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Analysis of aromatic sulfonates in water by solid-phase extraction and capillary electrophoresis

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Abstract

The separation of 14 different aromatic sulfonates of environmental concern by capillary (zone) electrophoresis (CZE) is presented. A new off-line solid-phase extraction (SPE) enrichment procedure, that is compatible with CE analysis, was developed, using the styrene–divinylbenzene adsorbent LiChrolut EN. The combined method of SPE and CE allows the determination of aromatic sulfonates in water samples in the low μg/l range. Separations are performed with a simple sodium borate buffer at pH 9.3. Analytes are detected by UV absorbance and fluorescence emission with a Xe-lamp excitation source, and both principles are compared. The recoveries for most of the sulfonates are >70% for the extraction from spiked tap and river water. The average method precision is <20% for replicate analyses. Very hydrophilic sulfonates cannot be extracted by this method. The detection limit of the combined method of SPE enrichment and CE analysis is approximately 0.1 μg/l for 200-ml water samples. The performance of the method was checked with the analysis of river and contaminated seepage water. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Solid-phase extraction; Seepage water; Water analysis; Aromatic sulfonates; Sulfonates, aromatic

1. Introduction

Aromatic sulfonates like benzene-, naphthalene-, anthrachinone- and stilbenesulfonates are widely used in industrial and domestic processes. For example, substituted benzene- and naphthalenesulfonates are used in the chemical industry for the production of pharmaceuticals and dyes [1–3]. Sulfonated azo dyes are extensively applied in the textile industry [4]. In the paper industry stilbenesulfonates are used as whiteners [5,6]. Tanneries emit polyphenolsulfonates, which have a widespread application as dispersants, wetting and suspending agents and stabilizers [7]. Ligno- and chlorolignosulfonates arise from the production of cellulose and are mainly discharged by pulp mills [8,9]. Alkanesulfonates and linear alkylbenzene sulfonates (LASs) are frequently used anionic surfactants in detergents [10–12].

Aromatic sulfonates are very acidic ($pK_a < -1$) and strongly hydrophilic. Contrary to LASs, most of the aromatic sulfonates without a hydrophobic alkyl chain (Fig. 1) are biologically persistent compounds [1,13–15]. Despite the widespread use of aromatic sulfonates, only little is known about their toxicology, ecotoxicology and environmental behaviour. Their aquatic toxicity appears to be small [13]. However, because of their low *n*-octanol–water

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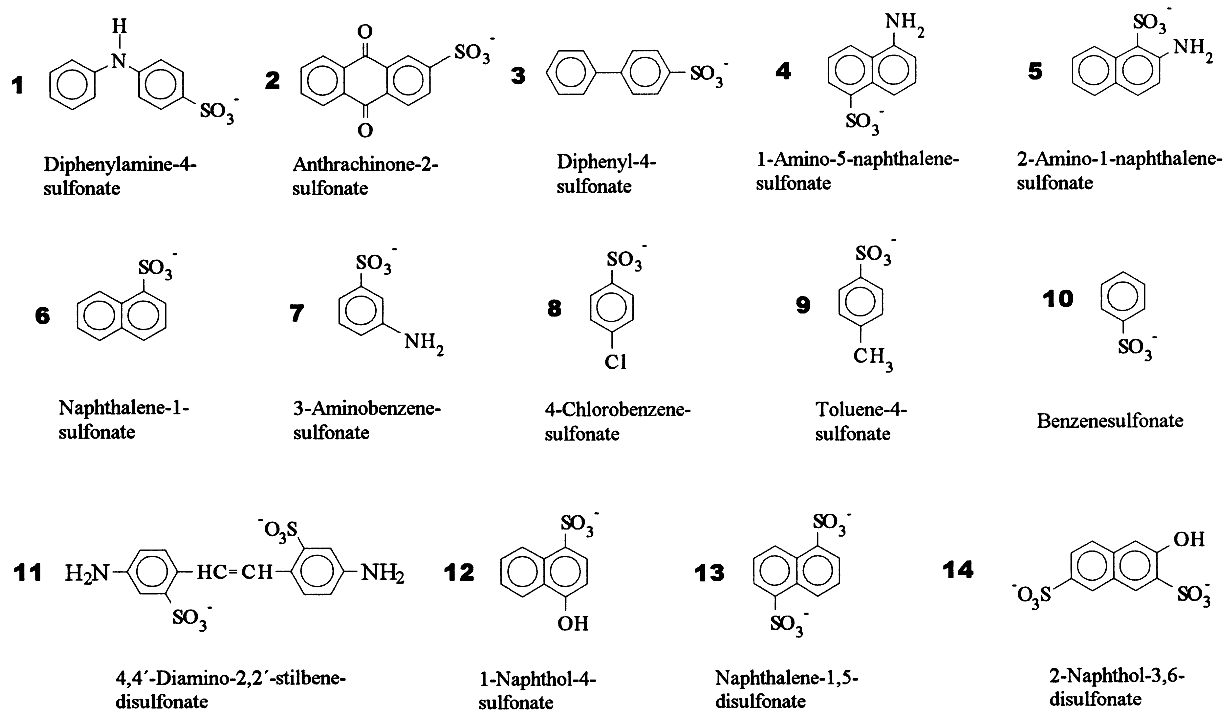

Fig. 1. Structures of aromatic sulfonates numbered in order of their migration times in Fig. 3.

partition coefficients (log $K_{OW}<2.2$) [7,13,15] they possess a high mobility within the aquatic system. Therefore, they can easily cause pollution of surface waters and pass the water treatment process. They are regularly found in natural waters [1,5,14,16–20] and even have been detected in tap and drinking water at levels of about 1 μg/l [21]. The concentrations encountered in waste waters from chemical industries and water treatment plants are much higher, values in the mg/l range have been reported [1,7,9,18,22–24]. During drinking water production, persistent aromatic sulfonates can only be removed effectively by means of frequently regenerated activated carbon. Therefore, they are so-called drinking water relevant pollutants. The surface activity of sulfonated compounds, in combination with their non-biodegradability in water and soil, enables the desorption and mobilization of soil adsorbed hydrophobic, xenobiotic, toxic compounds and their subsequent release into groundwater [25,26]. Knowledge about the presence and concentration of such compounds in the environmental compartments is therefore of great importance for the protection of our natural waters.

