# Separation and Direct Photometric Determination of Inorganic Anions by Capillary Zone Electrophoresis Using Suppressed Electroosmosis

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A simple, sensitive and separative method for the photometric determination of inorganic anions was developed on the basis of suppressed electroosmosis by using a common silica capillary and a simple migrating solution. During the analysis of analyte anions by capillary zone electrophoresis, electroosmotic flow in a silica capillary was suppressed by using low-pH migrating solutions containing sodium sulfate. The stacking effect of sulfate ion was utilized for analyte concentration. Four kinds of inorganic analyte anions examined were detected in sharp signals, and the separation of a nitrate and a nitrite ion was improved by using the low-pH migrating solution with no decrease in detection sensitivity. Calibration graphs for nitrate and nitrite ions showed good linearity in the concentration ranges from about $10^{-5}$ to $10^{-4}$ mol dm$^{-3}$, with the detection limit for nitrate ion $4\times10^{-6}$ mol dm$^{-3}$. Separations of organic anions, such as aromatic sulfonate and carboxylate ions, were also examined; many of them were well separated by the proposed migrating solution. Those organic anions did not interfere with the determination of the inorganic anions.

**Keywords** Capillary zone electrophoresis, inorganic anion, direct photometric detection, suppressed electroosmosis, stacking effect

In a common capillary electrophoresis using a silica capillary, separation and detection of small-molecular anions, such as halides, sulfate, carbonate and organic anions, are difficult, because such small anions can migrate very fast in the direction opposite to an electroosmotic flow (EOF). In order to solve this essential difficulty, some methods have been developed. They are based on lowering or reversing EOF effectively with a dynamic coating of the surface of silica capillary with a long-chained quaternary ammonium ion[1,2,6], using a polymer-coated capillary[3,4], or with the addition of macrocycle[5] or butanol[6] in the migrating solution. In most cases, analyses of anionic substances in capillary electrophoresis have been done by an indirect photometric method, using a chromate ion.[6-17] Such an analytical system is now called "Capillary Ion Analysis".[18] As a photometric reagent, phthalate[13,17,19], 2,3-naphthalene-dicarboxylate[17], and pyromellitate[17,20] ions have been investigated. However, the methods with a coating reagent and an indirect photometric reagent possess undesirable problems, such as the noisiness and unstability of baseline, less reproducibility, and low sensitivity of detection, which result in a poorer detection limit of analytes. Such undesirable problems are caused by the fact that the background absorbance of an indirect photometric reagent is large and the dynamic coating is unstable and less reproducible.

The authors considered that less light-absorbing migrating solution containing no dynamic coating reagent should stabilize the baseline and enhance the detection limits. To reduce and control the electroosmotic flow, as well as to utilize the effect of stacking reagent, the effects of pH and ionic strength of the migrating solution were examined. Analyte anions were measured by a direct photometric detection. Effective separation and a reproducible and sensitive detection method of inorganic anions was achieved in this study.

## Experimental

### *Apparatus*

An Applied Biosystems 270A-HT Capillary Electrophoresis (CE) System with a UV-detector was used. A fused silica capillary was purchased from GL Sciences. It was attached to the CE system after being cut to that required length and after a light-transmission detection window was made by burning a small portion of polyimide coating. The capillary used was 72 cm in total length, 50 cm in effective length to the UV detector, and had a 50 μm inner diameter. A Corning Ion Analyzer M-250, calibrated with standard pH solutions, was used for pH measurements.

*Reagent*

The components of migrating solutions, such as sodium monochloroacetate, phosphoric acid, potassium dihydrogenphosphate, and sodium sulfate, were purchased from Wako Pure Chemical Industries, Ltd. Inorganic analyte anions, such as iodide, thiosulfate, nitrate, and nitrite ions, were all sodium salts of guaranteed reagent grade, and were also purchased from Wako Pure Chemical Industries, Ltd. Organic analyte anions were purchased from Tokyo Kasei Kogyo Co., Ltd. All these reagents were used without further purification and were dissolved in purified water. The water used had been deionized and distilled, followed by purification by Milli-Q Labo System (Millipore).

*Capillary electrophoresis measurement*

A migrating solution was poured into the capillary before each measurement. The migrating solutions contained buffer components, such as monochloroacetate, phosphate, or acetate buffer with a required pH value, and sodium sulfate at the concentration ranges up to $3\times10^{-2}$ mol dm$^{-3}$. Sample solutions were injected on a reduced pressure hydrodynamically from the cathodic end. The rate of the injection volume was about 3 nl/s. High voltages were applied on the capillary, and CE separations were made. Anionic analytes were directly detected photometrically at 220 nm on the anodic end. The electropherograms, as well as migration times, peak heights, and peak areas, were recorded by a Hitachi D-2500 Chromato-Integrator.

