

## Effect of high concentrations of salts in samples on capillary electrophoresis of anions

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### Abstract

The capillary electrophoretic separation of anions using direct UV detection was studied. The separation conditions, such as carrier electrolyte, pH and concentration of electroosmotic modifier, were optimized. The carrier electrolyte co-ion affected the peak shape because electromigration dispersion was non-negligible. An unsuitable anion form of the electroosmotic flow modifier would cause negative peaks on the separation baselines when the samples contain high concentrations of salts, and the separation could be adversely affected. In this work, sodium chloride with 2.0 mM tetradecyltrimethylammonium bromide was used as the carrier electrolyte solution, and a good separation was achieved. Taking sodium chloride to represent salts in samples, the experimental results showed that the peak height and width of a detected anion were affected by the ratio of sodium chloride concentration between the carrier electrolyte solution and sample, and correspondingly the detection limit and separation efficiency of the anion were also affected. The principle of electrostacking was used to explain this phenomenon theoretically. The reproducibility of migration time, peak height and peak area under different conditions was also examined.

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### 1. Introduction

Capillary ion electrophoresis (CIE) [1–4] is a recently introduced capillary electrophoretic technique optimized for the rapid determination of low-molecular-mass inorganic and organic ions. In the capillary electrophoretic separation of anions, an electroosmotic modifier is added to the carrier electrolyte solution to create a layer of positive charges at the capillary inner wall and reverse or slow the electroosmotic flow [1–4].

Without the electroosmotic modifier, anions move rapidly in the opposite direction to the electroosmotic flow, which results in a very long migration time and renders the method less attractive.

Because most anions lack a chromophore, universal indirect UV detection is widely used in CIE [3–7]. In comparison with indirect UV detection, there have been only a few reports [8–10] of the use of direct UV detection. However, direct UV detection is more selective and sensitive. In the determination of a specific trace-level anion in a sample containing high concentrations of other anions, it is preferable to use direct UV detection provided that the trace-level

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anion shows UV absorption whereas the high-concentration anions do not.

CIE has been used in various application fields and is now recognized as a less matrix-dependent method than ion chromatography (IC). When IC is used to determine anions in samples with high concentrations of salts, there is a risk of sample overloading and consideration must be given to the total ion-exchange capacity of the column. Although CIE has not been used to determine anions in samples with high concentrations of salts, Jones and Jandik [9] successfully determined anions with disparate concentrations, such as anion impurities in 99.9% pure solid terephthalic acid. When we tried to determine anions in subterranean water, samples with high concentrations of salts were encountered (there are high concentrations of chloride, sulfate, carbonate salts, etc., in subterranean water, in which the concentration of chloride is generally the highest and can reach as high as grams per litre levels; the cation with the highest concentration is generally sodium) and the potential of CIE in the determination of anions in such samples was therefore investigated.

The objective of this study was to develop a CIE method for the determination of trace-level UV-absorbing anions in subterranean water. For this purpose, the capillary electrophoretic separation of anions using direct UV detection was studied. The separation conditions, such as carrier electrolyte, pH and concentration of electroosmotic modifier, were optimized. The effect of high concentrations of salts in samples on the separation was especially studied both experimentally and theoretically.

## 2. Experimental

### 2.1. Apparatus

The CE system used was a Quanta 4000 (Waters Chromatography Division of Millipore, Milford, MA, USA) with a negative power supply. Direct UV detection was accomplished with a zinc lamp and a 214-nm optical filter. Data acquisition was carried out with a Waters

Millennium 2010 chromatography manager with a bus satellite interface and LAC/E modules connecting the data station with the CE system. Data collection was initiated by a signal cable connection between the Quanta 4000 and the bus satellite interface.

### 2.2. Carrier electrolyte solutions

Four carrier electrolytes, sodium sulfate, sodium acid phosphate, piperazine sulfate and sodium chloride, were used to give carrier electrolyte solutions in which tetradecyltrimethylammonium bromide (TTAB) purchased from Waters was used as electroosmotic modifier according to the literature [11] and added at a suitable concentration.

### 2.3. Standard solutions

Analytical-reagent grade sodium or potassium salts were purchased in China and used to prepare 1000 ppm stock standard anion solutions containing a single anion. The mixed anion solutions consisted of ten anions, fluoride, chloride, bromide, iodide, nitrite, nitrate, sulfate, phosphate, carbonate and thiocyanate, of which only five, bromide, iodide, nitrite, nitrate and thiocyanate, show UV absorbance at 214 nm. Five mixed anion solutions containing 1, 4, 10, 20 and 40 ppm of each anion were prepared to evaluate calibration graphs. Another five mixed anion solutions containing 10 ppm of each of the above-mentioned ten anions with an additional 20 ppm of thiocyanate, 10 ppm of nitrate, 10 ppm of nitrite, 20 ppm of bromide or 20 ppm of iodide separately were prepared for the purpose of peak identification in electrophorograms. Mixed anion solutions were prepared not only with deionized water, but also with 12.5, 50 or 100 mM sodium chloride solutions. Polyethylene containers were utilized for the mixed anion solutions.

### 2.4. Electrophoresis

A Waters Accu-Sep polyimide-coated fused-silica capillary was used throughout. The capil-

lary dimensions were 75  $\mu\text{m}$  I.D., 60 cm total length and 52 cm from the point of injection to the detector cell. The capillary was flushed with 1 M potassium hydroxide solution for 1 h and then equilibrated with carrier electrolyte solution before use. All injections were performed in the hydrostatic mode at a height of 10 cm for 30 s. The applied voltage for each run was 10 kV. A 2-min capillary purge with carrier electrolyte solution by a vacuum applied to the receiving electrolyte vial was performed prior to each injection.