The trace enrichment and analysis of smaller, highly water-soluble aromatic sulfonates without a hydrophobic alkyl chain is still under development and not yet routine practice. Because of their polarity, non-volatility and thermal lability gas chromatography–mass spectrometry (GC–MS) is only of limited value for their identification [27]. The majority of presently available methods for their determination is based on ion-pair reversed-phase high-performance liquid chromatography (RP-HPLC) [1,6,9,14,16,24,28–33]. In principle, their analysis is difficult because of the variety and similarity of isomers. Therefore, capillary electrophoresis (CE), with its extremely high separation power and its suitability for ionized water-soluble compounds, is particularly well suited for their determination [2–4,19,20,34–37]. The major advantages of CE over HPLC are its simple, inexpensive and routine operation, superior efficiencies and short analyses times. However, only few reports are described in the

literature for the analysis of sulfonate surfactants [12,38–41] and aromatic sulfonates without an alkylchain [2–4,19,20,34–37] by CE in the pure capillary zone electrophoresis (CZE) and the micellar electrokinetic chromatography (MEKC) mode. For example, Jandera and co-workers analysed some technological samples containing various sulfonates (mostly naphthalenesulfonates) by CZE with borate and phosphate buffers and the addition of cyclodextrins [2,3]. Kok and co-workers investigated the comprehensive separation of 21 different (isomeric) amino- and hydroxy-substituted naphthalenesulfonates, but did not succeed [in spite of sodium dodecyl sulfate (SDS) and acetonitrile addition] in the separation of all compounds in one run [19,20,34].

The major limitation of CE for environmental analysis is its relatively poor concentration sensitivity with stems from the limited sample volume (1–10 nl) that can be analysed without compromising separation efficiency. Therefore, an enrichment step in combination with CE determination is necessary. On-column enrichment procedures such as on-line solid-phase extraction (SPE) [42], sample-stacking (field amplified injection) [43] or isotachophoresis [44] have not yet gained acceptance for routine analysis. Classical SPE, which has become the preferred technique for sample preconcentration, common for LASs [10,11,25,33], is less straightforward for more water-soluble sulfonates lacking a hydrophobic alkyl chain (Fig. 1). With log K_{OW} values up to four-orders of magnitude below LASs, these substances exhibit no retention on RP-C_{18} material. However, ion-pair SPE with cationic ion-pair reagents and hydrophobic sorbents (RP-C_{18}) is suitable for the extraction of these very water-soluble sulfonates and is by far the most frequently applied method for their enrichment. Quite good recovery values for many aromatic sulfonates have been reported [6,7,9,14,16,24,28,29,34]. However, very hydrophilic amino- and hydroxysubstituted benzenesulfonates, as well as naphthalenesulfonates with two or three hydrophilic groups have not sufficiently high breakthrough volumes and cannot be extracted quantitatively by ion-pair SPE [1,14,34]. In addition, ion-pair SPE suffers from further major drawbacks: (i) co-extraction of interfering dissolved organic compounds (e.g., humic substances) is an even larger problem than in conventional SPE. (ii) High contents of inorganic salts might substantially affect recoveries [9,14,26]. (iii) Ion-pair SPE is incompatible with CE analysis, as the non-volatile ion-pair reagents interfere with CE separation. Trace enrichment of aromatic sulfonates also has been reported by extraction with anion-exchange phases, but this attempt was not very successful. As ion-exchange materials are susceptible to high inorganic salt contents, this approach is not suited for the analysis of real water samples [9,16,24]. Altenbach and Giger presented a SPE procedure for aromatic sulfonates based on graphitized carbon black adsorption. They found that molecules with larger aromatic structures such as stilbene- and anthraquinonesulfonates are difficult to elute from carbon, whereas the recovery of very hydrophilic amino-hydroxysulfonates was also not satisfactory [1].

In the past it has often been impossible to extract very polar, hydrophilic, water-soluble organic compounds (e.g., pesticide metabolites) with log K_{OW} values <2 quantitatively by means of classical SPE with apolar RP materials (C_{18}). A revolutionary breakthrough in this field occurred with the development of the styrene–divinylbenzene (PS–DVB) copolymers, e.g., PLRP-S (Polymer Labs., Amherst, MA, USA), LiChrolut EN (Merck, Darmstadt, Germany) and Isolute ENV (International Sorbent Technology, Cambridge, UK). Especially LiChrolut EN, which has been designed for environmental analysis, is a microporous, crosslinked adsorbent material (ethylvinylbenzene–divinylbenzene copolymer) with a very large accessible specific surface area (1200 m^2/g) and small pore size diameter (0.5–10 nm) and even exhibiting a slightly hydrophilic character. It contains multiple binding sites with mixed mechanistic adsorption properties. These outstanding characteristic properties of LiChrolut EN are gained by a special post-cross-linking reaction in the production process. Because of these features, LiChrolut EN has a very high adsorption capacity for micropollutants and shows higher retention and recovery for polar analytes [45–47]. More detailed manufacturer's data about the sorbents' characteristics is not available.

Because of the inherent limitations of ion-pair extraction, graphitized carbon black or ion-exchange

adsorption, SPE with LiChrolut EN was examined for aromatic sulfonate extraction from different types of water. Analyses were performed by CZE.

2. Experimental

2.1. Chemicals and reagents

Diphenyl-4-sulfonate (sodium salt) is a product of Bayer (Leverkusen, Germany). All other sulfonates used were commercially available products of different quality obtained from Merck (Darmstadt, Germany), Sigma–Aldrich (Deisenhofen, Germany) or Fluka (Buchs, Switzerland). The structures of the sulfonates are given in Fig. 1. Sodium tetraborate ($Na_2B_4O_7$, anhydrous), sodium hydroxide, acetonitrile, hydrochloric acid (37%), ammonium acetate and ammonium hydroxide (>25% in water) all of analytical grade were purchased from Merck. The ion-pairing reagent tetrabutylammonium bromide (TBABr) was from Sigma–Aldrich. Methanol and acetone (purity for pesticide residue analysis) were supplied by Promochem (Wesel, Germany). Ultrapure water was prepared by ultrafiltration with a Millipore Q_{plus} apparatus (Millipore, Bedford, MA, USA).