In a standard procedure, a migrating solution containing $1\times10^{-2}$ mol dm$^{-3}$ of monochloroacetate buffer (pH 3.2) and $3\times10^{-2}$ mol dm$^{-3}$ of sodium sulfate was used. Sample solutions were injected for 3 s, which corresponded to the injection volume of about 9 nl. A voltage of 20 kV was applied, and analyte ions were separated and detected. The capillary was kept in a room thermostated at 30.0°C during the measurement.

## Results and Discussion

Separations of four kinds of inorganic anions; iodide, thiosulfate, nitrate, and nitrite ions, have been examined in the absence of and in the presence of sodium sulfate. The electropherograms obtained are shown in Fig. 1. Nitrite ion could not be detected within 10 min in the absence of $Na_2SO_4$, whereas it could be detected in the presence of $Na_2SO_4$ within 7 min of the migration time. Migration time for other anions were also shortened in the presence of $Na_2SO_4$. Peak shapes for those anions were improved by the addition of $Na_2SO_4$.

*Effect of pH and buffer composition of migrating solution*

To investigate the change in migration time, the velocity, $v$, was measured. To monitor the electroosmotic flow, an aqueous 3% ethanol solution was used as a monitoring marker.

The pH values of the migrating solutions were varied;

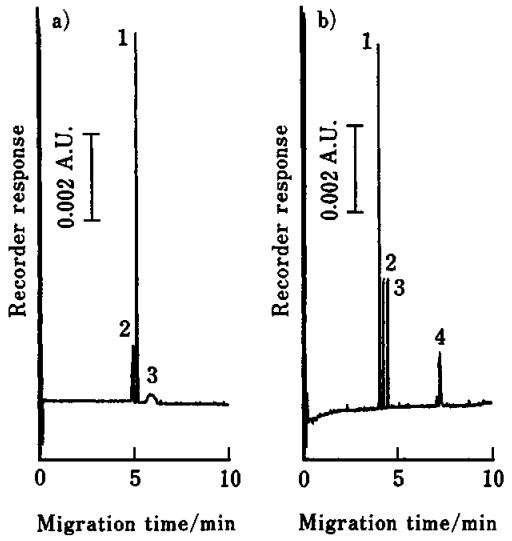


Fig. 1 Typical electropherograms for inorganic anions in the absence of and in the presence of sodium sulfate in the migrating solutions. Concentration of each analyte anion: $1\times10^{-4}$ mol dm$^{-3}$. Migrating solution: $1\times10^{-2}$ mol dm$^{-3}$ monochloroacetate buffer+$Na_2SO_4$ (pH 3.2). Concentrations of sodium sulfate in the migrating solutions: a) none, b) $3\times10^{-2}$ mol dm$^{-3}$. CE conditions: 20 kV, 30°C, 220 nm. Injection time: 3 s (9 nl). 1, $I^-$; 2, $S_2O_3^{2-}$; 3, $NO_3^-$; 4, $NO_2^-$.

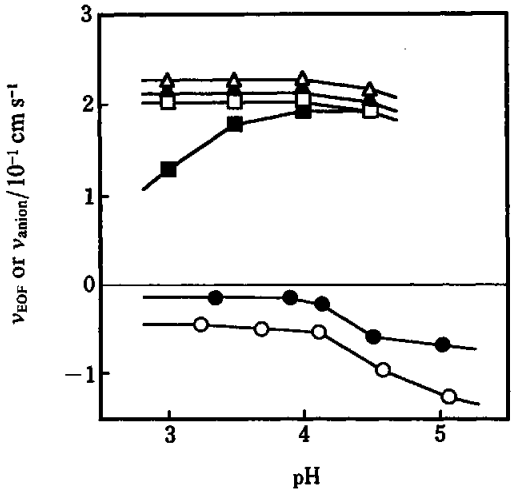


Fig. 2 Effect of pH on the migration velocity. Velocity of anions, including electroosmotic velocity, was measured in the presence of $3\times10^{-2}$ mol dm$^{-3}$ $Na_2SO_4$. A positive value of velocity corresponds to the migrating direction toward the anodic end. △, $I^-$; ▲, $S_2O_3^-$; □, $NO_3^-$; ■, $NO_2^-$. Velocity of EOF: ○, in the absence of $Na_2SO_4$; ●, in the presence of $Na_2SO_4$. Experimental conditions, except for pH, are the same as in Fig. 1(b).

