

## Wavelength-resolved laser-induced fluorescence detection in capillary electrophoresis: naphthalenesulphonates in river water

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### Abstract

Capillary electrophoresis (CE) is combined with wavelength-resolved laser-induced fluorescence detection for the environmental analysis of naphthalenesulphonates (NS) in river water samples. The method enables the detection and the identification of non- as well as hydroxy- and amino-substituted NS. An approximate 30-fold sample concentration is performed by off-line solid-phase extraction prior to the CE separation. The native-fluorescent NS are excited at 280 nm or 325 nm by an excimer dye laser combination providing tunable UV radiation. Wavelength-resolved fluorescence-emission spectra are recorded on-line by an intensified diode-array detector mounted on a spectrograph. Since the emission spectra are strongly affected by the type and pattern of substitution, they provide much structural information. In combination with migration times identification is possible, if standards are available. The low to sub- $\mu\text{g l}^{-1}$  detection limits achieved enabled the identification and quantitation of two trisulphonate and an amino-disulphonate NS in River Elbe samples. The data obtained satisfactorily agree with those obtained by ion-pair HPLC.

*Keywords:* Water analysis; Environmental analysis; Naphthalenesulfonates

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### 1. Introduction

Capillary electrophoresis (CE) has gained popularity in analytical chemistry, because of its extremely high separation power [1]. The technique is well suited for the determination of charged compounds, but can also be applied to neutral molecules by using micelles in the running buffer, a technique denoted as micellar electrokinetic chromatography (MEKC). Detection limits in terms of mass that can be achieved by using commercially available UV absorption detectors can be as low as 600 fg [2]. However, even in favorable cases limits of detection

in terms of concentration units are at best in the  $10^{-6}$ – $10^{-7}$  M range, since the injection volumes that can be used in CE without affecting the separation performance are typically a few nanoliters only. Therefore most applications of CE reported to date deal with biological and pharmaceutical samples [3]. Analysis of environmental samples for the presence of micropollutants generally has to be performed at much lower concentration levels and the applicability of CE for environmental analysis therefore seems rather limited. The use of CE for environmental monitoring purposes has been reviewed by Brumley [4]. Most of the quoted papers deal with the separation and detection of analytes present at relatively high concentrations. Examples are the use of CE for

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the monitoring of organic ions in rain [5] or wastewater [6,7], or of inorganic ions in rain or surface water [8–10]. Typical concentration levels considered in these studies are in the 1–100  $\mu\text{M}$  range. Another approach is to increase the concentration of the analytes concerned before performing separation, either by using an appropriate sample pretreatment procedure [2], or by on-column sample stacking [11]. Pertinent applications include the determination of phenols [12], benzidines [13], aromatic acids [14], polycyclic aromatic hydrocarbons (PAHs) [15] and organolead compounds [16].

Detection limits can of course also be improved by applying a more sensitive detection procedure. So far, the lowest limits of detection have been reported for laser-induced fluorescence (LIF); detection of only a few labelled molecules was effected by using an Ar-ion laser [17]. Unfortunately, most lasers only provide emission lines in the visible region of the electromagnetic spectrum; therefore in most instances LIF can only be applied if analytes are derivatized with a suitable fluorescent label. A relevant example in environmental analysis deals with phenoxy acid herbicides [18] but the procedure used was rather laborious. In general, derivatization of analytes present at very low levels in complex samples has some obvious disadvantages. It is time-consuming if possible at all because of first-order reaction kinetics [19,20]. Even more importantly, the unreacted label and also impurities present in the label easily interfere in the final separation [19,20]. Taking these facts into account, the most promising applications of LIF in environmental monitoring are for native-fluorescent compounds such as PAHs. Detection limits in the order of  $10^{-9}$ – $10^{-10}$   $M$  were reported for PAHs extracted from soil, using either MEKC [21] or capillary electrochromatography [22]. In the present study the CE separation and identification of naphthalenesulphonates (NS) is reported. These compounds are used extensively as intermediates in dye manufacturing, and can also be formed during degradation of dyes by microorganisms [23]. Furthermore, high concentrations of NS, both as such and as formaldehyde polymers, are used as plasticizers in concrete [24]. These compounds can be determined using ion-pair HPLC [25,26] but their charged nature makes them excellently suited for separation by CE. In a previous paper [27]

optimization of the CE parameters was reported and 23 (substituted) NS, including several isomers, were separated.