Sulfonate standard stock-solutions of 100 mg/l were prepared by dissolving 10 mg of each compound in 100 ml Milli-Q water. The working standard solutions were prepared by further dilution of the stock standard solutions with Milli-Q water. The standard mixtures were produced from these single-compound solutions. The sulfonate standard mixture contained 100 mg/l of each compound and was further diluted for CE analysis, calibration and preparation of fortified SPE samples. All solutions were stored at 4°C in the dark.

2.2. Solid-phase extraction

The solid-phase adsorption material LiChrolut EN was obtained from Merck. LiChrolut EN (200 mg) was filled in 3-ml glass cartridges (Merck ordering No. 1.19878.0001) between two PTFE frits (Merck, 1.19891.0001, porosity 10 μm). The adsorbent was activated and conditioned first with 5 ml of a methanol–acetone (3:2, v/v) solvent mixture and then with 5 ml water (without application of vacuum). For recovery studies, 1-l water samples (Milli-Q, tap or river water) were spiked with known volumes of a sulfonate standard mixture and were pH-adjusted with hydrochloric acid (37%) either to pH 2.0, 1.0 or 0.5 (or not adjusted for pH 7.5). The spike level for each sulfonate for the recovery study experiments was 2 μg/l. From the 1 l, 200 ml water was taken and filled into glass reservoirs connected to the extraction cartridge and was then drawn through with a flow-rate of 1–5 ml/min. It was demonstrated that the flow-rate did not influence the recovery rates. After the extraction, the cartridges were washed with 5 ml water and then dried with a stream of nitrogen to remove any remaining water. The aromatic sulfonates were eluted with 4 ml of the methanol–acetone (3:2, v/v) solvent mixture into glass vials. The solvent was evaporated under a gentle stream of nitrogen and the sulfonates redissolved in 100 μl water for CE analysis (enrichment factor: 2000). Absolute recoveries were determined using external calibrations. The mean values for recoveries were calculated from six determinations ($n=6$).

2.3. Samples and sample pretreatment

The tap water used was from the water supply system of Munich and the river water from the river Isar, the biggest river running through Munich. The samples were taken in May 1998. No special sample pretreatment was applied.

The seepage water analysed was from a municipal waste disposal plant in Augsburg (north-west of Munich). The water was taken in August 1997. Only 100 ml seepage water was extracted. Prior to the enrichment with LiChrolut EN, a clean-up step with RP-C_{18} material (LiChroprep RP-18 from Merck) was performed to remove interfering unpolar compounds. The C_{18} material (1 g packed in the same 3-ml cartridges of Merck) also was conditioned with methanol–acetone (3:2, v/v) and water (10 ml each). The seepage water was drawn through the C_{18} cartridge at its actual pH value (7.9) with a flow-rate of approx. 5 ml/min. The percolated water was collected, pH-adjusted to 2.0 and then extracted by the standard LiChrolut EN SPE procedure for the extraction of hydrophilic sulfonates as described

above. Finally, the samples were redissolved in 1 or 2 ml water (enrichment factor: 100 or 50).

2.4. Capillary electrophoresis

Separations were performed with a Crystal CE system equipped with a fixed-wavelength UV detector Model PU 4225 (both from Unicam Chromatography, Kassel, Germany) and a FD-300 dual-monochromator fluorescence detector (GTI/Spectrovision, Concord, MA, USA) which operates with a pulsed Xenon lamp and a CE flow cell. UV detection was performed at 210 nm (or at 230 nm in case of the real water samples) which is a reasonable compromise for a simultaneous detection of all investigated sulfonates. Fluorescence detection was performed with an excitation wavelength of 230 nm and emission wavelengths of 335 or 410 nm. Bare fused-silica capillaries of 57 to 70 cm (length to the detector 47–60 cm)×75 μm I.D.×375 μm O.D. were used (Unicam). A constant voltage of 25 kV was applied with the cathode end at the detector. The temperature of the capillary was maintained at 30°C by the instrument thermostatting system. Samples were pressure injected with 50 mbar for 12 s (cathodic injection). Data acquisition was performed with 4880 Unicam Chromatography data handling software.

CZE separations were routinely performed with a 25 m*M* sodium borate buffer at pH 9.3 (no pH adjustment necessary) and with a volatile 50 m*M* ammonium acetate buffer alkalized to pH 10 with ammonium hydroxide. For resolution improvement studies different amounts of acetonitrile were added to the 25 m*M* sodium borate buffer. The buffers are stable for five days at room temperature. They were filtered through 0.45-μm cellulose acetate filters (Sartorius, Göttingen, Germany). The capillary was conditioned every morning before starting a sequence of runs by rinsing in the high-pressure mode for at least 10 min with 0.1 *M* NaOH, 5 min with water and 5 min with running buffer. After every third run the capillary was rinsed for 5 min with 0.1 *M* NaOH, 3 min with water and 2 min with running buffer in order to remove adsorbed material from the walls of the capillary. Pre-run rinsing for equilibration was performed with running buffer for 2 min.

3. Results and discussions

3.1. Separations

For separation and recovery studies 14 aromatic sulfonates of a wide range of different structure, substitution and polarity were chosen (Fig. 1). These substances are of environmental concern and have partly been found in river water samples or effluents of industrial waste water treatment plants up to the mg/l range [1,5,7,9,14,16–20,22–24].

