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Ion-exchange-based eluent-free preconcentration of some anions

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Abstract

Preconcentration procedures based on ion-exchange methods are often used to enhance the sensitivities of analytical techniques where the eluent used for eluting the preconcentrated ions does not influence the subsequent analytical step. Until recently, only a limited use of ion-exchange-based sample preconcentration procedures has been found in those analytical techniques where the eluent components strongly influence the separation procedure [e.g., capillary electrophoresis (CE)]. In this paper, we present a preconcentration procedure based on (i) the preconcentration of anions on an ion-exchange resin, (ii) the subsequent elution of analytes, and (iii) on-line removal of eluent components by chemical suppression using an appropriate suppressor device (either packed-bed suppressor column or micromembrane suppressor). The adjustment of the system parameters, combined with a computer-controlled, sensing/switching system, resulted in a minimal additional dilution of the eluted preconcentrated anions. The efficiency of the proposed enrichment/matrix removal procedure was tested by using off-line CE analysis of collected preconcentrated samples, reaching a LOD of 1 μg/l for a selected anion.

Keywords: Preconcentration; Anions

1. Introduction

Modern analytical laboratories generally use ion chromatography (IC) and/or capillary electrophoresis (CE) for the determination of anions. Other available methods (e.g., ion-selective electrodes and conventional wet chemical methods) do not offer fast, reliable multi-ion determination in a reasonable time. IC and CE are also significantly less influenced by the matrix composition (e.g., color, presence of organic contaminants, etc.). Both techniques can be applied with no sample pretreatment or preconcentration in many areas. However, there are some applications where the determination of trace levels of target analytes is needed. The preconcentration of target analytes and/or the elimination of interfering sample matrix components is often necessary.

The best approach for IC is preconcentration of target ions on an appropriate anion-exchanger, because the eluent used for the elution from a preconcentration column to the analytical column does not usually disturb the separation process. The essential part of such a system is the preconcentration column that is usually installed in a separate injection valve. The above approach is widely used because it is simple, has reasonable enrichment

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factors, and is easy to operate (automation). It has been applied in chemically suppressed IC systems [1,2], HPLC systems [3], as well as non-suppressed IC systems [4]. Among other approaches for sample enrichment, Donnan dialysis [5,6] and the application of diffusion denuders [7] have been found to be significantly applicable, especially when the matrix effect has to be removed and/or gaseous analytes have to be analyzed.

Sample enrichment in CE is much more important than in IC because of CE's relatively high LODs compared to those obtained with modern IC systems. A very simple way to achieve a moderate sample preconcentration in CE is by electrokinetic injection, enabling up to a ten-fold increase in sensitivity. There are two severe drawbacks of this type of injection (and sample preconcentration): (i) high-mobility ions are driven into the capillary more efficiently than those with low mobility and (ii) samples of different ionic composition and conductivity are compromised [8]. The quantitative analysis in this case is very troublesome. On the other hand, sample stacking in the capillary can lead to a substantial enrichment. Sample volumes loaded onto a column can be very high because compressing the sample volume in the capillary in the initial phase of the separation, eliminates band broadening. Features of electrostacking are applicable, for example, in the determination of trace inorganic anions [9,10].

Capillary isotachophoresis (ITP) in combination with CE has also proved to be a very useful strategy, leading to a thousand-fold increase in concentration [11,12]. In this mode, ITP provides sample cleanup and substantial preconcentration, enabling matrix effects to be eliminated and achieving high reproducibility and excellent LODs.

Ion-exchange preconcentration, as part of a flow-injection analysis (FIA) system, offers an elegant sample pretreatment in routine applications [13,14] and can also be successfully used in combination with CE [15]. On-line sample preconcentration on an ion-exchange column in a FIA system is a very promising strategy because a substantial analyte enrichment is achieved and matrix effects are diminished. However, preconcentration based on ion-exchange has not found wide use until now due to the inherent elution step that leads to contamination of the sample with eluent components. Similar problems have been described when coupling IC to CE [16].

