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Capillary electrophoresis in determination of inorganic anions in microbial fermentation processes; comparison with high-performance liquid chromatography

Capillary electrophoresis (CE) and isocratic high-performance liquid chromatographic (HPLC) methods were optimized and compared for the analysis of several inorganic anions using ultraviolet (UV) and refractive index (RI) detectors, respectively. The chromatographic methods were then applied to the analysis of samples from fermentation processes aimed at microbial purification of flue gases, in particular, anions originating from sulfur and nitrogen oxides. In the CE method, the concentration of electrolyte had a notable effect on the resolution, migration order, and migration times of the anions. The best resolution was achieved in 10 mM chromate with 0.5 mM tetradecyltrimethyl-ammonium bromide (TTAB) as $S_2O_3^{2-}$, Cl^-, NO_2^-, NO_3^-, SO_4^{2-}, SO_3^{2-}, PO_4^{3-}, and CO_3^{2-} were separated within 10 min. With the HPLC method (ION-120 anion analysis column), the combination of 3 mM salicylic acid and 5 mM Tris showed the best resolution for nitrate and nitrite. Sulfite and sulfate did not separate from each other under any of the conditions tested while the total time of chromatography was 25 min. The UV detection of CE was about 10 times more sensitive than the RI detection used in HPLC. The reproducibility of quantitative analysis data with HPLC was generally slightly better than with CE. A distinct advantage of CE is that pretreatment of samples is unnecessary.

Key Words: Capillary electrophoresis; High-performance liquid chromatography; Microbial process mixtures; Inorganic anions

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1 Introduction

Nitrogen oxides (NO_x) and sulfur dioxide (SO_2) are the major air pollutants formed on combustion of fossil fuels. Purification procedures includes scrubbing, in which both SO_2 and NO_x are transferred to the aqueous phase with formation of ions such as sulfate, sulfite, nitrate, and nitrite. Novel biological approaches to detoxification of these anions apply biotechnological processes in which microbes convert the ions into elemental nitrogen and sulfur [1–5].

Monitoring of anions is critical for the operation of microbial purification processes of flue gases. Several chemical techniques have been used to determine small inorganic anions in bacterial media including spectrophotometric, gravimetric, and titrimetric methods [6–8]. The commonest method for the measurement of nitrate and nitrite involves azo-derivatization followed by photometry [9]. Thiosulfate is determined colorimetrically after cyanolysis [10] or by titration [11]. Sulfite is determined semiquantitatively with commercially available dip-sticks (Merck) or spectrophotometrically by the reaction of fuchsin and formaldehyde in sulfuric acid [12] or by iodometry [13]. Sulfate is determined turbidimetrically following precipitation with $BaCl_2$ [14].

In recent years, chromatographic techniques, in particular ion-exchange methods, have become competitive with chemical methods for the analysis of inorganic ions because most of the wanted ions can be analyzed simultaneously. The first method for analysis of inorganic ions by high-performance liquid chromatography (HPLC) was developed by Small et al. [15]. This technique used a combination of an analytical column and a suppressor column to decrease the conductivity of the mobile phase for conductometric detection. Later a number of alternative methods were developed without the suppressor column. Usually the analytical columns are based on ion-exchange or ion-interaction method [16]. Conductivity, indirect or direct UV photometry, amperometry, potentiometry, and refractive

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index (RI) have been employed for detection but conductometry has been the method of choice in HPLC [17–19].

The first publications dealing with the use of capillary electrophoresis (CE) in inorganic anion analysis were published more than 20 years ago (see a review [20]). Capillary zone electrophoresis (CZE) was first used in early 1980's in determination of nitrate, chloride and sulfate in drinking water. The experiments were carried out in an isotachophoretic column using cadmium acetate as a carrier electrolyte [21]. In the 1990's the interest in the use of CE techniques for the separation of inorganic anions has increased remarkably as provided in the recent review [22]. Detection in CE is commonly carried out optically (absorption or fluorescence), whereas amperometry or potentiometry has been explored comparatively little [23]. Conductivity detection has been used in many applications in CE [23–25] but in general it is rare for inorganic anions in CZE [22]. This is in contrast to HPLC which mainly employs conductivity detection in ion analysis [17–19, 23].