In general, for the separation of aromatic sulfonates by CZE a sodium borate/boric acid [34–38] or a simple sodium borate buffer [2,20] at pH between 8.3 and 10 is used. The pH of the buffer electrolyte is of essential importance and has to be above the analytes' pK_a values, in order to ensure dissociation of the acidic groups. In this work a simple 25 m*M* sodium borate buffer at pH 9.3 (without boric acid addition) was used. This buffer showed the best separation performance. No pH adjustment is necessary with this buffer. Very fast and efficient separations of aromatic sulfonates within 10 min were achieved with this buffer (Fig. 2). However, no complete baseline separation of all 14 test compounds depicted in Fig. 1 was attained under these conditions (60 cm capillary). Fig. 2 shows the

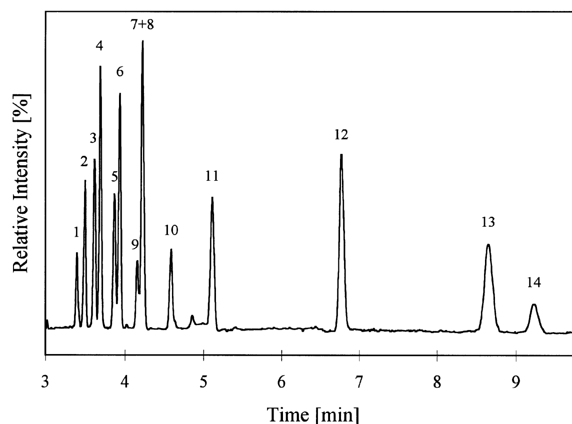

Fig. 2. Electropherogram of a 14-compound aromatic sulfonate mixture containing 5 mg/l of each compound. Conditions: running electrolyte 25 m*M* sodium borate, pH 9.3, capillary 60 cm (50 cm to detection window)×75 μm I.D., voltage 25 kV, temperature 30°C, pressure injection 50 mbar for 12 s, UV detection at 210 nm. For peak identification see Fig. 1.

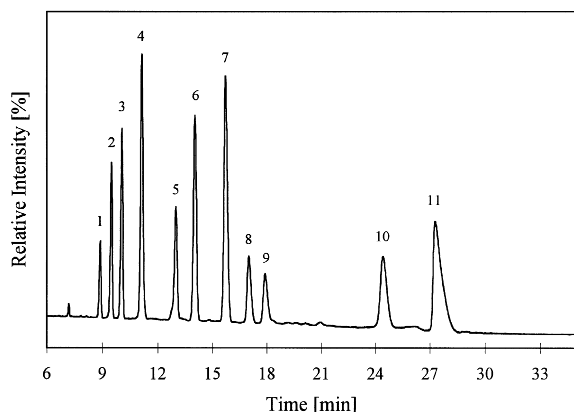

Fig. 3. Electropherogram of a 14-compound aromatic sulfonate mixture (10 mg/l of each compound) illustrating resolution improvement by acetonitrile addition to the buffer. Conditions as in Fig. 2 except for the running electrolyte, 25 m*M* sodium borate with 40% acetonitrile. For peak identification see Fig. 1.

separation of the 14-compound aromatic sulfonate test mixture at a concentration of 5 mg/l for each compound, 3-amino- and 4-chlorobenzenesulfonate coelute (peaks 7 and 8).

With 40% acetonitrile addition to the buffer the complete separation of all 14 test compounds was achieved. However, the last three very hydrophilic sulfonates are lost under these conditions (Fig. 3).

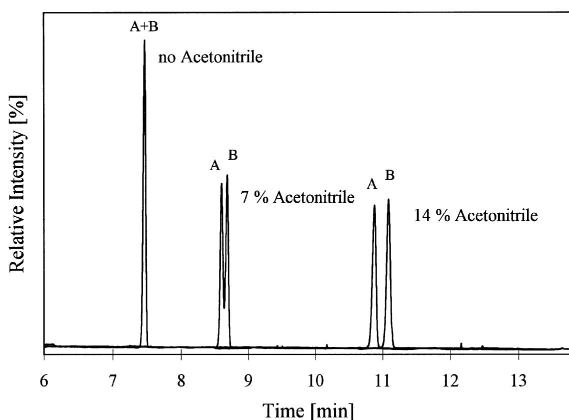

Fig. 4. Electropherograms of a naphthalene-1- and naphthalene-2-sulfonate mixture (A+B) (10 mg/l) illustrating the effect of acetonitrile addition. Conditions as in Fig. 2 except for capillary length: 70 cm (60 cm to detection window) and the running electrolyte, 25 m*M* sodium borate with 7 or 14% acetonitrile addition. Peaks: A=naphthalene-1-sulfonate, B=naphthalene-2-sulfonate.

The separation of very similar (isomeric) compounds like 1- and 2-naphthalenesulfonate (A and B) was achieved by addition of 7 or 14% acetonitrile to the sodium borate buffer (Fig. 4).

The migration order of the sulfonates in Fig. 2 can be explained as follows. The pK_a value of naphthylamine is about 4.1, therefore the amino groups will not carry any electrical charge at pH higher than 6, thus the presence of the amino groups does not essentially contribute to the separation (at pH 9.3). Conversely, the influence of the hydroxy substituents on electromigration will be considerable, with the pK_a value of 1-naphthol being 9.2 [34]. The largest compounds with just one SO_3^- group show up at the front of the electropherogram. The sulfonates with two SO_3^- groups have a larger average charge and therefore longer apparent migration times. The hydroxy substituents of the analytes will be partly dissociated, therefore these compounds (12 and 14) show up latest. The smaller benzenesulfonates appear in the middle between the one- and twofold negatively charged compounds. The reproducibility of migration times is fairly good. Relative standard deviations (R.S.D.s) are less than 5%. The theoretical plate numbers N were measured to be $>$100 000 for the first three peaks and $>$60 000 for peaks 1–11 of the standard electropherogram in Fig. 2.

As non-volatile buffers like sodium borate interfere with electrospray ionization (ESI), the use of volatile buffers like ammonium acetate is a prerequisite for coupling CE to MS detection. Therefore, separation of aromatic sulfonates was also investigated with an ammonium acetate buffer, which has to be alkalized to pH>9 by ammonium hydroxide. The migration order with this ammonium acetate buffer (at pH 10.0) is the same as with sodium borate (electropherogram not shown). At pH 10, the ammonium is buffering the pH.

3.2. Calibration

Calibration for the 14 aromatic sulfonates depicted in Fig. 1 was performed in the concentration range between 1 and 50 mg/l with UV detection at 210 nm. The calibration graphs are linear in this range. Regression data are not shown. The correlation coefficients r (n=6) are between 0.995 and 0.999.