In this paper, we present a preconcentration technique based on the preconcentration of anions on an anion-exchanger, subsequent elution of the analytes, and on-line removal of the eluent components by chemical suppression using a packed-bed and a micromembrane suppressor [17]. The procedure was optimized for the type of eluent (used for the elution of retained anions from the preconcentration column) and the overall enrichment factor. The system was completely automated through a computer-controlled sensing/switching system. For testing purposes, the enriched samples were analyzed by CE using direct UV detection. Due to complete matrix elimination, we also tested prolonged electrokinetic injection as an additional sample enrichment possibility in CE.

2. Experimental

2.1. Reagents

Na_2CO_3 solution (50 m*M*) and NaOH solution (100 m*M*, carbonate-free) were used as the eluent stock solutions. We made the appropriate dilution of the individual eluent on-line by applying the quaternary pump. Throughout the experiments, we used a composite standard of F^- (0.1 mg/l), Cl^- (0.1 mg/l), NO_2^- (0.5 mg/l), NO_3^- (0.5 mg/l), HPO_4^{2-} (1.0 mg/l), and SO_4^{2-} (1.0 mg/l). We prepared the appropriate diluted sample by diluting the stock solution with Milli-Q water. For the CE experiments, composite standards containing Br^-, I^-, NO_2^- and NO_3^- at concentrations of 1 mg/l, 100 μg/l, 10 μg/l, and 1 μg/l per analyte were used.

2.2. Apparatus

2.2.1. Anion preconcentration system

The sample preconcentration system, shown in Fig. 1, comprises a high-performance pump P1 (Spectra System 400, PEEK version, Spectra-Physics, San Jose, CA, USA), high-pressure pump P2 (LDC MiniPump®, Milton Roy, Chicago, IL, USA), peristaltic pump PP (Minipuls 3, Gilson, France), pneumatically driven injection valve Inj1

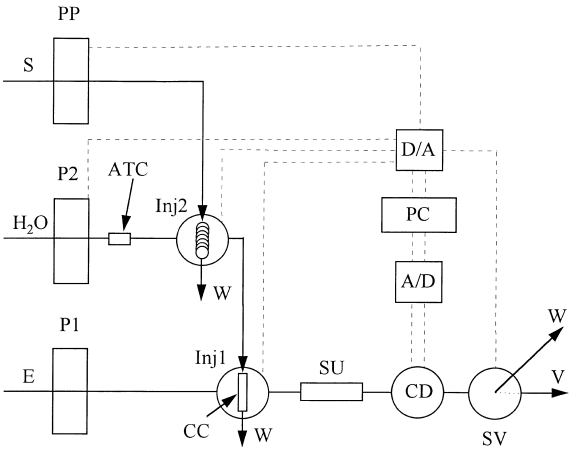

Fig. 1. A schematic diagram of the developed anion pre-concentration system. P1, P2, HPLC pumps; PP, peristaltic pump; E, eluent; H_2O, Milli-Q water; S, sample; CC, concentrator column; ATC, anion trap column; SU, suppressor unit; Inj1, Inj2, injectors; SV, selection valve; CD, conductivity detector; PC, personal computer with I/O ports (A/D and D/A); V, sample collecting vial; W, waste.

(Rheodyne 9010, Cotati, CA, USA), electrically driven injection valve Inj2 (LabPro™ PR-750-100-01, Rheodyne, CA), electrically driven selection valve SV (LabPro™ PR-100-105-01, Rheodyne, CA), and conductivity detector with a flow-through cell (Shodex CD-5, Japan). The IonPac TAC-LP1 (Dionex, Sunnyvale, CA, USA) anion-concentration column CC (4×35 mm) with a capacity of 25 μeq/column (20 μm styrene/divinylbenzene copolymer) and a void volume of approximately 250 μl was used through the experiments. An AnionTrap column ATC (Dionex, USA) was used to eliminate the possible contamination of the concentrator column by anions present in Milli-Q water. As the suppression unit SU, CTC-1 column(3 meq, DIONEX) and ASRS-ULTRA (4 mm, DIONEX) were selected.