the results obtained are shown in Fig. 2. The velocity change of EOF without sodium sulfate was quite similar to the one already reported.[21] The velocity of EOF decreased at every examined pH when $Na_2SO_4$ was

present in the migrating solutions. Migration velocities for iodide, thiosulfate, and nitrate ions were almost identical in the pH region from 3 to 4, and decreased in the region above pH 4. This is because the velocity of EOF in an inverse direction was decreased, as mentioned above. The migration velocity for nitrite changed dramatically even at low pH conditions. This change in migration velocity for nitrite can be attributed to the protonation of nitrite ions. The $pK_a$ value for an $HNO_2/NO_2^-$ couple is about 3.2, and partial protonation of nitrite ions occur and their apparent charges decrease. Therefore the velocity for the nitrite ion can decrease with decreasing the pH of the migrating solutions. The effective separation between a nitrate and a nitrite ion could not be attained with migrating solutions of pH above 4.5, because these two ions are very similar in ionic mobility. One of the advantages of the present method is to enhance the separation efficiency by using an acidic migration buffer.

Another buffer component of the migrating solution was also investigated by using a $1\times10^{-2}$ mol dm$^{-3}$ phosphate buffer (pH 3.2). The migration time and peak heights for analytes were almost identical and the baselines were equally stable in both buffer components, monochloroacetate and phosphate buffers.

*Effect of sodium sulfate concentrations in the migrating solution*

The effect of sodium sulfate concentrations has been examined; the results obtained are shown in Fig. 3. The velocity of EOF, $v_{EOF}$, decreased with an increase in the concentration of sodium sulfate. The change in $v_{EOF}$ should be attributed to an increased ionic strength, which can reduce the electric double layer near the internal surface of silica capillary. A $\zeta$ potential should be decreased with an decrease in electric double layer. The change in EOF velocity induced by the addition of $Na_2SO_4$ is a phenomenon similar to the one caused by the increase in buffer concentrations.[22]

The order of migration velocity and migration time for iodide and thiosulfate ion reversed at the concentrations of $Na_2SO_4$ above $1\times10^{-2}$ mol dm$^{-3}$. This was probably caused by the difference in the change in the hydration of the ions. The use of relatively high concentrations of sodium sulfate was very useful for the improvement of the peak shape, the mutual separation and for saving the analytical time.

Figure 4 shows apparent plate numbers of analyte anions as a function of $Na_2SO_4$ concentrations. The apparent plate numbers for the anions, as well as the peak heights, increased with increasing concentrations of $Na_2SO_4$. The apparent plate numbers increased up to about $10^5$. This increase in the plate numbers was attributed to the stacking effect occurring in the initial state of CE in the sample zone interposed by the migrating solution of a higher ionic strength. The concentration factor for anions at the sample zone-migrating solution boundary was increased about from 10-fold to 150-fold by the addition of sodium sulfate.

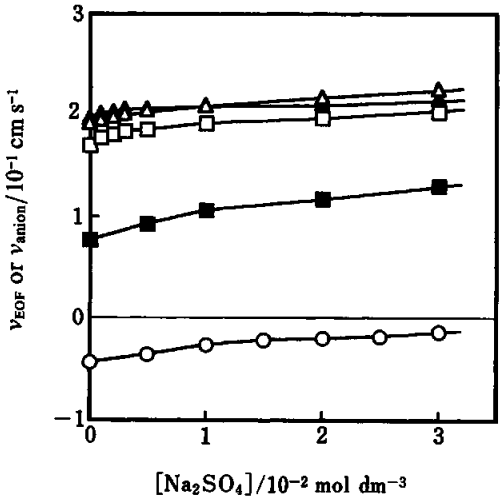


Fig. 3 Change in migration velocity as a function of sodium sulfate concentrations. △, $I^-$; ▲, $S_2O_3^-$; □, $NO_3^-$; ■, $NO_2^-$; ○, EOF. Experimental conditions, except for $Na_2SO_4$ concentrations, are the same as in Fig. 1(b).

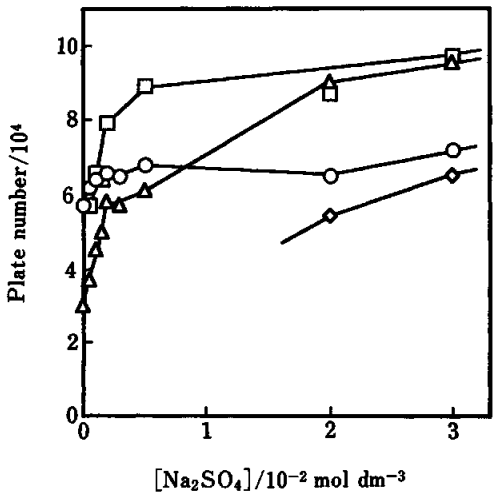


Fig. 4 Effect of sodium sulfate concentrations in the migrating solution on the apparent plate number of analyte signals. Experimental conditions are the same as in Fig. 1(b). ○, $I^-$; △, $S_2O_3^-$; □, $NO_3^-$; ◇, $NO_2^-$.