Many examples of the application of HPLC [19, 26–27] and CE techniques [28] to the analysis of anions in different environmental samples are reported. The present study describes convenient methods for analyzing several anions in microbial process media using CZE. The method is compared with conventional HPLC operating with a universal RI detector.

2 Materials and methods

2.1 Reagents

Ultrapure Milli-Q water (Millipore, Bedford, USA) was used for the preparation of electrolytes and standards. Standard solutions were prepared usually from sodium salts of various ions. The electrolyte was analytical-grade Na_2CrO_4 (J.T. Baker, Deventer, Holland). Tetradecyltrimethylammonium bromide (TTAB; Sigma chemical Co, St Louis, USA) was used as an electro-osmotic flow (EOF) modifier for the reversal of the direction of EOF. The HPLC elution buffer was prepared from TRIZMA Base and salicylic acid, which were of SigmaUltra grade (Sigma Chemical Co).

2.2 Instrumentation

2.2.1 Capillary electrophoresis

The capillary electrophoresis instrument was a HP^{3D}CE (Hewlett-Packard, Waldbronn, Germany). It was run with negative power polarity for separation of anions. A fused silica capillary with an extended detection window was used (Hewlett-Packard, Germany). The effective length of the 50 μm ID capillary was 72 cm. A separation potential of 20 kV at 10°C was applied during all separations. A sample or standard was introduced into the capillary by hydrodynamic injection at 50 mbar for 5 s. A short buffer plug was applied to the capillary at a pressure of 50 mbar for 1 s before the electrophoresis run. Detection was performed with a diode array detector at 254 nm. At this wavelength all the inorganic anions analyzed have low absorbances and therefore negative peaks were detected. The electropherograms were recorded and integrated with a HP^{3D}CE Chemstation software. The running electrolyte was prepared from a 100 mM Na_2CrO_4 stock solution.

TTAB was added to the running electrolyte from a 50 mM stock. The pH of the running buffer was adjusted with 0.1 M NaOH. The electrolyte solution was filtered through a 0.45-μm pore size filter and degassed by sonication for 15 min. The electrolyte solution remained stable for at least two days.

Anion standards were prepared by diluting stock solutions of individual ions (1 g/L) except S^{2-}, which was prepared from a stock containing 10 g/L of the ion. Sulfite and sulfide stock solutions were prepared daily because of their instability in aqueous solution. For carboxylic acid salts 100 or 500 g/L stock solutions were used. Stock solutions were filtered before use through a filter of 0.45-μm pore size.

2.2.2. Capillary conditioning

A new capillary was first washed with 1 M NaOH for 20 min, then with 0.1 M NaOH for 20 min, then with Milli-Q water for 3 min, and finally with the running buffer for 15 min. Between the runs, the capillary was flushed with 0.1 M NaOH for 4 min, Milli-Q water for 3 min, and with the running buffer for 5 or 10 min (10 if the buffer was changed between runs).

2.2.3. High-performance liquid chromatography

Inorganic anions were separated on an ION-120 anion analysis HPLC column (120 mm × 4.6 mm ID) containing a macroporous polymeric anion exchange material with the capacity of approximately 100 μeqv/g (Interaction Chromatography Inc. San Jose, CA, USA). The column was equipped with an IonGuard™ GA-100 guard column that contains the same polymer as the ION-120 column (Interaction Chromatography Inc.). The HPLC column was connected to a Model 2150 HPLC pump (LKB, Bromma, Sweden) equipped with a Rheodyne Model 7125 injector (20-μL sample loop; Cotati, CA, USA) and to a Model 2142 RI detector (LKB) with a Model C-R3A integrator (Shimadzu, Kyoto, Japan). The mobile phase was 3 mM salicylic acid and 5 mM Tris (Tris[hydroxymethyl]-aminomethane), which was filtered and degassed before

use. The column was operated at room temperature and the flow-rate of the mobile phase was 1 mL/min. Anion standards were prepared as for CE.