The preconcentration system was completely computer-controlled through a D/A converter (CIO-DDA06/Jr) and 16-channel, 12-bit A/D converter (CIO-DAS16/Jr) (both by ComputerBoards, Inc., Mansfield, OH, USA) using home-made software, written in GWBASIC.

2.2.2. Sample preconcentration procedure

In the ''start'' position both injectors (Fig. 1) were switched to ''load'' position, the selection valve SV was switched to waste, while the peristaltic pump PP (delivering sample S) was switched ON. In that position, the sample-loop installed in the injector Inj2, was loaded by the sample S and the concentrator column CC was cleaned by additionally purified (ATC column) Milli-Q water, delivered by a HPLC pump P2. HPLC pump P1 delivered eluent E through the suppressor unit SU and through the conductivity detection cell CD to waste W, or to the sample collection vial V, depending on the position of the selection valve SV.

During the second step, the peristaltic pump was switched OFF and the injector Inj2 was switched to ''inject'' position. The sample was flushed from the sample loop installed in the Inj2 through the concentrator column CC to waste. The preconcentration procedure continued with switching the injectors Inj2 and Inj1 to ''load'' and to ''inject'' positions, respectively. The anions preconcentrated on the concentrator column CC were eluted onto the suppressor column, where the eluent components were quantitatively converted to the low conductivity form (CO_2 and/or H_2O), while the the anions were converted to completely dissociated mineral acids. The conductance change was monitored using a PC controlled A/D converter (the necessary software was home-made, written in GWBASIC). Based on the peak intensity, the selection valve SV was set to V position (using PC-controlled D/A converter, Fig. 1). The selection valve SV was re-directed to waste after the level of the detector signal was decreased below the pre-determined level. The injector Inj1 was finally switched to ''load'' position and the pre-concentration cycle was finished.

2.2.3. CZE equipment

The instrument used for the CE experiments was a 270A-HT CE System (Applied Biosystems, Perkin-Elmer, equipped with Turbochrom software). A 50 μm I.D.×350 μm O.D. capillary (Supelco, Bellefonte, PA, USA) was used. The total length was 72 cm, and the section from the injection end to the UV detector was 50 cm. Anions were detected spectrophotometrically at 200 nm. The injection was performed hydrodynamically for 7 s at 0.169 bar or, in some cases, electrokinetically for 5 s at −10 kV. The separation voltage was negative, usually −16 kV (producing an electric field of −222 V/cm).

We conditioned the capillary with deionised (DI) water for 5 min on a daily basis, followed by 0.1 *M* NaOH (20 min), deionised water (5 min), and freshly prepared buffer (30 min). To achieve a reproducible analysis, after each completed run we rinsed the capillary with 0.1 *M* NaOH (5 min) and buffer (10 min). The buffer consisted of 20 m*M* sodium tetraborate (Kemika, Zagreb, Croatia). Cetyltrimethylammonium bromide (CTAB) ($9–10^{-5}$ *M*) was used to reverse the EOF under negative electrophoretic voltages.

3. Results and discussion

3.1. Adjusting the preconcentration procedure

3.1.1. Selection of a suppression unit

For the eluent suppression two types of suppressor columns were tested: (1) a CTC-1 packed-bed suppressor column [9×24 mm], packed with sulfonated cation-exchange resin, 3.0 meq/column total capacity and column void volume of 500 μl, and (2) a 4 mm ASRS-Ultra self-regenerating suppressor working in the external water mode (both Dionex, USA).

The CTC-1 column was prepared (regenerated) by pumping 100 m*M* HCl or H_2SO_4 through it for 1 h at a flow-rate of 1 ml/min and subsequent rinsing for 1 h with Milli-Q water at the same flow-rate. This column was later replaced by ASRS-ULTRA continuously regenerated suppressor (DIONEX, 4 mm).

