Simultaneous Determination of Inorganic Anions and Cations in Water and Biological Samples by Capillary Electrophoresis with a Capacitive Coupled Contactless Conductivity Detector Using Capillary Filling Method

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An analytical method for concurrent analysis of inorganic anions and cations has been developed using a capillary electrophoresis (CE)–capacitively coupled contactless conductivity detector (C4D) system. Although hydrodynamic and electrokinetic injection techniques have been widely used in CE, we employed a capillary filling method (CFM) for the analysis of inorganic ions. The procedure is relatively simple and has the advantage that CMF does not require pressure control and vial exchange. Three anions (chloride, sulfate, nitrate) and five cations (ammonium, potassium, sodium, magnesium, calcium) were successfully separated and detected at ppm levels within 80 s using a 9 mM histidine/15 mM malic acid (pH 3.6) containing 50 mM N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate as background electrolyte. Applying this analytical condition, the electroosmotic flow is negligible and anions and cations were migrated concurrently to different polarities according to their electrophoretic mobility. Obtained raw data showed stepwise increases in detected conductivity due to the migration of sample components, which expresses as peak profiles by differentiation of electropherograms. The RSD values of the peak area and migration times for the anions and cations were satisfactory and were less than 5.15 and 2.04%, respectively. The developed method was applied for the analysis of inorganic anions and cations in commercial mineral waters, tap water, urine, and exhaled breath condensate. These results indicate that the CE-C4D system with CMF is suitable for the rapid analysis of inorganic anions and cations in various samples.

Keywords Capillary electrophoresis (CE), capacitively coupled contactless conductivity detector (C4D), capillary filling method (CMF), water analysis

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between sample components and background electrolyte (BGE) was established in the middle of the separation channel, and an electric field was applied to drive the separation of the charged analytes in the sample. Each analyte was detected as a boundary between the zone containing that analyte (sample side) and the zone without that analyte (BGE side). Gradient elution moving boundary electrophoresis (GEMBE) is a continuous injection separation technique performed using a simple miniaturized fluidic geometry consisting of two fluid reservoirs; one for the sample to be interrogated and another containing BGE solution. With GEMBE, analytes are detected as moving boundaries, in a manner similar to conventional MBE. By applying voltage, sample components move to the electrode of opposite charge and their separation generated a stepwise signal profile. The differentiation of stepwise output yields the familiar electropherogram. The GEMBE is an extremely robust analytical method, delivering quantitative results with minimal sample preparation across a broad range of BGE solution conditions and complex sample matrices, but this method needs pressure-driven counter-flow opposing electroosmosis. In addition, the gradual decrease of applied pressure is required for non-linear and step by step, and this control of pressure is very difficult.

In this work, we describe a novel CE-C4D method for concurrent determination of cations and anions using CFM. The procedure is relatively simple and has the advantage that CFM does not require pressure control. In the adopted analytical condition, the electroosmotic flow is negligible and anions and cations were migrated concurrently to different polarities according to their electrophoretic mobility. Obtained raw data shows stepwise increases in detected conductivity due to the migration of the sample, and the amount of sample components automatically expressed as a series of peaks by differentiation of the stepwise profile. The method is simple and it needs only filling the capillary with the sample and applying high voltage to both capillary ends. The developed method was applied for the analysis of inorganic anions and cations in commercial mineral waters, tap water, urine, and EBC.

**Experimental**

**Reagents and chemicals**

Ammonium sulfate, potassium chloride, sodium chloride, magnesium sulfate, calcium chloride, urea and calcium nitrate were obtained from Nacalai Tesque (Tokyo, Japan). Dodecyl-N,N,N,N-diethyl-1-ammonio-1-propanesulfonate (DDAPS) was obtained from Sigma-Aldrich Japan K.K. (Tokyo, Japan). 18-Crown-6-ether was obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). 1-Histidine was obtained from Kishida Chemical Co., Ltd. (Osaka, Japan). Malic acid was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The ultrapure water used in the CE experiments was obtained from a Milli-Q system of Merck Millipore (Bedford, MA). Other reagents and solvents were of the highest commercial grade. The commercial mineral waters were purchased from a local supermarket. A prepared artificial urine solution consisting of 2.5% (w/v) urea, 0.1% (w/v) ammonium sulfate, 0.12% (w/v) potassium chloride, 0.85% (w/v) sodium chloride, 0.05% (w/v) magnesium sulfate, and 0.05% (w/v) calcium chloride was used for separating a sample of anion group and cation group by C4D. The buffer concentration in the separation buffer solution and further optimized BGE experiments indicated that good separation of anions and cations (data was not shown). However, mobility of Ca2+ is very close to that of Mg2+. The lengths of the capillaries