LIF detection of NS requires the use of UV-emitting lasers, which to date are rarely used. Some applications of continuous wave (cw) lasers have been reported in which either large-frame Ar-ion lasers, operated at 275 nm [28,29], 350–360 nm [30], or frequency-doubled Ar- or Kr-ion lasers [31,32] were used. The main disadvantage of these systems is the limited number of discrete wavelengths they provide: they can not be tuned to the wavelength of maximum extinction of the analyte of interest. The 257 nm line from a frequency-doubled Ar-ion laser can be used for most compounds containing an aromatic ring, but to improve selectivity and reduce interferences from contaminants in the detector cell and fluorescent impurities, the use of longer wavelengths is advantageous. As an alternative to cw lasers, pulsed lasers like KrF-excimer lasers can deliver the required UV radiation [33]. In combination with a dye laser and a frequency-doubling crystal they provide tunable UV radiation. Though it is well known that in terms of LIF detection limits pulsed lasers perform less well than cw lasers [34], they nevertheless should be quite useful in real practice where nanomolar detection limits have to be realized. In the present study a pulsed excimer dye-laser system is utilized; the wavelength was optimized for the detection of the NS which show maximum absorption wavelengths in the deep UV.

On-line spectral identification of analytes is a point of interest in CE because repeatability of migration times still remains a problem, especially as these may depend on minor changes in pH or buffer temperature. Since the pH of the buffer vials changes during analysis because of electrolysis of water, and furthermore heat is produced as a result of the current through the capillary, maintaining constant separation parameters is difficult to achieve. The most commonly used approaches for the on-line identification of migrating compounds are to record absorption spectra using a diode-array UV detector [35] or mass spectra [36]. On-line recording of fluorescence emission spectra, which was reviewed recently [37], is another method that will provide structural information, provided that the analytes

show native fluorescence [32]. The analytes of interest in this study indeed show different emission spectra, caused by the difference in nature and/or position of the substituents on the aromatic rings. Wavelength-resolved detection after CE was first shown in the literature using a CCD-camera mounted on a spectrograph for non-fluorescent analytes provided with a fluorescent label [38] and later for DNA sequencing, with different labels [39,40]. This detector has also been combined with an incandescent lamp as excitation source [41]. Although CCD-cameras provide 2D-spatial information, in practice the output of different pixels collecting light of the same wavelength generally is combined, so that effectively only one spatial dimension is utilized [40,42]. Therefore, an (intensified) diode-array detector mounted on the spectrograph is a good alternative [43]. In this study the latter approach was followed in order to identify the compounds of interest and to discriminate analyte signals from matrix interferences.

## 2. Experimental

### 2.1. Materials

The naphthalenesulphonates used in this study were purchased from Aldrich (Steinheim, Germany), Kodak (Darmstadt, Germany) and Merck (Prodotti Gianni, Milan, Italy). Three trisulphonated naphthalenes were kindly gifted by from DVGW-Technologiezentrum Wasser (Karlsruhe, Germany). As outlined in a previous paper [27], the individual compounds are indicated by the substituent positions on the naphthalene skeleton as follows: positions of  $\text{NH}_2$  groups/positions of OH groups/positions of  $\text{SO}_3^-$  groups. The label "zero" indicates the absence of a particular group. The eluents used for CE were prepared from laboratory-distilled water, HPLC-grade methanol and acetonitrile from J.T. Baker (Deventer, Netherlands), and Baker-grade cetyltrimethylammonium bromide (CTMABr), sodium dodecyl sulphate (SDS) and boric acid. To buffer the eluents, sodium hydroxide was added to the boric acid solution.

### 2.2. River water samples

Three samples were taken at different sites along the River Elbe in Germany: at 4 km, 475 km and 629 km from the Czech border. Concentrations of individual organic contaminants had been determined in advance at DVGW-Technologiezentrum Wasser using ion-pair HPLC, with fluorescence detection as described by Lange et al. [25].

### 2.3. Sample pretreatment

Off-line solid-phase extraction was utilized for trace enrichment of the NS and to remove matrix interferences, as described before [27]. In the present study a sample volume of 150 ml was used, so that an enrichment factor of 30 could be achieved. The recoveries were in the 50–100% range, except for 4/3/2 and 4/5/1 which showed a much lower (<5%) value. The repeatability of the whole sample pretreatment procedure still has to be further attended; in some experiments the recoveries obtained for the individual analytes were a factor of 1.2–2.8 times lower than indicated above.

### 2.4. Capillary electrophoresis

CE separations were performed in a 90 cm (80 cm to the detector) capillary from Polymicro Technologies (Phoenix, AZ, USA) with an I.D. of 75  $\mu\text{m}$ , using a Prince injection and high-voltage system from Lauerlabs (Emmen, Netherlands). A voltage of 30 kV was applied over the capillary. Samples were pressure-injected at 100 mbar for 6 s. The separation capillary was inserted in a 5-mm I.D. polyethylene tube, through which air of ambient temperature was led at a speed of 17 m/s. In this way, efficient thermostating was achieved.