### 3. Results and discussion

#### 3.1. Optimization of capillary electrophoretic separation of anions using direct UV detection

Three carrier electrolytes, sodium sulfate, sodium acid phosphate and piperazine sulfate, were chosen to examine the effect of carrier electrolyte on the capillary electrophoretic separation of anions. The three carrier electrolytes contained two anions, sulfate and phosphate, and two cations, sodium and piperazine, in which phosphate and piperazine could be protonated and had buffering ability. We believed that both the anion and cation of a carrier electrolyte have an effect on the separation. Carrier electrolyte solutions were prepared from 10 mM of sodium sulfate, phosphoric acid or piperazine with 0.5 mM TTAB separately, and the pH of the carrier electrolyte solutions was adjusted to 9.3 with sodium hydroxide or sulfuric acid by using a pH meter. Capillary electrophoretic separation of anions using direct UV detection was achieved as shown in Fig. 1.

It was clear that when sodium sulfate was used, peak 2 was separated from peak 3 (Fig. 1A); when sodium acid phosphate was used, peak 2 overlapped partly with peak 3 (Fig. 1B); when piperazine sulfate was used, peak 2 nearly overlapped with peak 3 (Fig. 1C). Apart from this, peak 1 was also affected. When sodium acid phosphate and piperazine sulfate was used, a negative peak occurred prior to peak 1 and rendered the calibration graph for peak 1 of

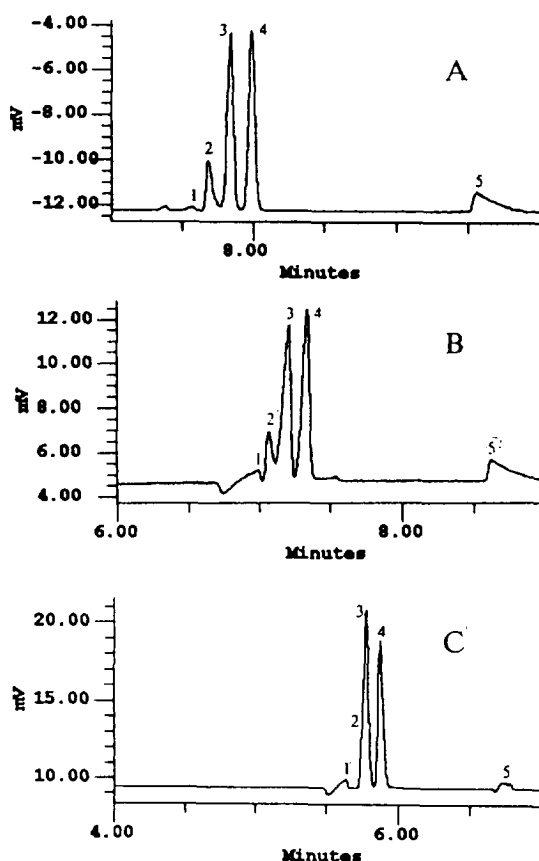


Fig. 1. Capillary electrophoretic separation of anions using direct UV detection. Carrier electrolyte: (A) 10 mM sodium sulfate; (B) 10 mM sodium acid phosphate; (C) 10 mM piperazine sulfate. Current: (A) 15  $\mu\text{A}$ ; (B) 16  $\mu\text{A}$ ; (C) 6.5  $\mu\text{A}$ . Peaks: 1 = bromide; 2 = iodide; 3 = nitrite; 4 = nitrate; 5 = thiocyanate. The concentration of each anion in the sample was 10 ppm.

peak height or area versus concentration non-linear.

Another effect of the carrier electrolyte on separation was to change the peak shape. Fig. 2 is an enlarged view of peaks 3 and 4 of Fig. 1. With sodium sulfate, relatively symmetrical peaks 3 and 4 (Fig. 2A) were observed; with sodium acid phosphate, fronting of peaks 3 and 4 (Fig. 2B) was seen; with piperazine sulfate, slight tailing of peaks 3 and 4 (Fig. 2C) occurred. This phenomenon has been reported previously in CIE when using indirect detection, and was caused by electromigration dispersion [12–14].

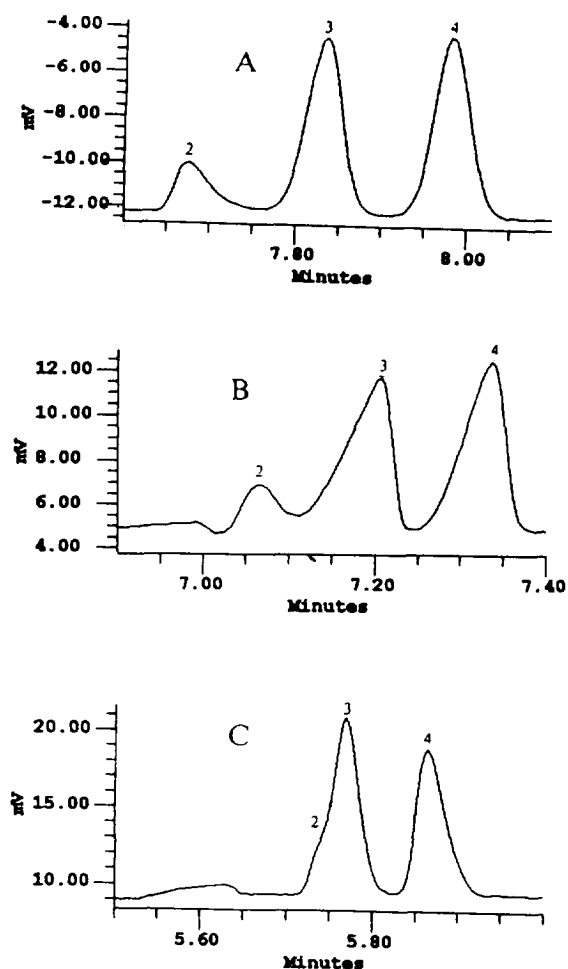


Fig. 2. Enlarged view of nitrite and nitrate peaks in Fig. 1. Peak identification as in Fig. 1.