As previously described [17], a packed-bed suppressor was applied in the first experiments to remove eluent during the anion preconcentration procedure. In Fig. 2 the base-line stability and the dynamic capacity of CTC-1 column at eluent concentration 30 meq/L is presented. The obtained dynamic capacity was significantly shorter than the theoretical one (about 1.2 meq at eluent flow-rate 1 ml/min). The same dynamic capacity was reached regardless of the eluent composition (NaOH or Na_2CO_3). However, there was a significant difference in the baseline stability between them. The baseline noise obtained using the NaOH eluent was much lower, so the peak threshold can be set to a lower value, which can improve the detection accuracy of the eluting peak. The noisy baseline, when

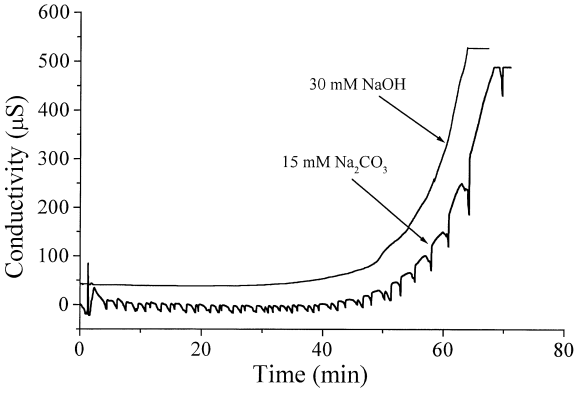

Fig. 2. The dynamic capacity and the base-line stability obtained using CTC-1 suppressor column. Individual base-line was obtained with the eluent as marked in the Figure.

Na_2CO_3 eluent is used, can be attributed to CO_2 evolving during the ion suppressor neutralization. Consequently, NaOH was chosen as the eluent component for further experiments.

The main problem connected with the packed-bed suppressor remains unresolved — its dynamic (breakthrough) capacity. Thus we decided to replace the CTC-1 packed-bed suppressor with a micromembrane ASRS-ULTRA self-regenerator suppressor that works continuously in the recycle mode (eluent is electrolyzed) or the external water mode (Milli-Q water is electrolyzed). In this study, the ASRS-ULTRA suppressor was operated only in the external water mode. Again NaOH-based eluent was found to be superior to the Na_2CO_3-based eluent, even the baseline noise obtained with Na_2CO_3 was significantly lower in comparison to that obtained by packed-bed suppressor (approximately 150 times). The baseline stability for the NaOH-based eluent was very high having an absolute value of about 20 nS. The application of ASRS-ULTRA suppressor additionally improved the overall stability of the proposed preconcentration system also due to its continuous mode of operation.

3.1.2. Selection of the sample loading flow-rate

During the method development phase, preconcentrated anions were not collected in the sample vials (Fig. 1), but were pumped through the detector cell into the waste. Sample preconcentration on ion-exchangers consists of two very important and time

consuming steps: loading the preconcentration column and elution of the retained anions from the preconcentration column. To increase the sample throughput, the sample preconcentration column should be loaded as often as possible. For that purpose, either a syringe or an additional valve with built-in sample loop is generally used. In our experiments, we used an additional injection valve carrying a sample loop of about 10 ml. According to the manufacturer's instructions, the sample can be loaded onto the TAC-LP1 concentrator column either manually or with a positive sample displacement pump. In either case, the sample flow-rate should not exceed 3 ml/min. Throughout our experiments a peristaltic pump was used for sample-loop loading, operating at a sample flow-rate of 2.5 ml/min.