### 2.5. LIF detection

The CE set-up in combination with wavelength-resolved LIF detection is schematically depicted in Fig. 1. Laser lines at 280 and 325 nm were provided by a frequency-doubled Lambda Physik (Göttingen, Germany) LPD 3002 dye laser, pumped by a Lambda Physik LPX 110i excimer laser operated on XeCl gas (308 nm). For frequency-doubling of either

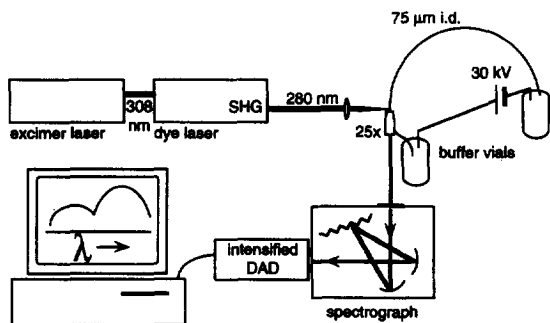


Fig. 1. Experimental set-up for CE with UV-laser induced fluorescence detection with on-line recording of emission spectra.

the 560-nm Rhodamine 6G or the 650-nm Rhodamine B dye laser output, a thermostatted KDP crystal was inserted. To avoid saturation of analytes or, even more important, damage to the detector cell, the 10 mW average output of the laser (operated at 10 Hz) was attenuated by a potassium nitrite (Merck) solution in water in a quartz cuvette. The beam was focused into the capillary by a 3-cm focal length quartz lens. Fluorescence emission was collected with a microscope objective (40 $\times$ , N.A.=0.6) and focused onto the entrance slit of a MonoSpec 18 spectrograph (Scientific Measurement Systems, Grand Junction, CO, USA) equipped with a holographic 600 g/mm grating. Fluorescence spectra were recorded at 1 Hz with an intensified diode-array detector Model 1420 from EG and G (Princeton, NJ, USA) equipped with a Model 1463 detector controller, and subsequently processed by a Model 1460 OMA III console. The 3D-data thus obtained were further analysed using the Igor (WaveMetrics, Lake Oswego, OR, USA) software programme run on an Apple Macintosh (Cupertino, CA, USA) computer. In this way, a spectral range of 311–512 nm was covered. The readout of adjacent pixels of the array could be combined to enhance the signal-to-noise ratio, and moreover to decrease the amount of data produced. To record detailed reference spectra, the output of four pixels was combined, which resulted in an overall resolution of 2 nm (full width at half maximum). For practical reasons, recording a complete electropherogram of up to 25 min was performed using spectra of only 18 points (to cover the 311–512 nm range) so that a low overall resolution of 11 nm was achieved. Under these conditions

computer data files of up to 300 kbytes are dealt with, which can easily be handled with commonly applied personal computers.

## 2.6. Room-temperature fluorescence spectra

Room-temperature fluorescence excitation spectra of standard solutions of the individual NS both in water and in the buffer used for CE were recorded on a Perkin Elmer (Norwalk, CT, USA) LS-50 luminescence spectrometer.

## 3. Results

### 3.1. CE separation

The optimization of the separation of the NS concerned was studied before [27]. A complete CE separation of 23 naphthalenesulphonates, could be achieved in only two 28-min runs, using a 50 mM borate buffer at pH 8.7 containing either 100 mM SDS or 15% acetonitrile (v/v). For the complete separation of the isomers containing a single sulphonate group, the SDS buffer has to be applied, while the addition of acetonitrile is required to separate the disulphonates. In this study, three trisulphonates were included. As expected they migrate slower than the mono- and disulphonated NS. These three compounds could easily be separated from each other using the buffer system with 15% acetonitrile (v/v). Fig. 2 shows a typical electropherogram, recorded using a borate buffer at pH 8.75 (without addition of acetonitrile or SDS); the regions in which the mono-, di- and trisulphonated NS migrate are marked. For this electropherogram the total emission in the 311–512 nm range was combined.

### 3.2. Excitation

Whereas the presence of sulphonate groups hardly affects the fluorescence excitation and emission spectra, the influence of the NH<sub>2</sub> and OH groups is pronounced. As an example, in Fig. 3 the excitation spectra of three monosulphonates are compared. These spectra were corrected for the wavelength dependence of the lamp intensity, while differences in fluorescence quantum yield have been ignored by