As indicated in the literature [12-14], electromigration dispersion occurs during the migration of sample ions that possess effective mobilities different to that of the background electrolyte co-ion; the higher the concentration of the sample component is in its zone, the more pronounced the electromigration dispersion will be. Generally, electromigration dispersion is considered to be negligible when the concentration of the carrier electrolyte co-ion is two orders of magnitude higher than that of the sample ions.

In capillary electrophoresis of biological macromolecules, generally no tailing or fronting

peaks occur because the concentration of the carrier electrolyte co-ion can be easily adjusted to be two orders of magnitude higher than that of the biological macromolecules. In capillary electrophoresis of anions using indirect UV detection, generally tailing and fronting peaks occur because the carrier electrolyte co-ion used will generate a UV absorption background and its concentration cannot be too high [14]. Under the experimental conditions of Fig. 2, the concentration of carrier electrolyte was 10 mM and that of the sample anions was 10 ppm. After the units of concentration had been converted to be the same, the ratio between the concentration of carrier electrolyte and that of the sample anions (nitrite and nitrate) was 46 (molecular mass of nitrite) and 64 (molecular mass of nitrate). Sodium sulfate is a strong electrolyte, so the concentration of the co-ion (sulfate) of the carrier electrolyte sodium sulfate at pH 9.3 was equal to the concentration of sodium sulfate, 10 mM. Phosphoric acid undergoes a three-step ionization with  $pK_{a1} = 2.12$ ,  $pK_{a2} = 7.20$  and  $pK_{a3} = 12.36$ , hence the concentration of the co-ion (phosphate) of the carrier electrolyte sodium acid phosphate at pH 9.3 was obviously lower than the original concentration of phosphoric acid, i.e., 10 mM. Piperazine is a base that undergoes a two-step ionization with  $pK_{a1} = 9.66$  and  $pK_{a2} = 5.20$ , hence the concentration of the co-ion (sulfate) of the carrier electrolyte piperazine sulfate at pH 9.3 was approximately half of the original concentration of piperazine, i.e., 5 mM. Therefore, the concentration of the carrier electrolyte co-ion in Fig. 2A-C did not reach two orders of magnitude higher than that of the sample ions (nitrite and nitrate). Hence electromigration dispersion was non-negligible. The main reason for this can be mainly attributed to the low molecular masses of nitrite and nitrate, whereas biological macromolecules have very high molecular masses and the situation will be the opposite. Of course, in order to suppress electromigration dispersion, the concentration of the carrier electrolyte co-ion could be raised, but the anion concentration in the sample may be even higher, so it was concluded that electromigration dispersion was inevitable in capillary

electrophoresis of anions using direct UV detection.

According to the literature [15], the equivalent conductances of sulfate, phosphate, nitrite and nitrate are 80.0, 33.0, 71.8 and 71.4, respectively. As the equivalent conductances relate directly to the mobilities of ions, the following discussion using mobilities of anions was based on the equivalent conductances of the anions listed above. In Fig. 2B, the sample anions (nitrite and nitrate) were more mobile than the carrier electrolyte co-ion (phosphate), so that fronting peaks occurred. In Fig. 2C, nitrite and nitrate was less mobile than the carrier electrolyte co-ion (sulfate), so that tailing peaks were observed. However, in Fig. 2A, although the carrier electrolyte co-ion was the same as in Fig. 2C, the peaks of nitrite and nitrate did not show obvious tailing. This is because the carrier electrolyte co-ion (sulfate) had a mobility close to those of nitrite and nitrate, and the concentration of sulfate in Fig. 2A, which was nearly double that in Fig. 2C, approached two orders magnitude greater than the concentrations of nitrite and nitrate. Hence the conclusion is that the selection of a co-ion with a mobility close to those of anions in the sample is necessary in CIE not only when using indirect UV detection but also when using direct UV detection. Because iodide and thiocyanate are known hydrophobic anions that could interact with TTAB, they were not used to examine the effect of the carrier electrolyte on the peak shape.

The relationship between the response and the concentration of anions was also affected by the carrier electrolyte. Taking nitrate as an example, when sodium sulfate was used the relationship between peak height or area and concentration was linear from 1 to 40 ppm (least-squares fitting); when sodium acid phosphate was used, the relationship was linear only within the range 1–20 ppm; when piperazine sulfate was used, the relationship between peak height and concentration was linear only from 1 to 20 ppm and that between peak area and concentration from 1 to 40 ppm. The decreased linear range was caused by peak broadening and decreased separation efficiency.

The experimental results showed that as a carrier electrolyte sodium sulfate gave the best separation, but it is not a pH buffer salt. Sodium acid phosphate is a pH buffer salt, but we predicted that it would cause more fronting peaks at pH lower than 9.3. Piperazine sulfate is a pH buffer salt, and it could give relatively good separations; so piperazine sulfate seemed to be the best choice as the carrier electrolyte.

Carrier electrolyte solutions of 10 mM sodium sulfate with 0.5 mM TTAB at pH 5.8, 7.0, 9.3, 10.2 and 11.2 were used to examine the effect of pH on separation. The results showed that pH had only a slight effect on the resolution of iodide and nitrite.