**Experimental design**

Figure 1 portrays the experimental design of sample filling CE-C4D. In this method, all separations were performed in 30 mM malic acid containing 100 mM DDAPS, 3 mM of 18-crown-6-ether and 18 mM L-histidine (pH 3.6) as BGE. The DDAPS has a strong effect on the suppression of EOF and improves anion resolution. 18-Crown-6-ether is often used to improve cation resolution. Introduction of BGE containing DDAPS to the capillary causes dynamic adsorption and dramatically decreases EOF. Then, a sample of anions and cations dissolved in BGE is introduced into the entire capillary (Fig. 1(a)). Next, both ends of the capillary are immersed in BGE and the separation voltage is applied (Fig. 1(b)). Anionic and cationic sample components quickly moved to the anode and cathode, respectively based on their size-to-charge ratio. The boundaries broaden over time and the boundaries move independently and are separated because they move at different speeds and passed through the C4D detector. Then, the discontinuity in the detected signal profile (raw electropherogram) separates the anions and cations according to each electrophoretic mobility (Fig. 1(c)). The obtained raw electropherograms are automatically converted to differentials by the detection system and yields the familiar peak-shaped electropherogram (Fig. 1(d)). The composition of the separation buffer solution and further experimental conditions have been optimized from the points of view of the anion and cation separation. The preliminary optimized BGE experiments indicated that good separation of anions could be achieved using this composition of buffer and continuous sample introduction method. We applied this BGE for separating a sample of anion group and cation group by CFM and found that this BGE is suitable for separation of anions and cations (data was not shown). However, mobility of Ca2+ is very close to that of Mg2+. The lengths of the capillaries

**Results and Discussion**

The sampler for collection of EBC was constructed according to Kubán group’s work with slight modification. A 15-mL centrifuge polypropylene tube was kept in ethanol in a low-temperature water tank at –40°C. A straw with o.d. 6 mm and wall thickness 0.2 mm was used to exhale the air through the sampler. After the EBC sampling was completed, the 15 mL centrifuge polypropylene tube was centrifuged at 5000 rpm, then transferred to the sample vial for CE analysis.
were optimized in order to adjust the mobilities for Ca$^{2+}$ and Mg$^{2+}$ and to decrease their lengths to that enabling fast separations and baseline separations of analytes. In this CE system, C$^4$D head stage could be positioned to 11 – 22 cm from the inlet of the capillary. Accordingly, the detection position was set at 11, 15, 20 and 22 cm from the inlet and was used for cations, while for anions, positions at 24, 20, 15, 13 cm to the detector was used. Ca$^{2+}$ and Mg$^{2+}$ could not separate at the effective length of 11, 15 cm, while resolution of Ca$^{2+}$ and Mg$^{2+}$ was completely achieved over 20 cm of the effective length. We decided to use the capillary of 35 and 20 cm to the detector for cations, while for anions we selected 15 cm to the detector. We used these separation conditions for the separation of 10 ppm of SO$_4^{2-}$, NO$_3^{-}$, NH$_4^{+}$, K$^+$, Ca$^{2+}$, Mg$^{2+}$, 20 ppm of Na$^+$, and 40 ppm of Cl$^-$.

Figure 2 shows the separation of those anions and cations using 35 cm capillary at 30 kV. Peaks were identified by spiking the sample solution with each anion or cation.

We also investigated the repeatability of the peak area and migration times, linearity range, and LODs of eight anions and cations. The results are summarized in Table 1. It can be seen that the repeatability of the peak area and migration times for the anions and cations were satisfactory and were less than 5.15 and 2.04%, respectively. The linearity ranged from 1 to 300 ppm and the LOD (corresponding to a signal-to-noise ratio of 5) for these anions and cations were 0.4 or 0.6 ppm. These analytical parameters were almost the same as in previous work, aimed at measuring anions and cations separately utilizing the same C$^4$D detector. Inter-day repeatability of migration time and peak area was also investigated ($n = 3$) and was less than 4.38 and 6.20%, respectively. Our method is useful for decreasing the total analysis time.

**Application to the analysis of ions in commercial mineral water and tap water**

The developed method enables complete separation of the eight model anions and cations with high reliability. We applied this method for the analysis of ions in commercial mineral water and tap water. For analysis of the mineral waters, each aliquot taken from the bottle was diluted with ultrapure water to 5 times with concentrated BGE. Figure 3 shows the electropherograms for the hard mineral water (Fig. 3A), soft mineral water (Fig. 3B) and tap water in Osaka (Fig. 3C). Some peaks

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**Table 1**  Analytical parameters of the developed CE-C$^4$D method for simultaneous determination of inorganic anions and cations ($n = 6$)

|       | Cl$^-$ | SO$_4^{2-}$ | NO$_3^{-}$ | NH$_4^{+}$ | K$^+$ | Na$^+$ | Ca$^{2+}$ | Mg$^{2+}$ |
|-------|--------|-------------|------------|-----------|-------|--------|-----------|-----------|
| RSD (%) Peak area | 5.10   | 3.69        | 4.66       | 3.82      | 3.94  | 5.15   | 4.82      | 2.18      |
| RSD (%) Migration time | 2.04   | 2.04        | 1.74       | 1.37      | 1.38  | 0.834  | 0.956     | 0.716     |
| Calibration range (ppm) |       |             |            |           |       |        |           |           |
| $r^2$  | 0.9970 | 0.9961      | 0.9963     | 0.9983    | 0.9990 | 0.9979 | 0.9980    | 0.9986    |
| LOD (ppm) | 0.4    | 0.6         | 0.6        | 0.4       | 0.6   | 0.6    | 0.4       |           |
observed at 30 to 100 s in all electropherograms were identified as Cl–, SO42–, Na+, Ca2+, and Mg2+. K+ is also detected at 80 s in the hard mineral and tap water, but less than LOD for soft drinking water. For the quantitative analysis of mineral waters, we compared peak areas of these cations with figures reported by producer companies and the data published by Osaka city waterworks bureau. The results are summarized in Table 2.

