23. Holčapek M, Jandera P, Přikryl J. *Dyes Pigments* 1999; **43**: 127.
24. Lemr K, Holčapek M, Jandera P. *Rapid Commun. Mass Spectrom.* 2000; **14**: 1878.
25. Fuh MR, Chia KJ. *Talanta* 2002; **56**: 663.
26. Monaghan JJ, Barber M, Bordoli RS, Sedgwick RD, Tyler AN. *Org. Mass Spectrom.* 1983; **18**: 75.
27. Monaghan JJ, Morden WE. *Rapid Commun. Mass Spectrom.* 1990; **10**: 436.
28. Monaghan JJ, Barber M, Bordoli RS, Sedgwick RD, Tyler AN. *Org. Mass Spectrom.* 1982; **17**: 529.
29. Monaghan JJ, Barber M, Bordoli RS, Sedgwick RD, Tyler AN. *Org. Mass Spectrom.* 1982; **17**: 569.
30. Conneely A, McClean S, Smyth WF, McMullan G. *Rapid Commun. Mass Spectrom.* 2001; **15**: 2076.
31. Weisz A, Andrzejewski D, Fales HM, Mandelbaum A. *J. Mass Spectrom.* 2002; **37**: 1025.
32. Ansorgová D, Holčapek M, Jandera P. *J. Sep. Sci.* 2003; **26**: 1017.
33. Sakalis A, Ansorgová D, Holčapek M, Jandera P, Voulgaropoulos A. *Int. J. Environ. Anal. Chem.* 2004; **84**: 875.
34. Holčapek M, Volná K, Vaněrková D, *Dyes Pigments* 2007; in press.
35. Gottlieb A, Shaw C, Smith A, Wheatley A, Forsythe S. *J. Biotechnol.* 2003; **101**: 49.

DOI: 10.1002/rcm