Carrier electrolyte solutions of 10 mM piperazine sulfate with 1.0, 2.0, 2.6 and 3.0 mM TTAB were selected to examine the effect of the electroosmotic modifier concentration on separation. The relationship between the migration time of each anion and the concentration of TTAB is shown in Fig. 3. When the concentration of TTAB was 2.6 and 3.0 mM, the migration time of thiocyanate was too long, and was not measured and shown in Fig. 3; the

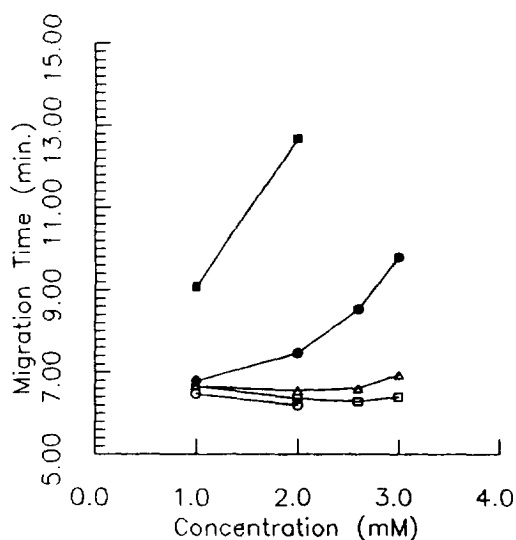


Fig. 3. Effect of concentration of TTAB on separation. ○ = Bromide; ● = iodide; □ = nitrite; △ = nitrate; ■ = thiocyanate. The concentration of each anion in the sample was 20 ppm.

bromide peak was covered by a negative peak prior to bromide, as shown in Fig. 1C, so its migration time also was not measured and shown in Fig. 3. It was observed that an increased concentration of TTAB caused longer migration times of iodide and thiocyanate and a higher resolution of nitrite and nitrate. A possible explanation of this effect involved ion pairing between nitrate and TTAB and hydrophobic interactions between hydrophobic anions (iodide and thiocyanate) and TTAB [16].

### 3.2. Effect of high concentrations of salts in samples on separation

It was shown above that the capillary electrophoretic separation of anions can be achieved by using sodium sulfate, sodium acid phosphate or piperazine sulfate as the carrier electrolyte. In each instance, TTAB was added to the carrier electrolyte solution as an electroosmotic flow modifier to reverse the electroosmotic flow. When these carrier electrolyte solutions were applied to separate anions in samples with high concentrations of salts, however, the separation became significantly poorer. In order to find the reason for this, sodium chloride was chosen to represent the salts in samples and the effect of high concentrations of sodium chloride in samples on the separation baseline was examined.

Four blank samples containing 0, 12.5, 50 and 100 mM sodium chloride and no analyte anions were injected and the separation baselines were monitored as illustrated in Fig. 4A and B when the sodium chloride concentration in sample was 12.5 mM. When sodium sulfate or piperazine sulfate carrier electrolyte solutions were used, an extremely broad negative peak followed by a very broad positive peak appeared on each baseline. Although the negative peak shown in Fig. 4A and B had different migration times, it appeared just before the bromide peak in the separation of anions and hence would seriously affect the separation. The peak width of the negative peak increased rapidly with increasing concentration of sodium chloride in the sample. Even when there was no sodium chloride in the sample there was still a small negative peak

followed by a small positive peak on separation baseline when piperazine sulfate was used as the carrier electrolyte solution, but they were not present when sodium sulfate was used as the carrier electrolyte solution. This can also be seen in Fig. 1C and A. When sodium chloride was used instead of sodium sulfate and piperazine sulfate as the carrier electrolyte, similar baselines were monitored again and are shown in Fig. 4C. It was observed that only a very sharp negative peak remained (the broad peak was generated by water). The width of the sharp negative peak increased linearly with increasing concentration of sodium chloride in the sample, but the separation of anions could still be accomplished. As the carrier electrolyte co-ion, chloride has an electrophoretic mobility close to that of sulfate [15] and would not cause too much tailing or fronting of peaks, hence piperazine chloride was the best choice as the carrier electrolyte for the separation of anions in samples with high concentrations of salts. Considering that subterranean water is generally neutral, and sodium chloride as carrier electrolyte would give a wider linearity range than piperazine chloride, we chose sodium chloride as the carrier electrolyte in this experiment.

The negative peaks on the baselines in Fig. 4 could originate from the use of TTAB as an electroosmotic modifier. Bromide in TTAB made the carrier electrolyte solutions generate UV absorption at 214 nm, and the presence of chloride in samples displaced bromide and caused a decrease in absorbance as observed in Fig. 4. However, the reason for the much wider negative peaks in Fig. 4A and B than in Fig. 4C seemed to be more complicated, and may originate from electromigration dispersion caused by the different electrophoretic mobilities between chloride and the carrier electrolyte co-ion (sulfate). It is believed that when the electroosmotic flow modifier is converted into a transparent anion form, such as chloride, the negative peak will disappear.

By using sodium chloride with TTAB as a carrier electrolyte solution, the capillary electrophoretic separation of anions was achieved as demonstrated in Fig. 5. In order to increase the