|       | Soft Ex. | Soft Di. | Hard Ex. | Hard Di. | Tap Ex. | Tap Re. |
|-------|----------|----------|----------|----------|---------|---------|
| Na+   | 28       | 29.5     | 6.2      | 7        | 18      | 20      |
| Ca2+  | 6.0      | 6.5      | 75       | 80       | 19      | 21      |
| Mg2+  | 3.4      | 3.7      | 26       | 26       | 7.1     | 8       |

The unit of the numerical value is ppm. Ex. = experimental data, Di. = display data, Re. = reported data.

Comparing experimental values and display or reported values of inorganic ions measured in soft, hard mineral water and tap water

The display or reported values compared to the experimental values were more than 92, 85, and 81% (w/v) composition for soft mineral water, hard mineral water, and tap water, respectively. In all the reported and calculated percent compositions, Na+, Ca2+, and Mg2+ were acceptable. K+ contained in tap water as reported by the Osaka city waterworks bureau was 3 ppm and detected in our experiment at 80 s. We also investigated the compared peak areas of K+ and calculated 3.0 ppm. It can be seen that this method is suitable for real sample analysis. The migration times of cations in Fig. 3 were largely different from Fig. 2. This is because samples formed boundaries in the capillary and moved independently to the anode or cathode. So, the component and amount of contained ion are different, and the electropherogram pattern greatly differs. Comparing the same ions contained in different samples, RSDs show a very high value. However, utilizing the same samples, analytical parameters are satisfactory as shown in Fig. 2.

Application to the biological sample analysis

To evaluate the applicability of the method, we first applied it to the analysis of ions in human urine, and compared values with artificial urine. Urine samples were diluted 200 times and applied to separation by CE-C4D. Figure 4 shows the separation of artificial urine (Fig. 4B) and the urine of healthy volunteers (Fig. 4A). Urine sample were diluted 100 times with water and 2 times with 2 times concentrated BGE and filtrated through PVDF membrane, and the filtrates were analyzed by the CE-C4D system. The identified peaks in the urine of healthy volunteers were Cl−, SO42−, NH4+, K+, Na+, Ca2+, and Mg2+ and their migration times were 50, 52, 70, 76, 107, 112, and 114, respectively. RSD values (n = 5) of migration time and peak area of seven ions of the urine samples was satisfactory and was less than 2.92 and 11.9%, respectively. The recovery rate was calculated by spiking with each anion or cation with the same sample concentration, and the range was between 89 to 108% (n = 5).

Next, we applied EBC analysis. EBC is the liquid obtained by cooling exhaled air. It mainly consists of water and CO2, but contains also aerosolized particles of airway lining fluid consisting of inorganic ions. Monitoring of EBC may indicate lung inflammation and provide insights into the pathophysiology of inflammatory lung diseases.31,32 We used donated EBC samples from healthy smokers and non-smokers to determine the concentration for the ionic contents. Figure 5 indicates typical examples of an EBC analysis of a non-smoker (Fig. 5A) and a smoker a few minutes after smoking (Fig. 5B). Cl−, SO42−, NO3−, NH4+, K+, Na+, Ca2+, and Mg2+ could be found in the EBC from a non-smoker. The main peaks of EBC from a non-smoker were Cl− and K+. On the other hand, we cannot detect SO42− and Mg2+ in the EBC from a smoker. The peak of Cl− and K+ were
in EBC and smokers. Therefore, our method may be rapid routine analysis of inorganic anions and cations in various hospital.

Conclusions

In this paper we demonstrated that CE-C4D with CFM can be used for rapid analysis of both inorganic cations and organic anions present in various kinds of samples. CFM is simple and sensitive because it requires only filling the capillary with the sample and applying the high voltage end of a capillary. Three anions (chloride, sulfate and nitrate) and five cations (ammonium, potassium, sodium, magnesium and calcium) were successfully separated and detected at ppm levels within 80 s using a 9 mM histidine/15 mM malic acid (pH 3.6) containing 50 mM N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate. Although migration time depends on the sample composition, the RSD values of the peak area and migration times for the same sample component were satisfactory and were less than 5.15 and 2.04%, respectively. The developed method was applied for analysis of inorganic anions and cations in various samples for applications such as environmental monitoring and assessment of a patient’s condition.

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