3.1.3. Selection of the eluent flow-rate

The next process that influences the overall sample enrichment procedure, is the elution of the retained anions from the TAC-LP1. Because the TAC-LP1 anion concentrator column was made to be used on-line with the appropriate analytical column, the recommended eluent flow-rate is the same as that used for the analytical column. We used the TAC-LP1 column as a self-standing concentrator column; therefore, the effect of eluent flow-rate was investigated at three different flow-rates (0.5, 1.0, and 2.0 ml/min). In Fig. 3, the influence of eluent flow-rate on the elution efficiency is presented. The normalized peak area for individual eluent flow-rate was (A) 7494 (0.5 ml/min), (B) 7591 (1.0 ml/min), and (C) 7484 (2 ml/min). Obviously increased eluent flow-rates increased peak dispersion (lower peak height at flow-rate 2.0 ml/min), but did not influence normalized peak area (the amount of the eluted analytes). At the same time increased eluent flow-rate decreased elution time which was almost 1.4 min at eluent flow-rate of 0.5 ml/min and 0.4 min at eluent flow-rate of 2.0 ml/min. The conductivity threshold limits used as the measure for the selection of the elution time intervals, were set to be 20 μS and 5 μS on the rising and on the falling part of the eluted peak, respectively, for this particular experiment. Higher eluent flow-rates were not tested because shorter elution times would not significantly shorten the over-all preconcentration cycle. Therefore eluent flow-rate of 2 ml/min was chosen as the most appropriate one.

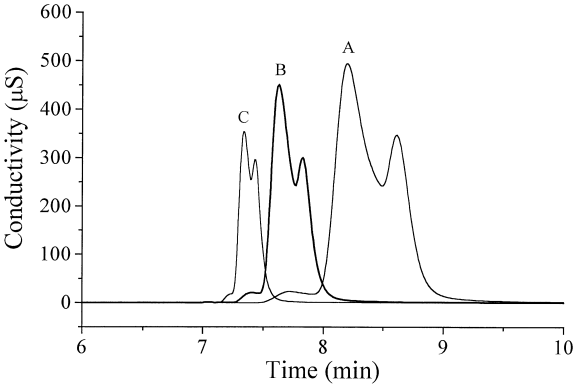

Fig. 3. The influence of the eluent flow-rate on the peak shape and peak area of the eluted pre-concentrated anions. Sample volume was 10 ml, sample carrier (Milli-Q water) flow-rate was 1 ml/min, while eluent (60 m*M* NaOH) flow-rate was 0.5 ml/min (A), 1 ml/min (B) and 2 ml/min (C). The sample was composed of F^- (0.1 mg/l), Cl^- (0.1 mg/l), NO_2^- (0.5 mg/l), NO_3^- (0.5 mg/l), HPO_4^{2-} (1.0 mg/l) and SO_4^{2-} (1.0 mg/l).

3.2. The repeatability of the preconcentration procedure

During the repeatability studies special attention was focused on the characteristic peak profiles. As shown in Fig. 3, a characteristic peak splitting was observed at all eluent flow-rates tested. This splitting was initially attributed to selective retention of the anions along the preconcentration column. We assumed that anions with low affinity to the stationary phase were eluted in the first part of the peak, while strongly retained anions are the main component of the second part of the peak. To confirm this, we ran an experiment in which only chloride (low affinity) and sulfate anions (high affinity) were preconcentrated separately. Contrary to our expectations, the same peak shape (split) was obtained in both cases. Thus, we finally assumed that peak-splitting appears due to gradient elution, established on the preconcentration column as a consequence of in-column eluent dilution.

The linearity of conductivity detector response in dependence on the analyte concentration was studied for the sample originally composed of: F^- (0.1 mg/l), Cl^- (0.1 mg/l), NO_2^- (0.5 mg/l), NO_3^- (0.5