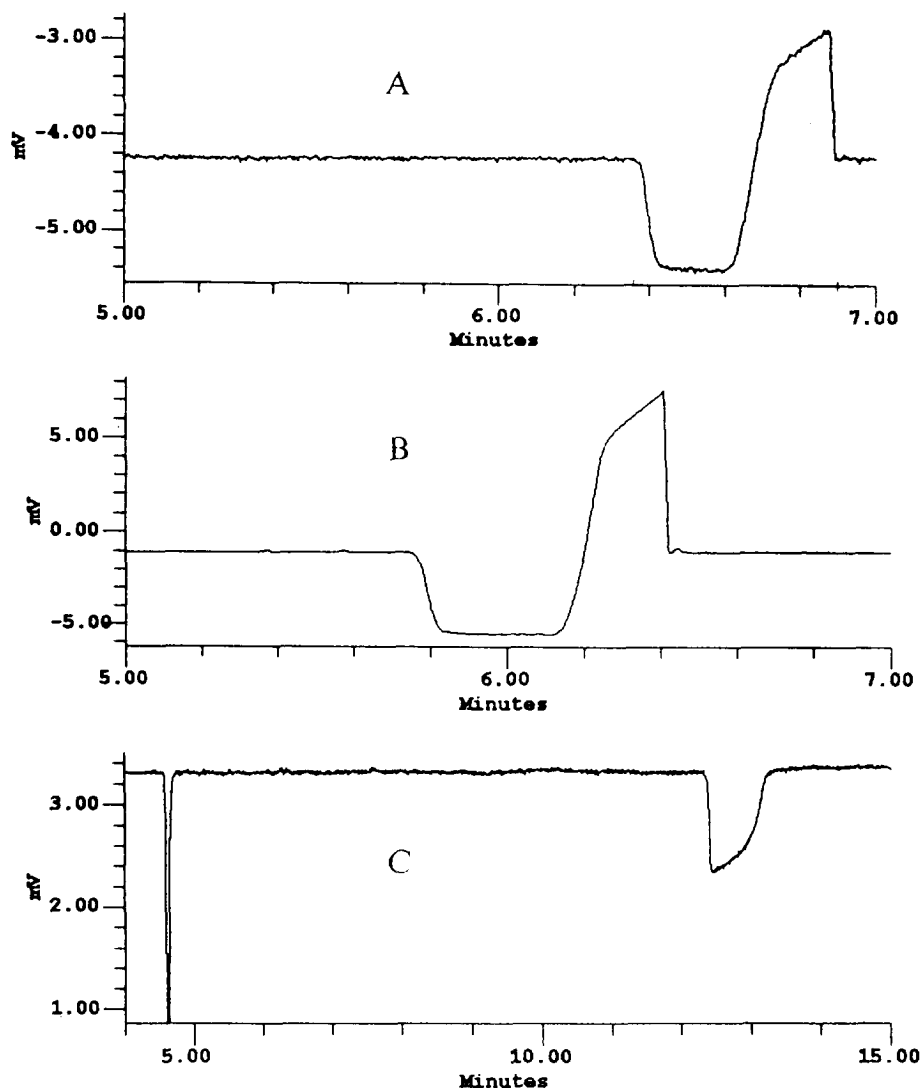


Fig. 4. Effect of 12.5 mM sodium chloride in the sample on the separation baseline. Carrier electrolyte solution: (A) 10 mM sodium sulfate with 0.5 mM TTAB; (B) 10 mM piperazine sulfate with 0.5 mM TTAB; (C) 25 mM sodium chloride with 2.0 mM TTAB.

resolution, 2.0 mM in addition to 0.5 mM TTAB was used. Five peaks belonging to bromide, iodide, nitrite, nitrate and thiocyanate were observed in the electrochromatograms when there was no sodium chloride in the sample (Fig. 5A–C). However, when there was high concentration of sodium chloride in the sample, the bromide peak disappeared owing to the negative peak caused by sodium chloride in the sample (Fig.

5D–F). It was clear that a successful separation depended on the ratio of sodium chloride concentration between the carrier electrolyte solution and the sample. When the concentration of sodium chloride in the carrier electrolyte solution was 25 mM, a successful separation of anions with 12.5 mM sodium chloride in the sample was achieved, except for bromide, but an unsuccessful separation of anions was obtained