3.3. Solid-phase extraction and recovery studies

Aromatic sulfonate concentrations in environmental waters are very low, therefore an enrichment step in combination with CE determination is required. As ion-pair extraction is incompatible with CE analysis and also exhibits limited extraction efficiency for very hydrophilic amino- and hydroxy-substituted aromatic sulfonates [1,14,34], SPE with LiChrolut EN for sulfonate enrichment was examined. The ion-pair reagents like TBABr are non-volatile and cannot be removed during the final evaporation step and consequently are still present in the final sulfonate extract. TBABr changes the ionic strength of the sample solution, influences the electroosmotic flow (EOF) in the capillary and thus prevents a successful CE separation. Kok et al. solved this problem by an additional clean-up step. They removed the ion-pair reagent with an aromatic sulfonic acid cation-exchanger SPE column [34]. Because of the same reason, ion-exchange SPE also is incompatible with CE analysis as the exchange ions of the sorbent alter the ionic strength of the sample.

Milli-Q, tap and river water samples were spiked with 2 μg/l for each of the chosen aromatic sulfonates (Fig. 1), acidified to either pH 2.0, 1.0 or 0.5 (or not adjusted for pH 7.5) and were extracted by SPE with LiChrolut EN. Analysis was performed by CZE and recovery studies were carried out. Recovery data for Milli-Q, tap and river water at different pH values are given in Table 1. Recovery from Milli-Q water was compared at pH 1.0 and 0.5, recovery from tap water at pH 1.0, 2.0 and 7.5. The highest recoveries for most of the sulfonates were obtained at pH 2.0. However, for benzenesulfonate (10) the optimum extraction pH was 1.0. The recoveries for most of the sulfonates are quite good, ranging from 38% for benzenesulfonate (10) to 106% for naphthalene-1-sulfonate (6) (extraction from Milli-Q water at pH 1.0), from 33% (10) to 104% for toluene-4-sulfonate (9) (tap water, pH 1.0) and from 72% for 4-chlorobenzenesulfonate (8) to 132% for 2-amino-1-naphthalenesulfonate (5) (river water, pH 2.0). At pH 7.5 recoveries are worst. At pH 2.0 recoveries of compounds 1, 2, 3, 6 and 12 were found to be superior than at pH 1.0 (for spiked tap water). These results indicate that the adsorption capacity of LiChrolut EN deteriorates at pH values below 2.0. Consequently, the river and seepage water samples were extracted at pH 2.0 only. The average precision of recovery of the method indicated by the R.S.D.s was determined to be between 7 and 18% for Milli-Q water and between 4 and 23% for tap water both at pH 1.0. At pH 2.0 the R.S.D.s are between 5 and 14% for tap water and between 6 and 15% for river water. Very hydrophilic aromatic sulfonates like 3-aminobenzenesulfonate (7) and the twofold negatively charged compounds 4,4′-diamino-2,2′-stilbenedisulfonate (11), naphthalene-1,5-disulfonate (13) and 2-naphthol-3,6-disulfonate (14) were not extracted from water with LiChrolut EN.

Adsorption of the aromatic sulfonates onto LiChrolut EN and recovery can be explained as follows. Compound 5 (relative to 4) is better retained because of the compensated charges and the substituent-free aromatic naphthalene structure on one side of the molecule which enables π–π binding interactions to the polystyrene sorbent. Hydrophobic alkyl groups attached to aromatic molecules increase retention considerably, as the recovery of toluene-4-sulfonate (9) is much better than of benzenesulfonate (10). Also a chloro-substituent [4-chlorobenzenesulfonate (8)] increases retention. The most important parameter for adsorption onto LiChrolut EN is the specific ionic charge of the analyte molecule and its water-solubility or hydrophilicity. Acidic, negatively charged substances are usually protonated before extraction with styrene–divinylbenzene copolymers by pH-adjustment.

The advantages of the new SPE method with LiChrolut EN for the extraction of hydrophilic aromatic sulfonates are its simplicity, fastness and CE compatibility. However, the conventional ion-pair SPE methods yield better recoveries mostly for multiple negatively charged sulfonates [9,14,16,28,34]. The main drawback of LiChrolut EN is its inability to retain very hydrophilic aromatic sulfonates with more than one SO_3^- groups. But similarly graphitized carbon black also exhibits problems in the adsorption of very hydrophilic amino-hydroxysulfonates [1]. Advantageous is the consumption of only 200 mg LiChrolut EN adsorbent per extraction.

Fig. 5 shows two electropherograms of spiked tap and river water samples after the optimized SPE

Table 1
Recovery of aromatic sulfonates from 200 ml fortified water at 2 μg/l (for each compound) with 200 mg LiChrolut EN ($n=6$)

No.	Compound	Milli-Q water pH 0.5		Milli-Q water pH 1.0		Tap water pH 1.0		Tap water pH 2.0		Tap water pH 7.5		River water pH 2.0	
		Recovery (%)	R.S.D.	Recovery (%)	R.S.D.	Recovery (%)	R.S.D.	Recovery (%)	R.S.D.	Recovery (%)	R.S.D.	Recovery (%)	R.S.D.
1	Diphenylamine-4-sulfonate	90.0	19.3	100.5	12.7	76.0	9.4	103.7	11.2	77.4	3.2	90.6	7.5
2	Anthrachinone-2-sulfonate	63.0	9.4	78.7	15.9	75.1	16.9	95.0	9.3	72.4	4.4	89.4	12.6
3	Diphenyl-4-sulfonate	84.6	7.9	100.6	8.4	90.3	11.6	96.4	9.0	72.7	4.7	96.4	8.2
4	1-Amino-5-naphthalenesulfonate	9.3	3.8	2.5	2.8	9.8	2.4	4.9	4.8	16.7	1.3	8.6	1.2
5	2-Amino-1-naphthalenesulfonate	42.1	4.1	70.9	10.1	64.8	4.2	65.0	5.9	54.5	9.8	131.6	12.8
6	Naphthalene-1-sulfonate	93.6	9.9	105.8	9.3	92.7	9.3	99.4	5.2	87.2	10.0	113.0	8.8
7	3-Aminobenzenesulfonate	–	–	–	–	–	–	–	–	–	–	–	
8	4-Chlorobenzenesulfonate	79.2	1.0	96.3	9.4	95.0	10.4	80.2	13.1	20.6	4.5	72.2	8.6
9	Toluene-4-sulfonate	94.9	15.7	102.7	8.5	103.6	9.6	105.6	13.9	58.2	8.0	112.0	6.3
10	Benzenesulfonate	17.5	10.6	38.4	18.4	33.3	23.1	–	–	–	–	–	–
11	4,4′-Diamino-2,2′-stilbenedisulfonate	–	–	–	–	–	–	–	–	–	–	–	–
12	1-Naphthol-4-sulfonate	68.8	9.8	89.1	7.2	79.6	8.6	82.9	6.6	8.8	1.8	74.8	15.1
13	Naphthalene-1,5-disulfonate	–	–	–	–	–	–	–	–	–	–	–	–
14	2-Naphthol-3,6-disulfonate	–	–	–	–	–	–	–	–	–	–	–	–