2.3 Bacterial cultures

A denitrifying bacterium, No. GII-6, isolated from soil was cultivated in a mineral medium containing 1.1 g/L KH_2PO_4, 0.11 g/L $MgSO_4$, 0.56 g/L NaCl, and 1 mL/L of a solution of microelements. In addition, varying amounts of nitrite or nitrate and glucose were added in the medium as growth substrates. The pH of the medium was adjusted to 9–10 with Na_2CO_3.

A sulfate reducing microorganism, No. 37-2-C, was also isolated from soil. It was cultivated in a mineral medium containing 0.82 g/L $MgCl \times 6H_2O$, 1 g/L KH_2PO_4, 0.34 g/L NaCl, 1 g/L NH_4Cl, 5 g/L Na_2SO_4, 1 g/L sodium lactate, 0.1 g/L thioglycolic acid, 0.2 g/L ascorbic acid, and 1 mL/L microelements. The pH was adjusted to 7.2–7.5 with Na_2CO_3.

2.4 Samples

Fermentation samples were centrifuged in 1.5-mL polypropylene tubes at 16000 *g* for 10 min. The supernatant was boiled for 8 min and centrifuged again. The supernatant was then diluted with MQ-water and analyzed with CE and HPLC.

3 Results and discussion

3.1 Optimization of the CE method

3.1.1 Electrophoretic conditions

Chromate was chosen as a carrier electrolyte for CE because it provides a suitable UV absorbance background over a wide range of wavelengths [29]. The wavelength of 254 nm was chosen because all the analyzed anions have low absorbance at this wavelength, and hence strong negative peaks can be detected [30–35]. The concentration of 0.5 mM TTAB was enough to fully reverse the EOF.

3.1.2 pH optimization

The effect of pH on 10 mM electrolyte solution was tested at 0.5-unit intervals from 8 to 10 with a standard solution containing 20 mg/L of each anion. Six injections were performed at each tested pH value. Below pH 8, chromate started to precipitate and hence these pH values were not applicable. The pH did not seem to have any effect on the resolution or on the migration times of tested anions. Since chromate has poor buffering capacity in the pH range studied, we chose pH 9 because it is near the initial pH value of the electrolyte solution.

3.1.3 The effect of chromate concentration

The concentration of chromate was varied from 2.5 mM to 15 mM. The concentration of electrolyte affected both the resolution and migration times of anions (**Table 1**). When the chromate concentration was raised, migration times of anions increased. The increase in the electrolyte concentration caused a decrease in EOF and thus the overall movement of ions slowed down [36–38]. Sulfate and nitrate ions migrated together in the same peak with 7.5 mM chromate. Chloride and thiosulfate migrated together both in 12.5 and in 15 mM chromate, while in 2.5 mM chromate thiosulfate could be detected as a shoulder in the chloride peak. As a rule, in other electrolyte concentrations all the ions shown in Table 1 were sufficiently resolved from one another to be quantified. Sulfate, nitrite, and nitrate separated only partially in 5 mM chromate, as exemplified by **Figure 1.a**. The same behavior was observed with sulfate and nitrite in 2.5 mM chromate. The migration order of sulfate ion changed from ahead of nitrite and nitrate to behind them when the chromate concentration was increased from 2.5 mM to 10 mM. A similar migration order of ions was also seen in other studies when chromate was used as a carrier electrolyte [37–38]. In our work, however, sulfate was seen to migrate slower than nitrite at 5 mM chromate (Table 1). The best resolution was achieved in 10 mM chromate, which is optimal for sensitivity and separation efficiency [38], although thiosulfate and chloride migrated quite close to each other (**Figure 1.b**). In 10 mM chromate, pH 9, all the desired anions ($S_2O_3^{2-}$, SO_3^{2-}, SO_4^{2-}, NO_2^-, and NO_3^-) could be detected while chloride, phosphate, and carbonate did not interfere with the detection.

Table 1. Effect of chromate concentration on the capillary electrophoretic migration times of anions in a standard solution of various ions (means of 6 replicate injections).