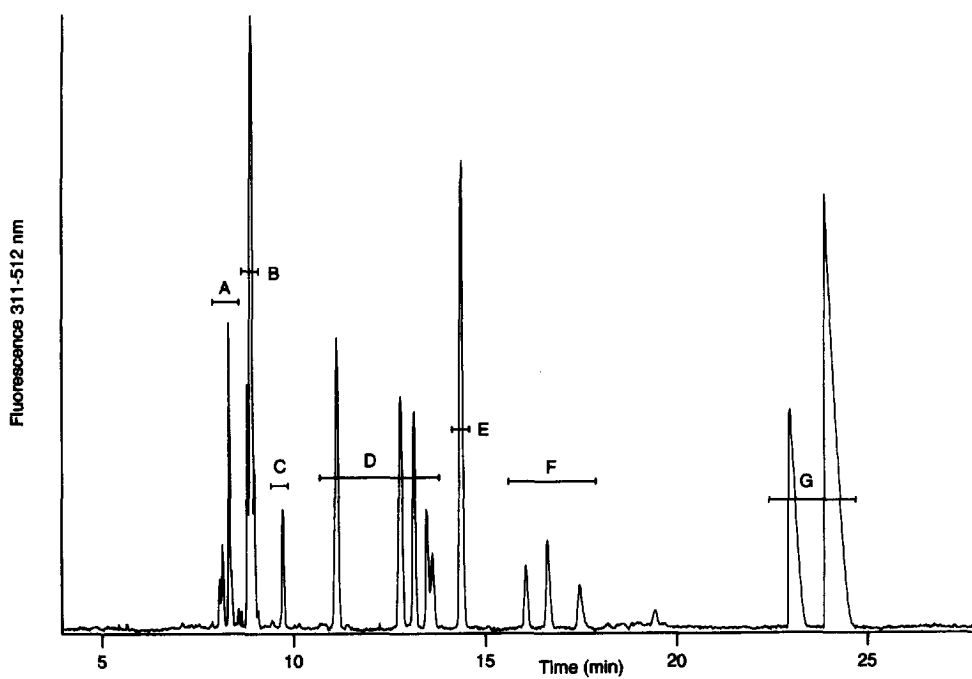


Fig. 2. Electropherogram of a standard solution of the test NS using a 50 mM borate buffer at pH 8.75. The regions of migration for the variously substituted compounds are indicated. A: Monosulphonates and amino-monosulphonates; B: amino-hydroxy-monosulphonates; C: hydroxy-monosulphonates; D: amino-disulphonates; E: disulphonates; F: hydroxy-disulphonates; G: trisulphonates.

expressing the y-axis in relative units. As can be seen, substitution of  $\text{NH}_2$  or OH causes a red-shift, which can be readily rationalized [44]. The inclusion of a lone-pair-containing substituent introduces  $n\pi^*$  character (charge transfer from the lone-pair to the ring system) in the first excited electronic state. The

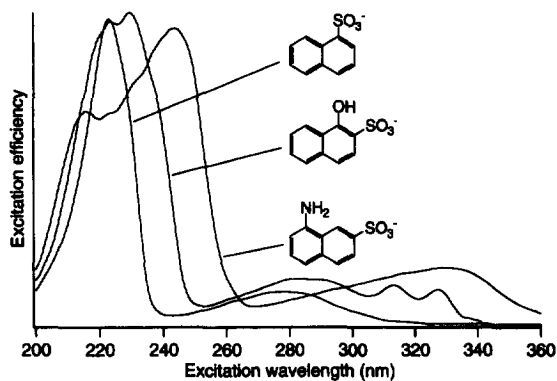


Fig. 3. The effect of hydroxy- and amino-substitution on the excitation spectra of NS.

position of this level will be influenced by both the position of the substituent in the aromatic system and the solvent cage surrounding the molecule. Therefore, extinction coefficients will depend on the solvent, dependencies that should be accounted for when recording calibration curves. At first sight, Fig. 3 suggests that the optimum excitation wavelength for the NS series is around 230 nm. However the use of such a short wavelength will cause strong fluorescence of minor impurities in both the CE capillary and the optics applied [45]; in other words the wavelength of the laser line should be as long as possible. In this study the two wavelengths optimal for the excitation of non-substituted, and  $\text{NH}_2$ - or OH-substituted NS; that is, 280 and 325 nm (see Fig. 3) were applied. It was found (data not given) that at 325 nm excitation, only the  $\text{NH}_2$ - and some OH-substituted NS showed up in the electropherograms, which illustrates the extra selectivity that can be obtained by means of the laser system involved. The signal-to-noise ratios were, however, less favorable than for 280 nm excitation. For this reason in the

other CE experiments only 280 nm excitation, suitable for the complete class of NS was performed.

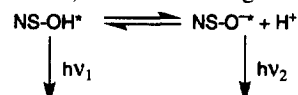
Extremely high laser powers should be avoided in LIF detection [34] because excitation saturation of the analytes is generally reached at much lower irradiances than the saturation of the background signal caused by scatter and impurity fluorescence. Therefore the signal-to-noise (*S/N*) ratio was optimized by attenuating the output (the peak power is 70 kW at an average pulse width of  $15 \cdot 10^{-9}$  s) with potassium nitrite solutions in water at several concentrations. The best *S/N* ratios were obtained if the laser power was 50-fold reduced.