However, heat generation increased with an increase in the ionic strength, analyte zones were broadened during the separation, and the plate numbers did not so much increase. In the present CE procedure, the effect of broadening caused by the heat generation was considered to be smaller than the increase in concentration factor.

The apparent plate number for iodide ion did not increase so much with an increase in the concentration of $Na_2SO_4$. This is because it possesses large electrophoretic mobility and can be well stacked even in the absence of $Na_2SO_4$. Therefore, the stacking effect of $Na_2SO_4$ is more useful for the anions which possess small

electrophoretic mobility.

Sodium chloride was also examined as a stacking reagent, instead of sodium sulfate. The use of $6\times10^{-2}$ mol dm$^{-3}$ NaCl also realized sharp signals for analyte anions. However, the electric current became larger, the stability of the background was worse, and the peaks were broader than those with $3\times10^{-2}$ mol dm$^{-3}$ $Na_2SO_4$.

*Effect of applied voltage and sample size*

How applied voltage affected the separation was examined. The apparent mobility for anions increased along with the increase in the voltage. For example, the apparent mobility for nitrate ion increased about 20% in the voltage change from 15 to 30 kV. This result indicates that the temperature in the capillary tube increased with an increase in voltage. A high voltage was not adequate for the improvement of separations and sensitivity.

Effect of sample size was also examined. Peak heights increased almost proportionally with an increase in the injection time. The plate numbers, however, decreased gradually with an increase in injection time. For example, the plate number for nitrate ion decreased to 60% when the injection time increased from 1 to 5 s.

*Calibration graphs and detection limits*

Calibration graphs were obtained by the standard procedure described in the experimental section. The graphs for iodide, nitrate, and nitrite ions showed good linearity in the concentration ranges from about $10^{-5}$ to $10^{-4}$ mol dm$^{-3}$ by using peak height. Detection limits corresponding to the signal to noise ratio of 3 were $1\times10^{-6}$, $4\times10^{-6}$, and $8\times10^{-6}$ mol dm$^{-3}$ for iodide, nitrate, and nitrite ions, respectively. The detection limit for nitrate, $4\times10^{-3}$ mol dm$^{-3}$, for example, was almost equal to that in ref. 17, and better than those in refs. 3 and 23, applying indirect photometric methods, although the injection volume in this study was smaller than that in ref. 17.

In the present CE system, detection limits will be further improved by using a capillary with a larger internal diameter and a longer injection time, as the separation of each analyte was good. For example, the detection limits of anions could be lowered to about one fourth by using a 75 μm i.d. capillary and a sampling time of 6 s; that is, anions of a $10^{-7}$ mol dm$^{-3}$ level could be detected.

*Reproducibility of peak height and migration time*

Reproducibility of peak heights and migration times was examined for each analyte anion. The values of relative standard deviations (RSD) for 10 measurements are summarized in Table 1. These results show that the RSDs of the peak heights are almost the same as those in ion chromatography, and they are all satisfactory in practical use.

*Application to the separation and detection of organic anions*

The proposed method was applied to the separation and detection of aromatic sulfonate compounds; the typical electropherogram is shown in Fig. 5. Most of the anions were well separated, but 1,5- and 2,6-naphthalenedisulfonates could not be separated. This is because both anions possess the same molecular weight and charge. The separation of position isomers of such as naphthalenedisulfonates must be carried out by some modification of the migrating solutions. The use of ion association reagent will be a good modification.[24]

Separation of carboxylate compounds was also examined in this system, where the phosphate buffer was used as a migrating solution instead of monochloroacetate buffer (Fig. 6). Eight kinds of anions were detected and well separated. However, seven other kinds of carboxylate ions could not be detected in the separation

Table 1 Results in reproducibility test

| Anion[b] | RSD[a], % | |
|---|---|---|
| | Peak height | Migration time |
| $I^-$ | 2.60 | 0.43 |
| $S_2O_3^{2-}$ | 2.52 | 0.45 |
| $NO_3^-$ | 1.97 | 0.44 |
| $NO_2^-$ | 1.68 | 1.56 |

a. Values obtained in 10 measurements.
b. Concentrations are $1\times10^{-4}$ mol dm$^{-3}$.