Ion	Chromate concentration (mM)					
	2.5	5.0	7.5	10.0	12.5	15.0
$S_2O_3^{2-}$	5.78	6.08	6.46	6.48	6.68	6.75
Cl^-	5.98	6.25	6.58	6.55	6.68	6.75
SO_4^{2-}	6.13	6.49	6.93	6.97	7.18	7.29
NO_2^-	6.17	6.44	6.80	6.78	6.92	6.98
NO_3^-	6.25	6.54	6.93	6.88	7.03	7.10
SO_3^{2-}	6.94	7.22	7.73	7.70	7.89	8.05
PO_4^{3-}	7.95	8.32	9.00	8.94	9.22	9.43
CO_3^{2-}	8.66	9.25	10.02	9.77	10.11	10.31

Other conditions: 0.5 mM TTAB, pH 9.0, separation voltage 20 kV at 10°C and hydrodynamic injection of 50 mbar for 5 s.

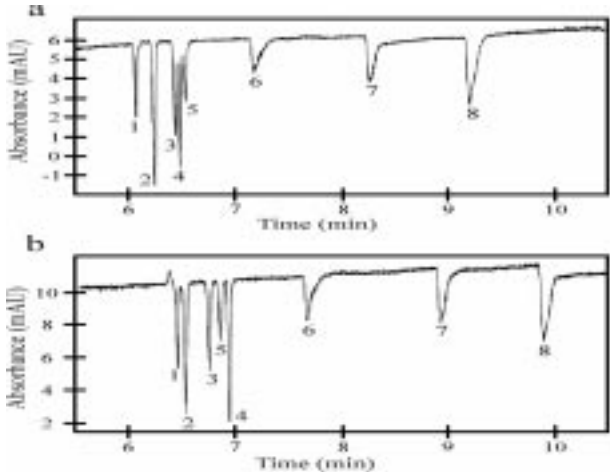

Figure 1. Effect of chromate concentration on the migration of various ions. The concentrations were (a) 5 mM chromate and (b) 10 mM chromate. Numbers refer to: (1) thiosulfate, (2) chloride, (3) nitrite, (4) sulfate, (5) nitrate, (6) sulfite, (7) phosphate, and (8) carbonate. CE conditions as in Table 1.

3.1.4 Migration of interfering substances

When analyzing inorganic anions, it is essential that carboxylic acids do not disturb the detection since these compounds are often present in fermentation media [39]. Some of the shortest carboxylates (formate, acetate, and lactate) were tested in detail in optimal electrolyte conditions since the longer ones clearly migrate behind the inorganic anions. None of the tested carboxylates interfered with the anions. Formate migrated first [migration time (MT.) 8.46 min], between sulfite and phosphate. Acetate and lactate migrated after carbonate (MT. 10.66 min and 11.79 min, respectively).

Sulfide might interfere with the analysis of fermentation media of flue gas purification processes, since this compound is an intermediate in microbial desulfurization and it is produced in significant amounts. Sulfide perhaps has a long migration time because in test runs it was not observed after 30 min although the concentration was as high as 100 mg/L or because sulfide absorbs at 254 nm it was perhaps not detected against the background [30]. Sulfide can be detected if the running conditions are changed. However, this was not essential since sulfide evaporates rather rapidly and it is more conveniently determined by other, sulfide-specific techniques [14, 40].

Process liquids may contain proteins and other macromolecules but these compounds migrate far behind the inorganic anions and will not interfere with the analysis.

3.2 Optimization of the HPLC method

With a RI detector the mobile phase should show a high refractive index relative to that of the solute to be analyzed [41]. Initially, the mobile phase was 4 mM Tris-salicylate, pH 7.8. Ions CO_3^{2-}, Cl^-, NO_2^-, NO_3^-, PO_4^{3-}, and $S_2O_3^{2-}$ were separated well, but the column could not separate SO_4^{2-} and SO_3^{2-} from each other. Different elution conditions were tested for sulfate and sulfite. The concentration of salicylic acid and Tris was varied between 2–4 mM and 2–8 mM, respectively, but the resolution was not improved. Nor did changing the pH of elution buffer affect the separation of sulfate and sulfite. However, the combination of 3 mM salicylic acid and 5 mM Tris showed a good resolution for nitrate and nitrite. The elution orders and retention times in minutes were following: CO_3^{2-}, 3.93 ± 0.01; Cl^-, 4.91 ± 0.02; NO_2^-, 5.55 ± 0.02; NO_3^-, 8.74 ± 0.02; PO_4^{3-}, 13.45 ± 0.08; SO_3^{2-}, 16.58 ± 0.18; SO_4^{2-}, 16.88 ± 0.10 and $S_2O_3^{2-}$, 24.64 ± 0.47. Short chain organic acids do not interfere with the analyses but proteins can bind to the column. To minimize the binding of proteins to the matrix the samples were treated as shown in Section 2.