### 3.3. Emission spectra

The influences of the  $\text{NH}_2$  and OH groups on the emission spectra are even more pronounced than on the excitation spectra, as can be seen in Fig. 4. Whereas, again, the sulphonate groups hardly have any effect on the spectral features (see Fig. 4A), the amino-substituted NS spectra show a single broad band in the wavelength region from 410 to 480 nm, indicating a substantial Stokes shift. This can be readily understood because the charge separation in the  $n\pi^*$  first excited state in these molecules will

cause a reorientation of the polar solvent cage compared with the ground state. Again the shift will depend on both the polarity and temperature of the solvent [44]. The pertinent spectra of 5/0/2 and 2/0/1 are given in Fig. 4B. As can be seen, the Stokes shift also depends on the position of the  $\text{NH}_2$  group on the aromatic ring system, which agrees with literature data [44]. Unfortunately the increase in Stokes shift is accompanied by a decrease in quantum efficiency of fluorescence [46]: the values for 2/0/1 and 8/0/1 in water are 0.21 and 0.016, respectively. Obviously low quantum yields limit the sensitivity of the LIF detection scheme.

Some OH-substituted NS show an extra emission band at long wavelength in aqueous solutions [47]. This phenomenon is probably caused by the competition between deprotonation of the molecule during its residence in the excited state and emission of a photon. In other words, the following reaction



scheme plays a role:  $\text{NS-OH} \rightleftharpoons \text{NS-O}^- + \text{H}^+$

The  $\text{pK}^*$  values for OH–NS in the first excited electronic state,  $\text{pK}^*$ , are much lower than those in the ground state: for instance 9.1 versus 1.6 for

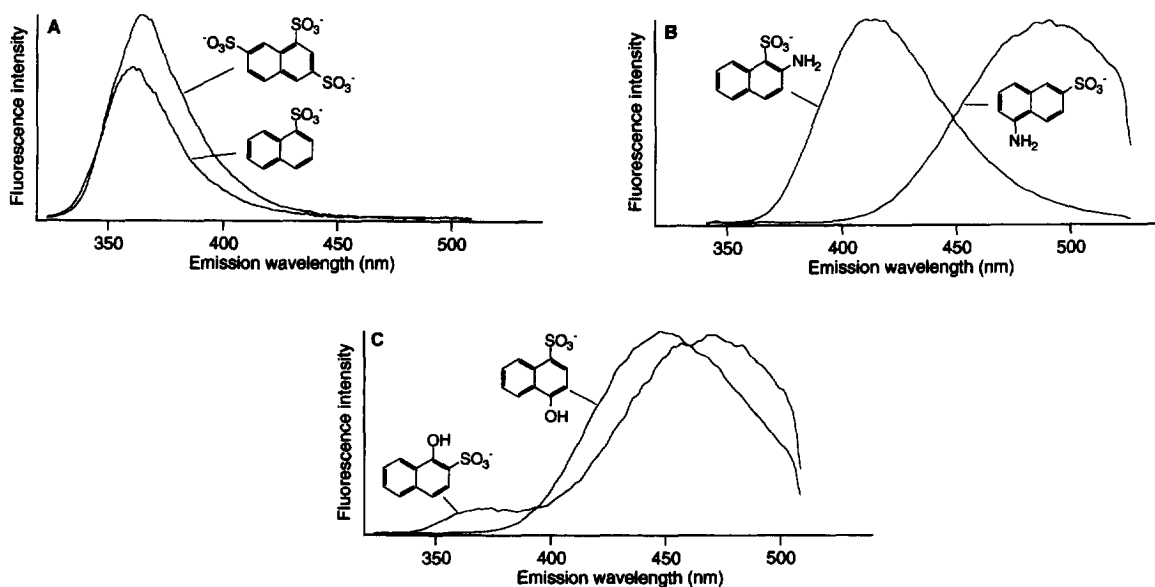


Fig. 4. Comparison of emission spectra for some selected NS. (A) 0/0/1,3,7 and 0/0/1; (B) 2/0/1 and 5/0/2; (C) 0/1/2 and 0/4/1. Concentrations:  $10^{-6}$  M range in borate buffer pH 8.7.

0/5/2, and 9.2 versus 0.5 for 0/5/1 [48]. Similar values were found for other OH-NS [49]. Consequently, under the present experimental CE conditions, deprotonation in the excited state tends to be complete, whereas in the ground state it will hardly play a role. This can easily be demonstrated by comparing the emission spectra of 1/0/2 in ethanol and water [47]: in the latter solvent the intensity of the bands at wavelengths around 350 nm ( $\nu_1$ ), also observed in ethanol, are lower, and a new broad band appears at 460 nm ( $\nu_2$ ) which is not observed in ethanol. Similar effects were reported for 0/2/0 (2-naphthol) at very low and high pH [48]. In view of the above reaction scheme, it will be obvious that the intensity ratio of the short- and long-wavelength emissions will be dependent on the  $pK^*$  values and therefore on the positions of the individual substituents in the aromatic ring system. From an analytical point of view this is quite interesting because it may enable on-line identification of

migrating NS. In Fig. 4C spectra of two OH-NS are depicted. As can be seen from the spectrum of 0/1/2, bands originating from both the protonated and deprotonated compound occur. Of course, this also indicates that reference spectra and calibration curves have to be recorded under the same (solvent composition, temperature) conditions.