enrichment procedure with LiChrolut EN. The tap water (lower plot) was extracted at pH 1.0 and the river water at 2.0. Therefore, benzenesulfonate (10) is only detected in the spiked tap water samples. Sample matrix effects are observed in both water types. The signal background resulting from interfering matrix compounds such as humic substances is much higher in the river water. In addition, the electropherogram of the river water sample contains additional peaks, marked by the letters A–E. High recoveries of compounds 5 (132%) and 6 (113%) for the extraction from spiked river water indicate the presence of interfering compounds in the river water with identical retention times. Further experiments will show whether these signals are caused by the corresponding sulfonates. The retention time of peak 12 is quite variable for the analysis of real water samples, but peak identification was consistent with further spiking the river water with the standard compound.

3.4. Fluorescence detection

Due to increased excitation intensity, fluorescence detection usually offers the best performance in sensitivity and selectivity. With laser-induced fluorescence (LIF) even the detection of a single molecule in a capillary has been reported [48]. Since many aromatic sulfonates possess a native fluorescence, no chemical derivatization of the analytes is required. Fig. 6 shows two electropherograms of a sulfonate mixture containing the 14 test compounds using fluorescence detection with a pulsed Xenon lamp fluorescence detector. Excitation is performed at 230 nm, emission detection at 335 nm (lower plot) and 410 nm. The electropherograms show that fluorescence detection is more selective than UV detection, as only fluorescent compounds are detected. This enhanced selectivity is advantageous for the analysis of real samples. Table 2 gives the measured optimum fluorescence excitation and emission wavelengths for the chosen aromatic sulfonates. Fluorescence detection at approximately 335 nm is very selective for naphthalene sulfonates without other substituents (compound Nos. 6 and 13), whereas detection at 410–445 nm is appropriate for most naphthalenesulfonates carrying additional amino and/or hydroxy groups (compound Nos. 5, 12 and 14) [14,19]. The baseline in the electropherogram with emission detection at 410 nm is quite bad. The reason for this are severe background noise problems with the fluorescence detector and its light alignment interface. The latter problem is explained below.

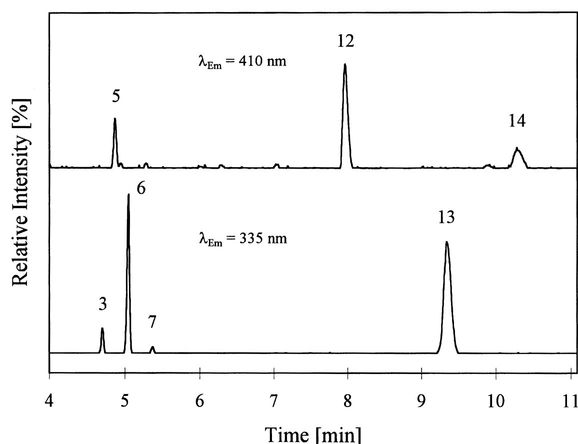

Fig. 6. Electropherograms of a 14-compound aromatic sulfonate mixture containing 10 mg/l of each compound using fluorescence detection, λ_{EX}=230 nm, λ_{EM}=335 nm for the lower plot, λ_{EM}= 410 nm for the upper plot. Conditions: running electrolyte 25 m*M* sodium borate, pH 9.3, capillary 65 cm (55 cm to detection window)×75 μm I.D., voltage 25 kV, temperature 30°C, pressure injection 50 mbar for 12 s. For peak identification see Fig. 1.

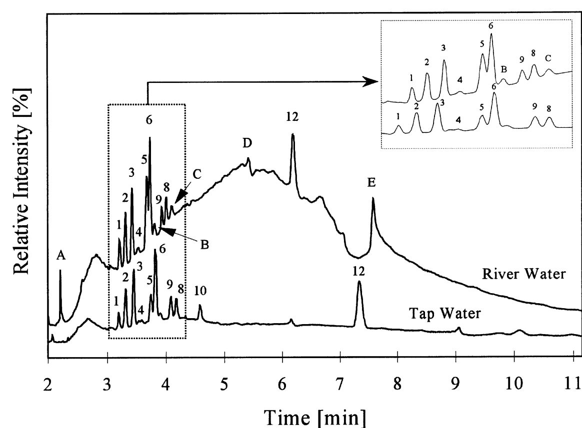

Fig. 5. Electropherograms of 200-ml spiked tap and river water samples at 2 μg/l for the 14 aromatic sulfonate test compounds after SPE with LiChrolut EN (enrichment factor 2000). Conditions as in Fig. 2. For peak identification see Fig. 1.