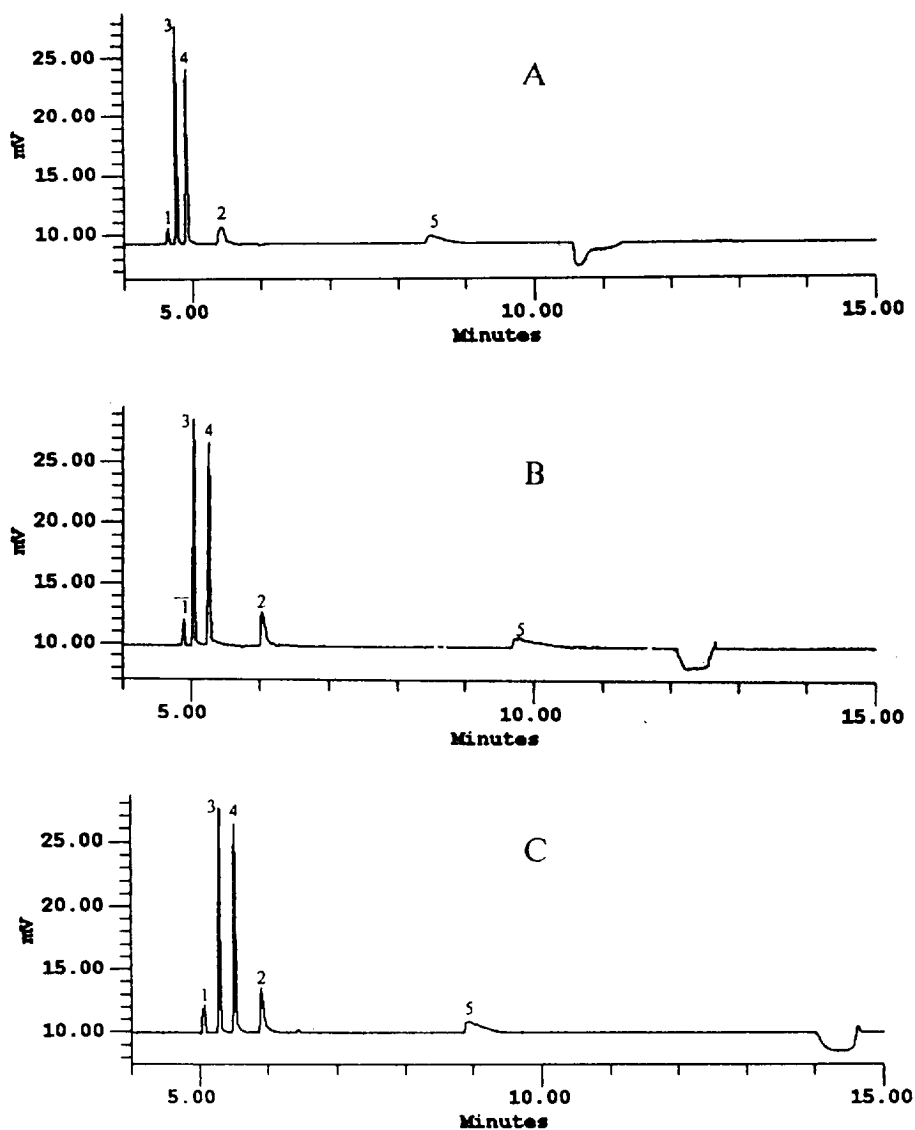


Fig. 5.

with 100 mM sodium chloride in the sample. This was because the peak height and width of anions depended on the ratio of sodium chloride concentration between the carrier electrolyte solution and the sample. Although the sodium chloride concentration in the sample was very high, its concentration in the carrier electrolyte solution was limited by Joule heating, so capillary electrophoresis of anions in samples with sodium chloride concentrations higher than 100

mM still remained a problem. It should be noted that high concentrations of sodium chloride in samples did not cause serious Joule heating problems in electrophoresis, which was concluded to be due to the slight current change caused by the high-concentration sodium chloride in the sample.

The detection limit was defined as the detectable concentration of an anion when the anion gave a peak twice as high as the noise ( $N$ ). For a



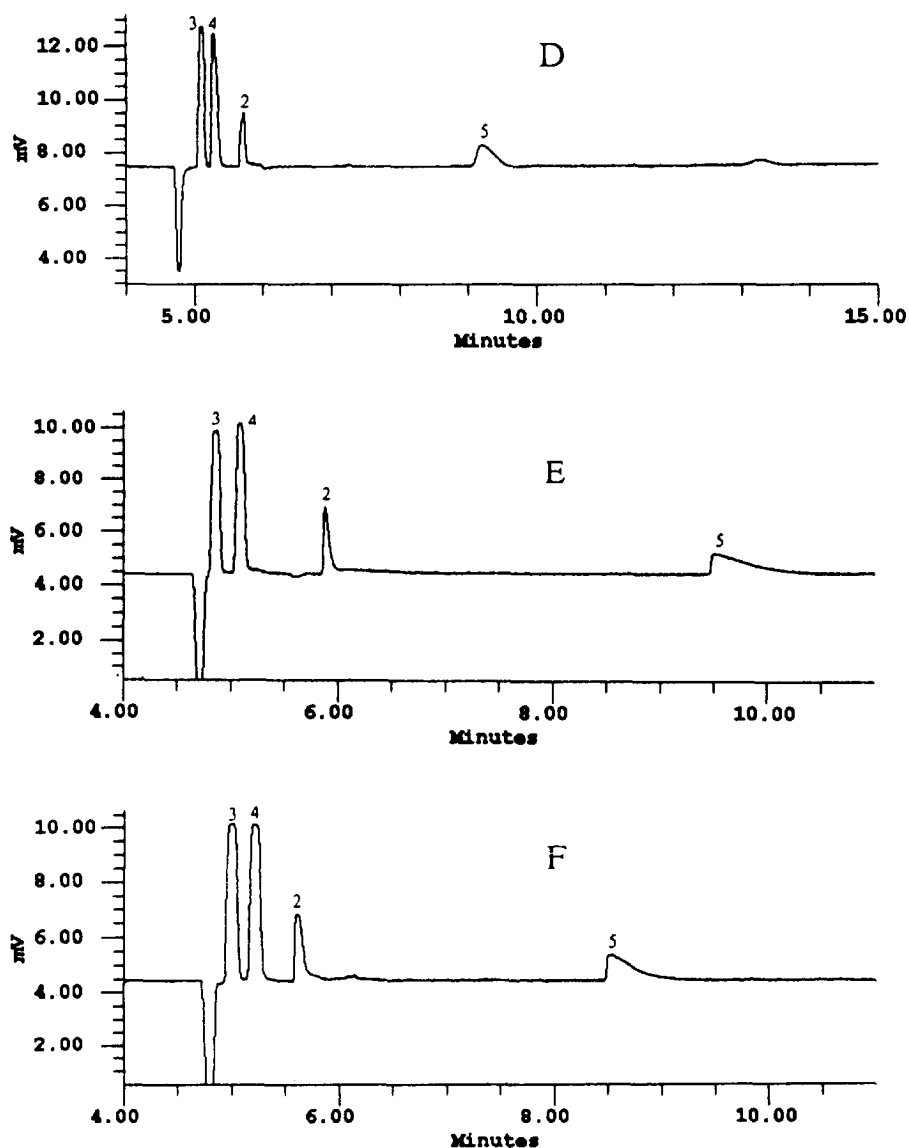


Fig. 5. Capillary electrophoretic separation of anions by using sodium chloride with 2.0 mM TTAB as carrier electrolyte solution. Ratio of sodium chloride concentration (mM) between the carrier electrolyte solution and sample: (A) 25:0; (B) 50:0; (C) 100:0; (D) 25:12.5; (E) 50:50; (F) 100:100. Peaks: 1 = bromide; 2 = iodide; 3 = nitrite; 4 = nitrate; 5 = thiocyanate. The concentration of each anion in the sample was 10 ppm.