Fig. 5 shows the 7 to 9 min part of an electropherogram covering the monosulphonated NS, see Fig. 2, in a 3D representation which provides complete fluorescence emission spectra of the migrating compounds. The injected concentrations are in the  $10^{-6}$  M range. As is to be expected, the emission spectra of the individual compounds are rather different, which illustrates the potential of wavelength-resolved detection for identification purposes. A single emission band of 0/6/2 is found around 440 nm, while the emission spectrum of 0/1/2 exhibits the two bands mentioned above, at 360 and 460 nm, respectively. The separation for

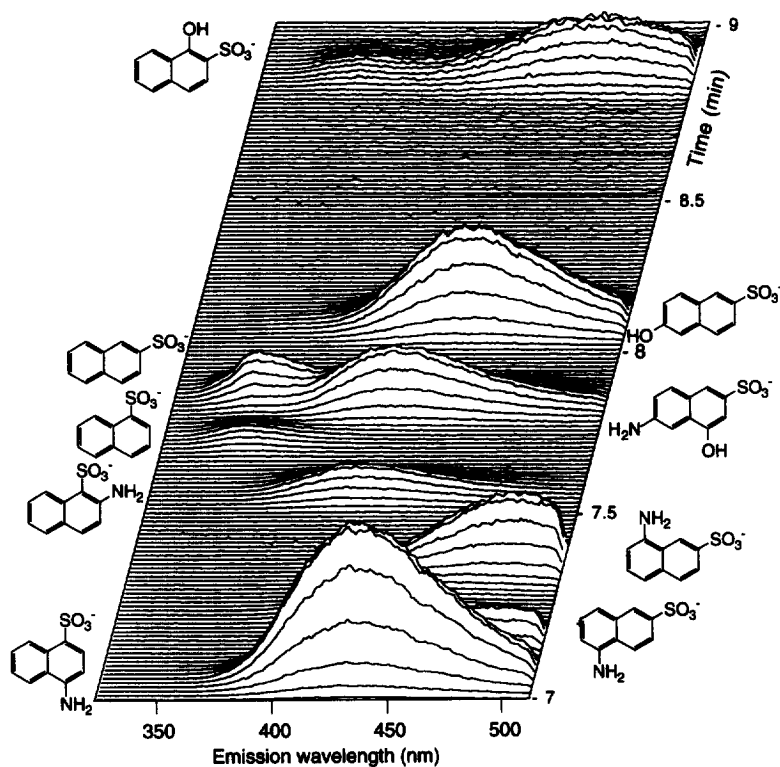


Fig. 5. Three-dimensional electropherogram of naphthalene monosulphonates, recorded using a borate buffer of pH 8.75 with 100 mM SDS. The structures of the nine NS are shown on the y-axis.

6/4/2 and 0/0/2 can be seen to be poor, but as the wavelengths of the emission maxima differ as much as 90 nm, the analytes can be clearly distinguished.

#### 3.4. Analytical data

The detection limits of the NS in standard mixtures are in the 0.4–10  $\mu\text{g/l}$  range, indicating the performance of the detection system as such. For the spiked river water samples, the sample pretreatment was used and detection limits in the 0.1–2  $\mu\text{g/l}$  range ( $S/N$ , 3:1) were found; obviously, these figures reflect both recoveries and differences in background of real samples compared to standard solutions. A presentation of all individual data is beyond the scope of this paper, but it may well be said that these values are rather good for a CE-based procedure, which underlines the potential of the excimer LIF detection. Differences in detection limits originated mostly from differences in fluorescence quantum yield. The dynamic range of the system was found to be three orders of magnitude, and was limited on the high concentration side by the dynamic range of the detector. If necessary, this can be improved by using a higher repetition rate of collecting spectra.

#### 3.5. River water samples

To investigate the applicability of the present CE–LIF procedure for environmental analysis, three River Elbe water samples taken at sites with different pollution levels, were analysed. The electropherogram of the river water sample taken at the most up-stream site is presented in Fig. 6A, showing the total 311–512 nm emission. Three major peaks are observed, i.e., at 13:06 min, 22:42 min and 28:04 min. Of course, the selectivity can be enhanced by narrowing the emission window. By applying a 349–380 nm window only the NS which do not have an OH or  $\text{NH}_2$  group show up in the electropherogram. This is illustrated in Fig. 6B, in which peaks resulting from di- and trisulphonated NS can be seen; major peaks are found at 13:55 min, 14:15 min, 24:16 min and 25:27 min. The identity of the various peaks in the electropherograms was studied by spiking the samples with reference NS and comparing both migration times and emission spectra. In this way, it was found that the peak at 13:06 min