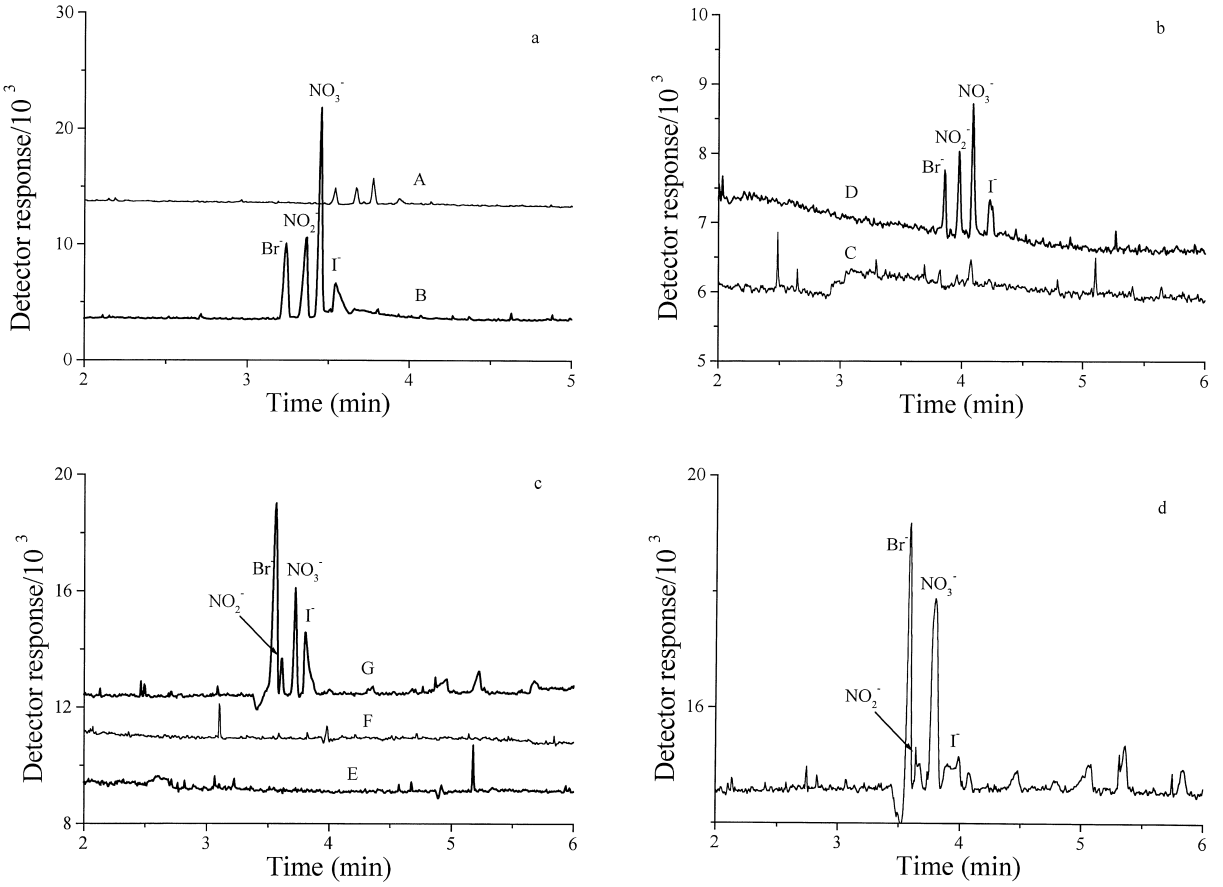

Fig. 4. (a–d) Electropherograms of Br^-, I^-, NO_2^- and NO_3^- (a) 1 mg/l each, without (curve A) and with (curve B) pre-concentration; (b) 100 μg/l each, without (curve C) and with (curve D) pre-concentration; (c) 10 μg/l each, also using electro-kinetic injection (EK), without (curve E) and with (curve F) pre-concentration and with electro-kinetic injection (curve G); (d) 1 μg/l each, EK injection only after sample pre-concentration. Other conditions: 50/72 cm capillary, 50 μm ID, direct detection at 200 nm, injection 5 s at 0.169 bar or 5 s at −10 kV (EK), run at −16 kV.

mg/l), HPO_4^{2-} (1.0 mg/ l) and SO_4^{2-} (1.0 mg/l). Lower concentrations of the analytes were prepared by dilution of this stock solution by Milli-Q water. Dilution factors were 5, 25, 125 and 625. From the log(peak area)−log(total molar concentration) plot it was determined that using the proposed system the detector response was linear down to 0.2 μ*M* of total molar concentration of the analytes. With increased dilution factors, the detector responses did not decrease properly, most probably because of the impurities present in the dilution water.