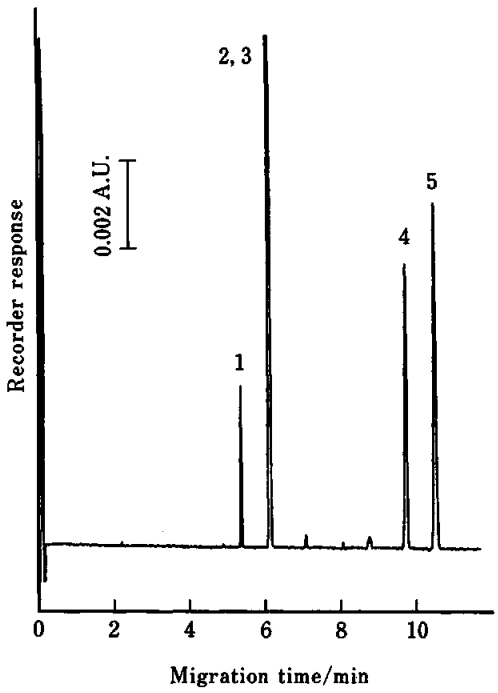


Fig. 5 A typical electropherogram for aromatic sulfonates. Sample solution contains five kinds of $1\times10^{-4}$ mol dm$^{-3}$ organic anions. Experimental conditions are the same as in Fig. 1(b). 1, 1,3-benzenedisulfonate; 2, 1,5-naphthalenedisulfonate; 3, 2,6-naphthalenedisulfonate; 4, *p*-toluenesulfonate; 5, *p*-ethylbenzenesulfonate.

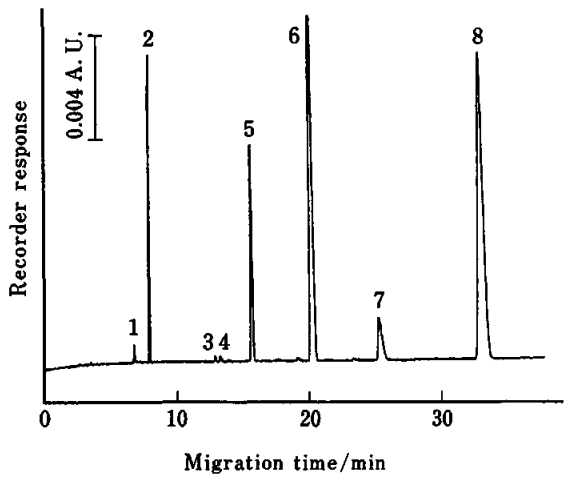


Fig. 6 A typical electropherogram for carboxylates. Sample: fifteen kinds of $1\times10^{-4}$ mol dm$^{-3}$ anions. Migrating solution: $1\times10^{-2}$ mol dm$^{-3}$ phosphate buffer (pH 3.2)+$3\times10^{-2}$ mol dm$^{-3}$ $Na_2SO_4$. Detection wavelength: 210 nm. Other experimental conditions are the same as in Fig. 1(b). 1, oxalate; 2, maleate; 3, citrate; 4, propionate; 5, phthalate; 6, 2,3-naphthalenedicarboxylate; 7, terephthalate; 8, isophthalate. Succinate, butyrate, gluconate, malate, valerate, tartrate, and acetate could not be detected.

time (less than 35 min) and in the sensitivity at 210 nm. The migration times of the carboxylates were affected by the change in their apparent charges, associated with their protonation. In the carboxylate anions, the small difference in acid dissociation of weak acids was favorably used for the improvement of separation without any modifier in the migrating solutions. For example, position isomers of three phthalate compounds were well separated. The migration order of such analytes agreed with the order of p$K_a$ values; that is, a longer migration time was required for the detection of the compound possessing larger p$K_{a1}$ value.

The electropherograms in Figs. 5 and 6 also indicate that such organic anions as sulfonates and carboxylates do not interfere with the analysis of the inorganic anions.

In conclusion, a rapid and sensitive analysis of inorganic and organic anions has been achieved by using low-pH migrating solution containing a stacking reagent. The proposed migrating solution is very simple compared with those already reported using a dynamic coating reagent and an indirect photometric reagent. The migrating solution used in this system can stabilize the baseline. Although the proposed analytical system can be used only for UV-absorbing analytes, it is very useful for nitrate and nitrite ions in environmental samples.

This work was financially supported by the Okayama Foundation for Science and Technology.

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(Received March 14, 1996)
(Accepted May 10, 1996)