originates from 7/0/1,3 (cf., insert in Fig. 6A). The other major peaks could not be identified because of a lack of reference compounds. The migration times and the spectra (shown in the other insert), however, give some information regarding their structure: for the 22:42 min and 28:04 min peaks threefold negatively charged compounds can be expected with, possibly, OH or  $\text{NH}_2$  substituents. In the electropherogram recorded at short emission wavelengths (Fig. 6B) three peaks could be assigned. The peak at 14:15 min was attributed to 0/0/1,5 and that at 24:16 to 0/0/1,3,6. The peak at 25:27 min may be due to either 0/0/1,3,5 and/or 0/0/1,3,7, because these two analytes could not be separated with the borate buffer. The concentrations of the NS found at the downstream sites (475 km and 629 km from the Czech border) were distinctly lower than at 4 km. Still, even then the present CE–LIF set-up could be used successfully. This is evident from Fig. 7 which shows the traces for both the wide (311–512 nm) and the small (349–380 nm) emission range. Unexpectedly, the peaks which show up in this figure are the same as those observed in Fig. 6. The concentrations found for the identified peaks are summarized in Table 1, and compared with the data found using an established HPLC procedure [25]. If the peak at 25:27 min is attributed to 0/0/1,3,5 only, the concentrations obtained by CE–LIF are about 2-fold higher, a quite satisfactory result in view of the strong difference in the two approaches. The remaining discrepancy should be mainly attributed to the difference in sample handling procedures applied. The method for separation and detection/identification as such show only small standard deviations (typically 4%).

## 4. Conclusions

Capillary electrophoresis combined with excimer-laser-induced fluorescence detection is an appropriate technique for the trace-level detection of the (substituted) naphthalenesulphonates considered in this study. When reference compounds are available, the combination of wavelength-resolved emission spectra and migration times enables the unambiguous identification of the target analytes. Discrimination