The repeatability of the entire preconcentration procedure was studied using the sample composed of F^- (1 μg/l), Cl^- (1 μg/l), NO_2^- (5 μg/l), NO_3^- (5 μg/l), HPO_4^{2-} (10 μg/l) and SO_4^{2-} (10 μg/l). The relative standard deviation of ten consecutive measurements was determined to be 1.7%.

3.3. CE Analysis of the preconcentrated samples

After the optimization procedure was completed, we tested the efficiency of the proposed anion preconcentration procedure using CZE. Because of the high quality of the eluent pump, the anions were always eluted in the same time frame at a constant

eluent flow-rate. Therefore, the selection valve SV (Fig. 1) was programmed to be switched into the sample collection mode (V) according to the time interval elapsed from switching the injector Inj1 into the ''inject'' position. When an eluent flow-rate of 2 ml/min was used, selection valve SV was in the sample collection mode between 7.3 and 7.8 min. Having known the eluent flow-rate and sample loading volume, we expected an enrichment factor of 10.

In the CE experiments, Br^-, I^-, NO_2^-, and NO_3^- ions in a dilution series of 1 mg/l, 100 μg/l, 10 μg/l, and 1 μg/l per analyte, respectively, were subjected to the optimized preconcentration procedure and subsequently analyzed by CE. The CE method was based on a report by Guan et al. [18]. The results of the sample enrichment-anion determination on the CZE system are summarized in Fig. 4a–d, and the calculated enrichment factors are given in Table 1.

The preconcentration factor obtained was in the range of 5.8–8.8. The preconcentration step was sufficient for CE analysis of samples containing more than 0.1 mg/l of each analyte (Fig. 4a and b). For lower concentration ranges, the proposed preconcentration was not efficient enough because of the relatively low sample volume. In the present study we did not test the effect of the increased sample volume. Instead, one additional benefit of the proposed sample enrichment was exploited. Due to completely matrix-free enriched samples obtained by the proposed procedure, additional sample enrichment was achieved by the application of the prolonged electrokinetic injection. Prolonged electrokinetic injection, as one of possible preconcentration procedures in CZE, was applied because of its simplicity, although this way of injection/preconcentration results in the discrimination of the enrichment factors for individual ion (different ion mobilities). The results of this experiment are presented in Fig. 4c. Individual analytes in concentration range of 1 μg/l were detected only after electrokinetic injection of the preconcentrated sample (Fig. 4d).

Table 1
Enrichment factors calculated on the basis of the quotient of peak integrals with and without sample enrichment

Anion	Peak area without preconcentration	Peak area after preconcentration	Enrichment factor
Br^-	1912	11014	5.8
NO_2^-	1939	12820	6.6
NO_3^-	2898	25479	8.8
I^-	905	7489	8.3

4. Conclusions

The efficiency of the preconcentration procedure based on ion-exchange has been demonstrated. Preconcentrated anions were not contaminated by the eluent because it was removed on-line by chemical suppression on a suppressor column. We found the micromembrane suppressor to be superior when compared to a packed-bed suppressor because of the continuous mode of operation and lower baseline noise. The application of NaOH-based eluent resulted in additional baseline stabilization. In our case, the enrichment factors of 5.8 to 8.8 were obtained (Table 1). The sample enrichment factors are determined by dividing the time interval during which the preconcentrated anions were eluted from the column by the eluent flow-rate. The sample loading flow-rate (up to 3 ml/min) and eluent flow-rate (up to 3 ml/min, limited by the suppressor's technical characteristics) did not affect the reproducibility of the preconcentration. This fully computer-controlled sensing/switching system enabled us to collect a predetermined section of the eluted peak, which resulted in higher enrichment factors. The fact that the eluted anions were completely matrix-free was additionally exploited by electrokinetic injection in the applied CE system, which resulted in additional sample enrichment. The proposed system can be used for cation preconcentration as well. Due to its automation, this sample enrichment system can be used for on-line coupling with separation systems like IC and CE.

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