3.3 Comparison of CE and HPLC methods

3.3.1 Reproducibility

The reproducibility of the optimized CE method was determined by injecting five times a standard mixture containing 20 mg/L of each anion. For HPLC, injections were repeated similarly, but 10 times higher concentrations were used. Reproducibilities in HPLC and in CE were determined for peak areas, peak heights and for migration and retention times as relative standard deviations (R.S.D.; **Table 2**). In CE, peak heights turned out to be more reproducible than peak areas (Table 2; columns "1 and 2"). With thiosulfate and chloride, peak area analysis became

Table 2. Relative standard deviations (R.S.D.; %) for peak areas (P.A.), peak heights (P.H.), peak migration times (P.MT.) of CE and for peak retention times (P.RT.) of HPLC.

Ion	R.S.D.(%)					
	CE			HPLC		
	P.A.	P.H.	P.MT.	P.A.	P.H.	P.RT.
$S_2O_3^{2-}$	7.58	2.79	0.04	4.31	4.31	1.84
SO_3^{2-}	11.55	5.25	0.07	4.43	2.23	1.09
SO_4^{2-}	2.89	3.43	0.05	4.65	2.53	0.62
NO_2^-	3.61	0.84	0.06	3.06	1.62	0.40
NO_3^-	5.03	4.17	0.07	4.39	2.75	0.27
Cl^-	6.28	5.05	0.06	2.97	1.74	0.35
PO_4^{3-}	6.54	6.17	0.09	4.32	2.14	0.58
CO_3^{2-}	3.69	3.49	0.12	2.63	2.17	0.30

A standard solution contained 20 mg/L of each anion in CE and 200 mg/L in HPLC. Solutions were injected 5 times. CE conditions: 10 mM chromate, 0.5 mM TTAB, pH 9.0, separation voltage 20 kV at 10°C and injection of 50 mbar for 5 s; HPLC conditions: 3 mM salicylic acid and 5 mM Tris at room temperature.

more uncertain because both ions migrated quite close to each other. That is why the integrator program may not determine the peak areas as correctly as the peak heights. The chloride peak area was, however, more reproducible than that of thiosulfate. This may be due to the relatively larger peak of chloride compared to that of thiosulfate and because small distortions in the peak area of chloride do not exert much influence on the reproducibility. In the case of sulfite the reproducibility of peak areas was poor because the peak shape varied considerably from one injection to another, making the baseline quite diffuse. This again influenced the peak area more than the peak height. For nitrite, both the peak area and the peak height were well reproducible. The peak area of sulfate was slightly more reproducible than was the peak height. For nitrate, phosphate, and carbonate, there were very small differences between the reproducibilities of peak areas or peak heights.

Peak heights were also in HPLC more reproducible than peak areas (Table 2; columns "4 and 5"). In general R.S.D. values of peak areas and peak heights were smaller when compared to CE which indicates better reproducibility of the HPLC system. Increasing the concentration of ions 3 times decreased the R.S.D. values of peak areas and peak heights approximately to 1/3 and 1/2 of that when 0.2 g/L of ions was used in HPLC (results not shown). When the retention times were short like with the ions CO_3^{2-}, Cl^-, and NO_2^- the shapes of the peaks were sharper and the peak areas and heights were then more reproducible. The column started to corrode after about 150 injections with an influence on the resolution of ions and on R.S.D. values.