Table 2
Optimum fluorescence excitation and emission wavelengths for aromatic sulfonates

No.	Compound	$\lambda_{Excitation}$ (nm)	$\lambda_{Emission}$ (nm)
1	Diphenylamine-4-sulfonate	Not fluorescent	
2	Anthrachinone-2-sulfonate	Not fluorescent	
3	Diphenyl-4-sulfonate	203 or 258	314
4	1-Amino-5-naphthalenesulfonate	Not fluorescent	
5	2-Amino-1-naphthalenesulfonate	230	410
6	Naphthalene-1-sulfonate	230	335
7	3-Aminobenzenesulfonate	210	365
8	4-Chlorobenzenesulfonate	Not fluorescent	
9	Toluene-4-sulfonate	Not fluorescent	
10	Benzenesulfonate	Not fluorescent	
11	4,4′-Diamino-2,2′-stilbenedisulfonate	Not fluorescent	
12	1-Naphthol-4-sulfonate	230	430
13	Naphthalene-1,5-disulfonate	230	335
14	2-Naphthol-3,6-disulfonate	230	445

3.5. Sensitivity

The detection sensitivity by UV and fluorescence detection for the (fluorescent) aromatic sulfonates is in the same range. The limit of detection (LOD) for both principles is approximately 0.2 mg/l, or about $2 \cdot 10^{-6}$ M (S/N=3). The problem for CE with conventional fluorescence excitation sources (Xe-lamp) is the inherent difficulty in focusing a large amount of light from a divergent light source into the nanoliter detection volume of the narrow capillary while minimizing light scattering. Utilization of a laser as a fluorescence excitation source reduces such problems. Therefore, fluorescence detection in CE for detection limit improvement usually is performed with LIF. Unfortunately, the relatively short absorption wavelengths of 230 nm for aromatic sulfonates require the use of a rather expensive UV-producing laser [34,49]. The detection limit of the combined method of SPE enrichment and CE analysis is approximately 0.1 μg/l for 200-ml water samples.

3.6. Analysis of real water samples

To investigate the applicability of the present procedure for real environmental analysis, river Isar and seepage water samples from a municipal waste disposal plant were analysed by CZE using UV and fluorescence detection after analyte enrichment with LiChrolut EN. Fig. 7 shows an electropherogram of the extracted river Isar water at an enrichment factor of 2000. The lower plot represents for comparison the standard aromatic sulfonate mixture. A signal for compound 10 is not observed because the detection wavelength of the UV detector was set to 230 nm. The background signals of matrix compounds for the real water samples are significant lower at 230 nm compared to 210 nm. In the river water few small peaks marked by the letters A–F are observed. Retention times of peaks B and C agree by spiking with the sulfonates 4 and 6. These results might be an indication for the presence of similar aromatic

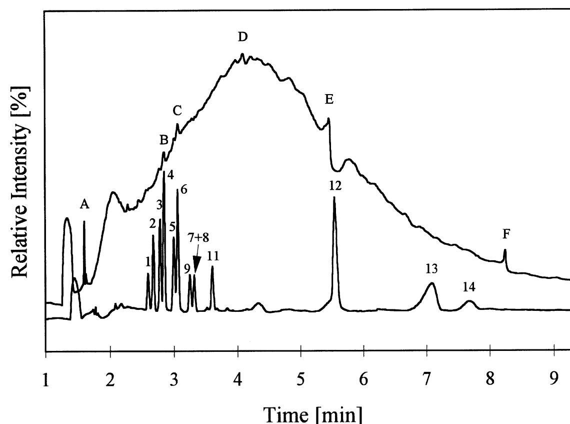

Fig. 7. Electropherogram of a river Isar extract after SPE with LiChrolut EN (enrichment factor 2000), UV detection. Lower plot: 14-compound sulfonate standard at 5 mg/l. Conditions as in Fig. 2 except for capillary length: 55 cm (45 cm to detection window) and detection wavelength 230 nm. For peak identification see Fig. 1.

sulfonates like the compounds 4 and 6 (isomeric amino-naphthalene-mono-sulfonates for peak B and naphthalene-mono-sulfonates for peak C) in the river Isar, because isomeric naphthalenesulfonates cannot be separated with the simple borate buffer. Unfortunately, certain confirmation of compounds without diode-array or MS detection is not possible. However, considering the enrichment factor of 2000 for the electropherogram in Fig. 7, the calculated concentrations for peaks B and C are very low (< detection limit of the method). The early eluting peak A (near the EOF peak) can be attributed to a more non-polar compound.

For seepage water analysis, prior to the LiChrolut EN extraction at pH 2.0, an additional clean-up step with a RP-C_{18} material was applied to remove interfering unpolar compounds. This C_{18} extraction is part of the sequential extraction scheme for non-target water analysis as proposed by Fiehn and Jekel [50]. A C_{18} phase selects all neutral hydrophobic compounds at neutral pH (7.9 for the seepage water). The percolated water was collected, acidified to pH 2.0 and finally extracted by LiChrolut EN. Fig. 8 shows the electropherogram with UV detection of this extract (in comparison to the standard mixture). The enrichment factor is only 50. Several peaks are observed, only the more intense are assigned (A–I). The highest peak D matches with peak 6 by spiking. In addition, peak B matches with the first eluting bigger sulfonates (1–3) like anthrachinone-2-sulfonate (2) and peak C can be attributed to a onefold negatively charged aminonaphthalenesulfonate. Peak E is in the elution region of the smaller benzenesulfonates (7–10), peak F is a good indication for the presence of twofold negatively charged stilbenesulfonates (like compound 11) and peaks H and I for the presence of twofold negatively charged naphthalenesulfonates with, possibly, OH or NH_2 substituents in the seepage water. To measure the concentrations of the compounds tentatively found in the seepage water, the peaks were integrated and calculated by an external calibration in relation to the sulfonate standard. The concentrations calculated for the peaks are summarized in Table 3.

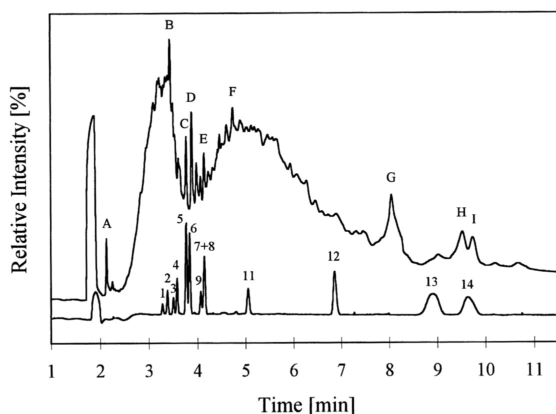

Fig. 8. Electropherogram of a seepage water extract after C_{18} clean-up at pH 7.9 and SPE with LiChrolut EN at pH 2.0 (enrichment factor 50), UV detection. Lower plot: 14-compound sulfonate standard at 5 mg/l. Conditions as in Fig. 2 except for detection wavelength 230 nm. For peak identification see Fig. 1.