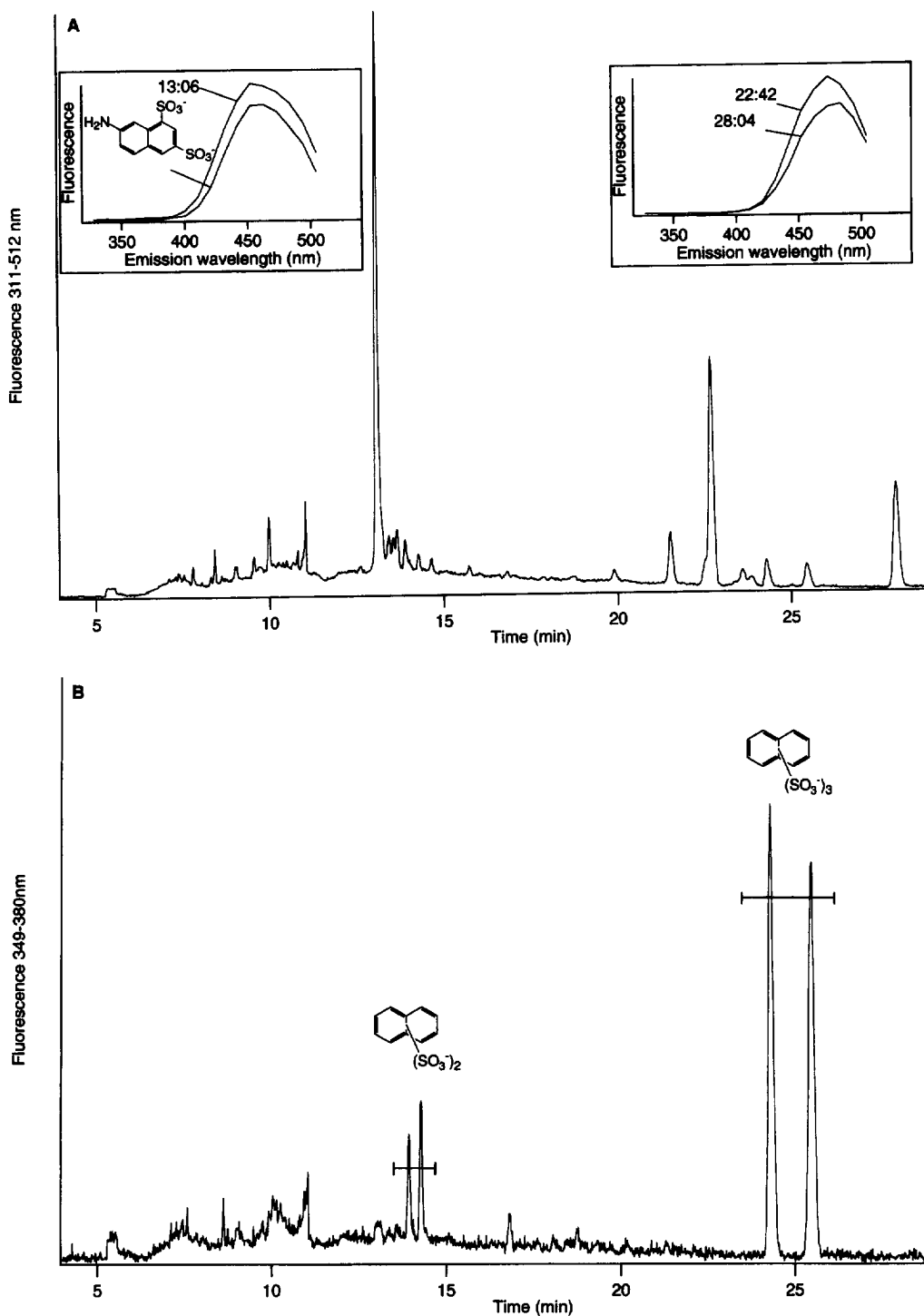


Fig. 6. Electropherogram of a River Elbe sample, recorded using a borate buffer of pH 8.75, after a 30-fold pre-concentration by means of off-line SPE. Excitation: 280 nm; emission range: (A) 311–512 nm or (B) 349–380 nm.

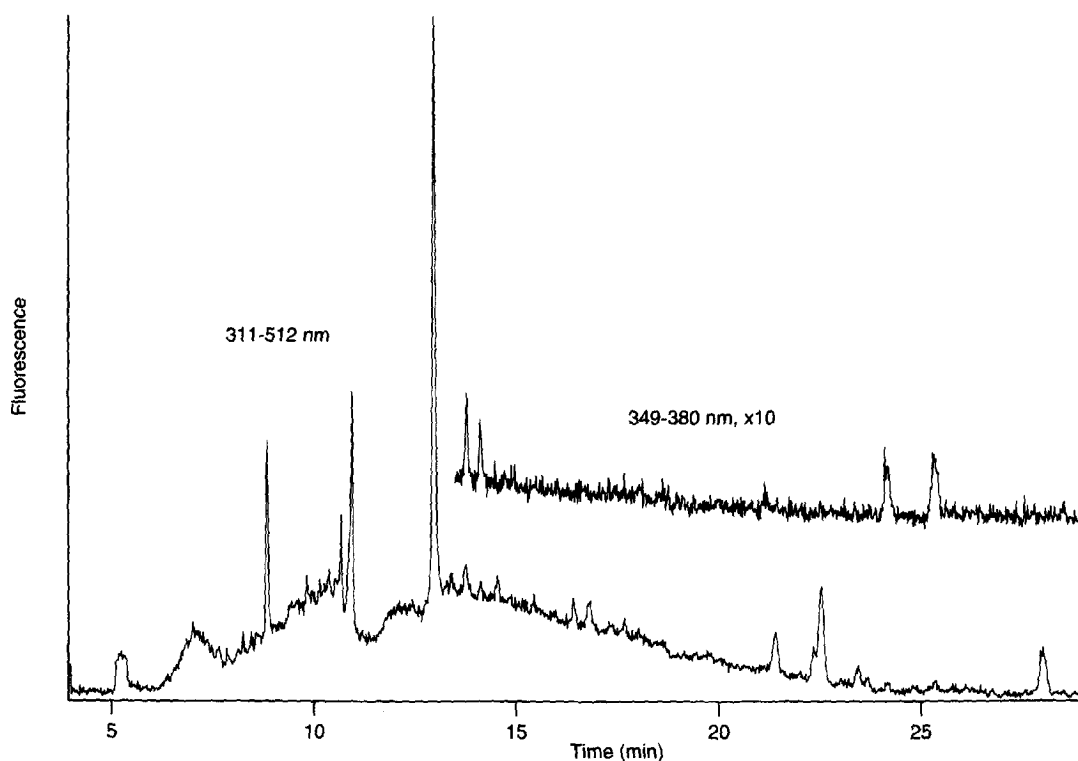


Fig. 7. Electropherogram of a River Elbe sample taken 629 km from the Czech border. Experimental conditions used same as in Fig. 6.

Table 1

Concentrations of three NS (in  $\mu\text{g l}^{-1}$ ) identified in River Elbe samples (taken in Germany) and analysed by CE-LIF or HPLC with fluorescence detection

Compound	Sampling site, from Czech border					
	4 km		475 km		629 km	
	CE-LIF	HPLC-Flu	CE-LIF	HPLC-Flu	CE-LIF	HPLC-Flu
7/0/1,3	6.4	n.d.	1.1	n.d.	1.6	n.d.
0/0/1,3,6	31	18	3.9	2.3	2.2	1.0
0/0/1,3,5	14 <sup>a</sup>	6.8	1.5 <sup>a</sup>	0.81	1.3 <sup>a</sup>	0.41

<sup>a</sup> Assuming a pure peak, see Section 3.5.

n.d., not determined.

from unknown (matrix) constituents and background scatter is relatively simple because these show non-structured spectral features. If the CE-LIF procedure is combined with pre-separation analyte enrichment, the limits of detection of most NS are in the low to sub- $\text{mg l}^{-1}$  range, which is sufficient for real-world monitoring studies. The frequency-doubled excimer dye laser system of course can be used for other classes of native fluorescent compounds as well [50].

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