defined change of anion concentration, its corresponding change in peak height was calculated from the slope ( $SL$ ) of the calibration graph for the anion between peak height and anion concentration from 1 to 40 ppm. The detection limit of each anion was calculated as  $2N/SL$  ( $N = 55 \mu\text{V}$ ) and results are given in Table 1 under

different conditions. It was observed that when there was no sodium chloride in the sample, the detection limit had an obvious tendency to decrease slightly when the concentration of sodium chloride in the carrier electrolyte solution from 25 to 100 mM (there was one exception). However, when the concentration of sodium

Table 1  
Detection limit of each anion under different conditions

Concentration of NaCl in carrier electrolyte solution (mM)	Concentration of NaCl in sample (mM)	Detection limit (ppb)				
		Bromide	Nitrite	Nitrate	Iodide	Thiocyanate
25	0	1724	130	148	1742	3644
50	0	1048	120	130	728	— <sup>a</sup>
100	0	1036	120	138	648	2982
25	12.5	— <sup>b</sup>	418	420	— <sup>c</sup>	— <sup>c</sup>
50	50	— <sup>b</sup>	420	390	— <sup>c</sup>	— <sup>c</sup>
100	100	— <sup>b</sup>	380	378	— <sup>c</sup>	— <sup>c</sup>

<sup>a</sup> Not measured.

<sup>b</sup> Not separated.

<sup>c</sup> Peaks showed strong tailing and the calibration graph between peak height and anion concentration was not linear.

chloride in carrier electrolyte solution was constant at 25, 50 or 100 mM, the detection limit increased with increasing concentration of sodium chloride in the sample.

The separation efficiency was described by the number of theoretical plates,  $n$ , which is calculated from  $n = 5.54(RT/WI)^2$ , where  $RT$  is the migration time and  $WI$  is the peak width at half-height. Theoretical plate numbers of bromide, nitrite and nitrate under different conditions were calculated and are given in Table 2. Theoretical plate numbers for iodide and thiocyanate are not given because their peaks showed strong tailing. Each value of theoretical plate number in Table 2 is the average of five values obtained with five mixed anion solutions

(1, 4, 10, 20 and 40 ppm) used to evaluate the calibration graphs, except the values with a superscript b, which are averages of five values obtained with five mixed anion solutions prepared for the purpose of peak identification in the electrophorograms as described under Experimental. It was observed from Table 2 that when the concentration of sodium chloride in the carrier electrolyte solution remained constant, an increased concentration of sodium chloride in sample resulted in a decreased theoretical plate number. When the concentration of sodium chloride in the sample remained at 12.5 mM, an increased concentration of sodium chloride in carrier electrolyte solution caused an increased theoretical plate number. However, when there

Table 2  
Effect of concentration of sodium chloride in carrier electrolyte solution and sample on separation efficiency

Concentration of NaCl in buffer (mM)	Concentration of NaCl in sample (mM)	Theoretical plate number $\times 10^4$		
		Bromide	Nitrite	Nitrate
25	0	22.27	20.60	15.50
	12.5	— <sup>a</sup>	1.99	2.05
50	0	24.34	18.20	13.85
	12.5	— <sup>a</sup>	5.78 <sup>b</sup>	6.10 <sup>b</sup>
	50	— <sup>a</sup>	2.14	2.34
100	0	17.61	17.79	16.56
	12.5	— <sup>a</sup>	13.60 <sup>b</sup>	11.24 <sup>b</sup>
	100	— <sup>a</sup>	1.64	1.70

<sup>a</sup> Peak not separated.

<sup>b</sup> See text.

was no sodium chloride in the sample, an increased concentration of sodium chloride in the carrier electrolyte solution sometimes caused a decreased theoretical plate number, which may be attributed to increased Joule heating.

The reason why different concentrations of salts in the samples caused different detection limits and separation efficiencies of an anion can be explained by the principle of electrostacking [17] as follows. Electrostacking occurs when the injected sample has a lower ionic strength than the carrier electrolyte solution. In this experiment, the sample and the carrier electrolyte solution contained the same salt (sodium chloride), so the length of the sample zone after electrostacking can be calculated by [17]

$$l_{ib} = l_{is} C_s / C_b \quad (1)$$

where  $C_b$  and  $C_s$  are the concentration of sodium chloride in the carrier electrolyte solution (background) and sample, respectively, and  $l_{ib}$  and  $l_{is}$  are the zone length of the sample ion in the carrier electrolyte solution and sample, respectively. Eq. 1 shows that the zone length of a sample ion in the carrier electrolyte solution is inversely proportional to the ratio of the sodium chloride concentrations in the carrier electrolyte solution and sample, and the peak width is also inversely proportional to this ratio whereas the peak height is proportional to this ratio. According to the definitions of separation efficiency and detection limit used above, different peak widths and peak heights resulted in different separation efficiencies and different detection limits, respectively. It had been considered theoretically that when  $l_{is}$  was small enough that the variance of a sample zone was controlled only by molecular diffusion, as described by Jorgenson and Lukacs [18], then the separated peak would have the same height and width regardless of the value of  $C_b/C_s$  and electrostacking. However, the experimental results showed that this is difficult and the injection plug length made a contribution to the variance of a sample zone. In this experiment,  $l_{is}$  was constant and calculated to be 3.3 mm, but  $l_{ib}$  decreased with decreasing  $C_s/C_b$ . As shown in Table 2, when  $C_s/C_b$  decreased from 100:100 through 12.5:100 to 0:100 and  $l_{ib}$

decreased from 3.3 through 0.41 to 0 mm (0 mm is a theoretical value; in practice  $l_{ib}$  would be affected by other factors and be greater than 0 mm), the separation efficiency increased from 16 400 through 136 000 to 177 900. That meant that even when the injection plug length was very small (theoretically 0 mm), it still contributed to the variance of a sample zone.