With CE the highest R.S.D. of a migration time was for carbonate (0.12) while R.S.D. values for other anions were below 0.10 (Table 2; column "3"). This high reproducibility of migration times made the identification of peaks quite reliable. In HPLC R.S.D. values for retention times were higher than for migration times in CE (Table 2, last column). In HPLC larger variations appeared with the peaks of long retention times like with $S_2O_3^{2-}$ and SO_3^{2-}. With CE, R.S.D. values for all ions were small and there were no difference between the ions although migration times were long (Table 2). The higher R.S.D. variation in HPLC was thus due not only to manual injection but also to other inherent properties of the system.

3.3.2 Quantification, linearity, and separation efficiency of the methods

Limits of detection (LOD) with CE were determined for thiosulfate, sulfite, sulfate, nitrite, nitrate, chloride, and phosphate. LOD was determined as the peak height which was three times the baseline noise. It was not reasonable to determine the LOD value for carbonate since water solutions absorb carbon dioxide readily from surrounding air. LOD for thiosulfate, sulfate, nitrite, nitrate, and chloride was 1 mg/L and for sulfite and phosphate 2.5 mg/L. With HPLC LOD was 20–30 mg/L which is 10 times higher than with CE. When the column was fairly new, smaller amounts than 20 mg/L were detected. However, the RI detector in HPLC is not as sensitive as the UV detector used in CE. When the same kind of detection systems are used methods may not differ so drastically.

The CE method was linear at least within 5–150 mg/L compared to the HPLC method of 300–1 000 mg/L of sodium salt of an anion.

Table 3 shows the number of the theoretical plates for CE and HPLC methods. It shows that CE owes distinctly higher resolution.

Table 3. The number of theoretical plates (calculated by Sigma 5 method) for various ions determined by CE and HPLC.

Ion	CE	HPLC "new column"	HPLC "old column"
$S_2O_3^{2-}$	435 000	–	420
Cl^-	235 000	–	200
NO_2^-	287 000	900	350
NO_3^-	253 000	1 600	530
SO_4^{2-}	353 000	2 200	650
SO_3^{2-}	47 000	–	370
PO_4^{3-}	54 000	2 200	480
CO_3^{2-}	80 000	1 300	370

CE and HPLC conditions were as in Table 2. "New column" indicates a column used for less than 100 injections of microbial cultivation samples while "old column" indicates to a column used for more than 200 injections.

3.3.3 Analysis of fermentation samples

In CE mere centrifugation and dilution with water were sufficient for the pretreatment of samples from fermentation processes. Migration times of actual samples did not vary more than shown for standard solutions in Table 2. That is why samples were not deproteinized and if samples contained small amounts of proteins they migrate much more slowly than small ions. In addition, proper rinsing procedures were used to flush proteins and other impurities out of the capillary between the runs. However, proteins do interfere with the HPLC column and that is why all samples were boiled and centrifuged before analyses. After boiling test samples were analyzed for changes in ion concentrations or in other quality. No changes were detected, and boiling was therefore used as a sample treatment.

Even though the samples were carefully pretreated to remove proteins, an increase in the back pressure of the HPLC column occurred especially when the samples con-

Table 4. Comparison of analysis results with CE and HPLC from fermentation samples.

Sample	CE (g/L)			HPLC (g/l)			Ratio (%)		
	NO_2^-	NO_3^-	SO_4^{2-}	NO_2^-	NO_3^-	SO_4^{2-}	NO_2^-	NO_3^-	SO_4^{2-}
GII-6 (2)	0.14	3.22	2.34	0.068	3.06	2.37	206	105	99
GII-6 (7)	3.34	1.52	0.96	3.78	1.23	0.80	88	124	120
GII-6 (16)	1.63	–	0.22	2.52	–	0.16	65	–	138
37-2-C (1)			3.52			3.07			115
37-2-C (2)			2.76			3.01			92
37-2-C (3)			3.32			2.76			120

Concentrations of nitrite, nitrate and sulfate were analyzed with CE and HPLC from two different bacterial fermentation processes. GII-6 was a denitrifying bacterium and 37-2-C a sulfate reducing bacterium (see Section 2). The number in parentheses shows the number of the sample taken from the fermentation process. The values for ratios were calculated comparing the amounts of ions analyzed by CE to the amounts analyzed by HPLC. CE and HPLC conditions as in Table 2.