The seepage water extract also was investigated with fluorescence detection. Fig. 9 shows in comparison to the standard mixture (lower plot) the electropherogram with emission detection at 335 nm (enrichment factor 100). Three small (A, C, D) and two large peaks (B, E) are detected. The retention time of the highest peak B exactly matches with naphthalene-1-sulfonate (6). By spiking, peak E corresponds to compound 13 and the small peaks A and D to the compounds 3 and 7. The concentrations for the two large peaks B and E measured by fluorescence detection were calculated by external calibration to be 112 μg/l for naphthalene-1-sulfonate 6 (or isomeric sulfonates) and 318 μg/l for non-substituted naphthalenedisulfonates. These results agree with the values found by UV detection (Table 3).

Fig. 10 shows the electropherogram of the seepage water with fluorescence emission detection at 410 nm for detection of substituted aromatic sulfonates (in comparison to the standard mixture). The baseline is very bad at this emission wavelength

Table 3
Calculated aromatic sulfonate concentrations in the seepage water with UV detection (Fig. 8)

Peak	Corresponding compounds	Concentration (μg/l)
B	Large-molecule-monosulfonates	73
C	Aminonaphthalenemonosulfonates	50
D	Naphthalenemonosulfonates	81
E	Benzenemonosulfonates	38
F	Stilbenedisulfonates	49
H+I	Naphthalenedisulfonates	114

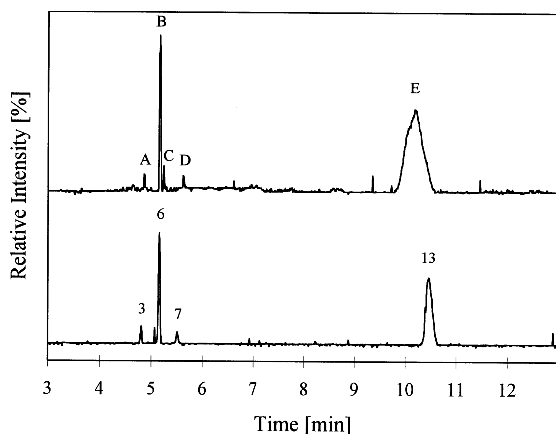

Fig. 9. Electropherogram of a seepage water extract after C_{18} clean-up at pH 7.9 and SPE with LiChrolut EN at pH 2.0 (enrichment factor 100), fluorescence detection, λ_{EX}=230 nm, λ_{EM}=335 nm. Lower plot: 14-compound sulfonate standard at 10 mg/l. Conditions as in Fig. 6 except for capillary length 67 cm (57 cm to detection window), For peak identification see Fig. 1.

of 410 nm (because of the light alignment interface) and also more background matrix signals are observed (compared to 335 nm). However, in the electropherogram, four larger peaks are detected (A–D). By spiking, it was shown that Peak B corresponds to 2-amino-1-naphthalenesulfonate (5) and

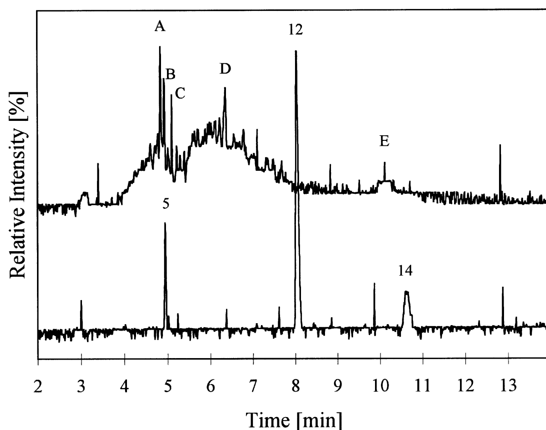

Fig. 10. Electropherogram of a seepage water extract after C_{18} clean-up at pH 7.9 and SPE with LiChrolut EN at pH 2.0 (enrichment factor 100), fluorescence detection, λ_{EX}=230 nm, λ_{EM}=410 nm. Lower plot: 14-compound sulfonate standard at 10 mg/l. Conditions as in Fig. 9. For peak identification see Fig. 1.

signal E to compound 14. Peak A probably corresponds to a similar or isomeric amino-naphthalenemonosulfonate such as 5. Peaks C and D are not identified because of the lack of standard substances.

4. Conclusions

CZE is very well suited for the analysis of ionized aromatic sulfonates. Very fast and efficient separations are possible with a simple 25 m*M* sodium borate buffer. Resolution improvement can be achieved by acetonitrile addition to the buffer electrolyte. Some aromatic sulfonates can be detected by fluorescence detection. However, because of severe noise background problems of the light alignment interface, a pulsed Xenon lamp fluorescence detector does not provide greater sensitivity compared to UV detection. A new CE-compatible procedure for SPE sample enrichment of hydrophilic aromatic sulfonates with the styrene–divinylbenzene sorbent LiChrolut EN was developed. The optimum pH value for SPE of such hydrophilic compounds with LiChrolut EN is 2.0. Below this value recoveries are worse due to solid-phase degradation. The limits of detection of the combined method of SPE enrichment and CE analysis are in the low μg/l range. This is sufficient for real-world applications. The performance of the method was checked with the analysis of river and contaminated seepage water. In seepage water, aromatic sulfonates were clearly identified for the first time by spiking with the appropriate reference standards and subsequent UV and fluorescence detection. The greater selectivity of fluorescence detection is advantageous for the analysis of real water samples and the identification of compounds. The simplicity and fastness of CE analysis in combination with LiChrolut EN SPE enrichment makes the presented method time efficient, economic and easy to handle.

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