When determining anions in subterranean water, it is reasonable to choose 100 mM sodium chloride as the carrier electrolyte because it gave the lowest detection limit and could match samples with higher concentrations of sodium chloride. As different concentrations of sodium chloride in subterranean water could cause different peak heights with a constant concentration of an anion, the simplest approach to achieve accurate quantification was to use the standard addition technique. In this technique, a constant volume of a sample was added to a set of standard anion solutions separately and then diluted to the same volume for the purpose of quantification, so the ionic strengths of these solutions were the same and controlled by the sample. Therefore, although electrostacking has been proposed to be used as an on-column preconcentration technique [10,17,19–23], it caused quantification difficulties when a sample contained high concentrations of salts.

### 3.3. Reproducibility of migration time, peak height and peak area

The 10 ppm mixed anion standard solution was separated by using 10 mM sodium sulfate with 0.5 mM TTAB as the carrier electrolyte solution. The reproducibilities of migration time, peak height and peak area after five successive injections are given in Table 3.

With different concentrations of sodium chloride in the carrier electrolyte solution and sample, the reproducibilities of the migration times were calculated using five mixed anion solutions as used to evaluate the calibration graphs as described under Experimental and are given in Table 4. When an R.S.D. value in Table 4 is not marked with a superscript, it was calculated from

Table 3  
Reproducibility of migration time, peak height and peak area after five successive injections

Anion	R.S.D. (%)		
	Migration time	Peak height	Peak area
Bromide	1.49	2.02	11.02
Nitrite	1.51	0.82	2.15
Nitrate	1.49	0.72	1.98
Iodide	1.52	2.40	6.49
Thiocyanate	1.78	2.16	8.03

five migration times, when it has a superscript c, it was calculated from four migration times, and when it has a superscript d, it was calculated from three migration times. Because of detection limit limitations, iodide and thiocyanate were not detected at low concentrations. It was observed

from Tables 3 and 4 that the reproducibility of the migration times of all peaks was not very good. When the migration times of anions were close to each other, it was difficult to identify a peak only from the migration time when a real sample was separated. Therefore, peak identification was supplemented by analysing five anion mixture solutions containing 10 ppm of each anion with additional individual anions as described under Experimental.

When the concentration of sodium chloride in sample was 12.5 mM, the reproducibilities of peak height and peak area at different concentrations of sodium chloride in the carrier electrolyte solution were calculated from five values obtained with five mixed anion solutions used for the identification of each peak in electrophorograms under the same conditions, and are given in Table 5. The RSDs in Table 5 are

Table 4  
Reproducibility of migration time with different concentrations of sodium chloride in the carrier electrolyte solution and sample

Concentration of NaCl in carrier electrolyte solution (mM)	Concentration of NaCl in sample (mM)	R.S.D. (%)				
		Bromide	Nitrite	Nitrate	Iodide	Thiocyanate
25	0	2.81	2.73	3.14	2.40 <sup>c</sup>	2.15 <sup>c</sup>
50	0	1.36	1.40	1.57	1.85 <sup>c</sup>	— <sup>a</sup>
100	0	1.82	2.07	1.97	2.45 <sup>c</sup>	3.16 <sup>c</sup>
25	12.5	— <sup>b</sup>	1.65	2.93	1.50 <sup>d</sup>	2.36 <sup>c</sup>
50	50	— <sup>b</sup>	0.22	0.05	0.63 <sup>d</sup>	1.81 <sup>c</sup>
100	100	— <sup>b</sup>	0.51	0.79	0.19 <sup>d</sup>	0.98 <sup>c</sup>

<sup>a</sup> Not measured.

<sup>b</sup> Peak not separated.

<sup>c</sup> See text.

<sup>d</sup> See text.

Table 5  
Reproducibility of peak height and peak area with a concentration of sodium chloride in the sample of 12.5 mM

Basis	Concentration of NaCl in carrier electrolyte solution (mM)	R.S.D. (%)			
		Nitrite	Nitrate	Iodide	Thiocyanate
Peak height	25	8.60	8.26	14.51	15.41
	50	6.71	6.04	9.89	17.13
	100	3.40	5.88	14.07	18.95
Peak area	25	5.68	3.50	15.03	23.93
	50	3.91	3.00	8.10	7.85
	100	5.94	5.93	6.27	9.39

not very good; the R.S.D.s for iodide and thiocyanate are higher than those for nitrite and nitrate, which is attributed to peak tailing.

Comparing Table 3 with Tables 4 and 5, it was found that the reproducibility of migration times in Table 3 was better than that in Table 4 in most instances, and the reproducibility of peak height and peak area in Table 3 was better than that in Table 5. It should be noted that more factors affect the reproducibility in Tables 4 and 5 than in Table 3, but the reproducibility in Tables 4 and 5 approached more closely the situation of quantification.

From the R.S.D.s of migration time, peak height and peak area shown in Tables 3–5, it is seen that the separation conditions studied in this work were not satisfactory for the determination of anions in subterranean water. An improved separation for the determination of anions in subterranean water has now been accomplished, and will be published in the near future.

#### 4. Conclusions

The selection of a carrier electrolyte co-ion with an electrophoretic mobility close to those of anions in the sample is necessary in CIE, not only when using indirect UV detection but also when using direct UV detection.

An unsuitable anion form of the electroosmotic modifier would cause negative peaks on the separation baselines when samples contain high concentrations of salts, and the separation could be adversely affected.

Although electrostacking has been proposed as an on-column preconcentration technique in the CIE determination of anions, it caused quantification difficulties when the sample contained high concentrations of salts. An increased concentration ratio of sodium chloride between the sample and carrier electrolyte solution resulted in lower and wider peaks of detected anions, and correspondingly higher detection limits and a lower separation efficiency for the anion.

From the R.S.D.s of migration time, peak height and peak area shown in Tables 3–5, it is

concluded that the separation conditions studied in this work were not satisfactory for the determination of anions in subterranean water.

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