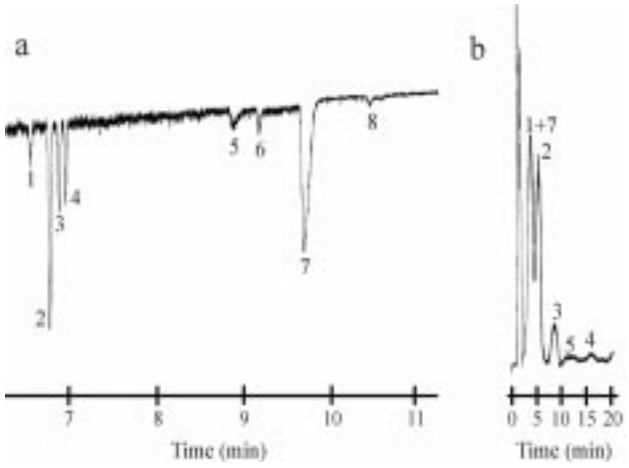

Figure 2. Separation of inorganic ions from a fermentation sample of a denitrifying bacterium GII-6 by CE (a) and by HPLC (b). Concentrations of identified ions are the same as for the sample GII-6 (7) on the Table 4. Numbers refer to: (1) chloride, (2) nitrite, (3) nitrate, (4) sulfate, (5) phosphate, (6) unknown, (7) carbonate, and (8) acetate. CE and HPLC conditions were as in Table 2.

tained high amounts of sulfate. The pressure may increase because sulfate formed an insoluble precipitate with other compounds. Boiling and centrifugation were enough for HPLC analysis when samples containing nitrate and nitrite were analyzed. The fast increase in the back pressure of the HPLC column was not seen when nitrate/nitrite were analyzed. In routine use HPLC seems to be the method of choice for nitrate and nitrite. For all sulfur oxides, especially for thiosulfate, retention times were long and that is why detection limits increased.

The concentrations of ions in fermentation samples analyzed by CE versus HPLC are shown in **Table 4**. In addition, **Figure 2** illustrates the separation of ions by CE (Figure 2.a) and by HPLC (Figure 2.b) from a fermentation sample. Usually the CE method showed higher amounts of ions. The recovery in HPLC was poor with low NO_2^- concentrations. That is in accordance with the poor linearity of the HPLC method below 0.3 g/L. Instead, CE was linear to as low as 5 mg/L. At higher concentrations CE showed less NO_2^- than HPLC (Table 4). That is, however, because CO_3^{2-} and Cl^- ions migrated near NO_2^- in HPLC and disturbed the analyses, especially when an old column is used (Figure 2.b). With CE, sulfate or chloride did not interfere with the determination of nitrite and nitrate in the fermentation analyses when samples were diluted to 1 : 100 (Figure 2.a). In CE even after 400 analyses peaks separated well enough from each other. If samples were not diluted overlapping of nitrate and sulfate peaks occurred. With a "new HPLC column" chloride, carbonate, nitrate, and nitrite separated from each other but with an "old HPLC column" chloride and carbonate eluated as one peak and nitrite eluated very close to chloride and carbonate (Figure 2.b). Nitrate and sulfate eluated as separate peaks and other ions did not disturb the elution (Figure 2.b). Usually, the recovery of nitrate and sulfate from culture samples ranged from 80% to 120%.

4 Concluding remarks

CE is the method of choice for the analysis of various inorganic ions from complex biological liquids, for example from industrial process samples. Resolution with CE is distinctly better than with HPLC, as is the reproducibility of the retention times, which makes the analyses of complex mixtures by CE more reliable. Preparation of samples for CE is less demanding than for HPLC since impurities can be readily rinsed from a CE column. For selected ions, HPLC analysis may be more applicable because routine applications of HPLC are still more common and developed, and validated procedures are available. The sensitivities of analyses of the ions cannot be directly compared because different types of detectors were used.

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