

## Short communications

### Simultaneous determination of inorganic cations and anions in capillary zone electrophoresis (CZE) with indirect fluorescence detection

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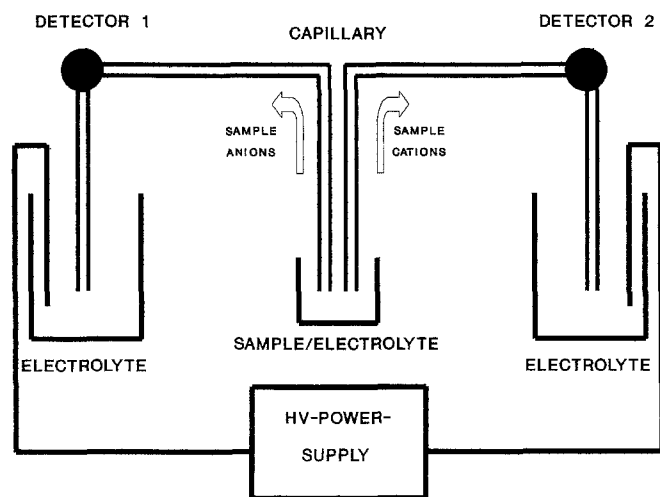
**Summary.** An instrumental configuration for the simultaneous determination of inorganic cations and anions in capillary zone electrophoresis (CZE) is described. The sample is injected into the central part of the capillary by hydrostatic injection. Indirect fluorescence detection is used. The electrolyte system consists of 2,5-dihydrobenzoate as fluorescent anion and cerium(III) as fluorescent cation. This method allows the analysis of both cations and anions without changing the electrolyte system in less than five minutes.

#### Introduction

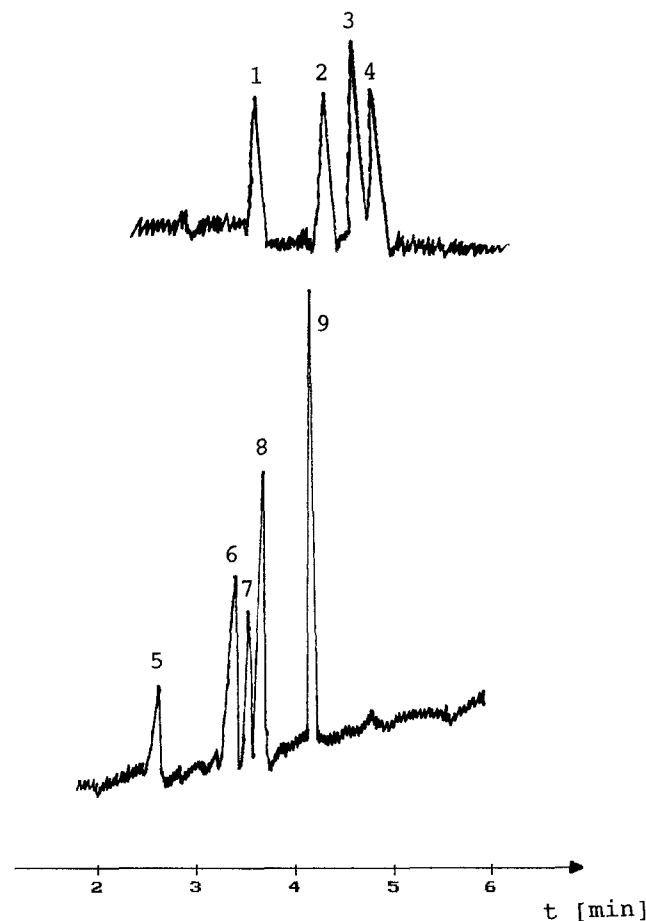
Over the past few years, the significance of the determination of inorganic ions in CZE has steadily increased [1–3]. In this case on-column UV-absorbance and fluorescence have been the most commonly used detection methods. Until now cations and anions had to be determined separately except if the velocity of the electroosmotic flow is high enough, then both cations and anions can be determined in one run [4]. In this special case, the anions are transported in a direction opposite to that in standard capillary electrophoresis by the electroosmotic flow and appear after the cations in the detector. However, when the electrophoretic mobilities of some anions are higher than the

electroosmotic flow mobility of the bulk electrolyte, these anions will escape detection. This drawback is not given in the method described in this paper. The simultaneous determination of cations and anions by injecting the sample in the central part of the capillary is shown. Two detectors, one for cation and one for anion analysis are used. The developed instrumental configuration shows some principal advantages.

Only one sample injection for both cations and anions is necessary. This is of special interest in cases where only small sample volumes – which do not allow more than one injection – are available. For example, in medicine, atmospheric chemistry or biotechnology it is necessary to handle small volumes (nl range). This implies reduced contamination risk during sample introduction. A further advantage is the saving of time, an important aspect especially for routine analysis. This means that changing the electrolyte and reversing the polarity become no longer necessary.



**Fig. 1.** Instrumental configuration for the simultaneous determination of cations and anions



**Fig. 2.** Electropherograms of a cation and anion standard. Anions: 1  $\text{Cl}^-$ , 2  $\text{ClO}_4^-$ , 3  $\text{ClO}_3^-$  and 4  $\text{ICO}_3^-$ . Cations: 5  $\text{K}^+$ , 6  $\text{Ca}^{2+}$ , 7  $\text{Na}^+$ , 8  $\text{Mg}^{2+}$  and 9  $\text{Li}^+$ , each  $10^{-4}$  mol/l. Electrolyte: 0.5 mmol/l cerium(III) sulphate, 0.5 mmol/l 2,5-dihydroxybenzoic acid, pH 3.4. Capillary: fused silica,  $2 \times 80$  cm total length, 55 cm to detector, column I.D.  $75 \mu\text{m}$ . Voltage: 30 kV,  $6 \mu\text{A}$ . Detection: indirect fluorescence, [cerium(III): 251/345 nm] (2,5-dihydroxybenzoic acid: 314/389 nm). Injection: hydrostatic, 10 cm (30 s)

## Experimental section

**Instrumentation.** The capillary electrophoresis instrument used is home-built (see Fig. 1).

It is a modified CZE-system with an capillary interrupt for sample introduction between both electrolyte vials which contain the electrodes. After the injection the sample vial is replaced by a third electrolyte vial before starting the electrophoresis. Sample introduction was carried out by hydrostatic injection.

We used polyimide-clad fused silica capillaries with an inside diameter of 75  $\mu\text{m}$  (SGE, Weiterstadt). Differences in retention times between cations and anions can be minimized using different capillary lengths. A high-voltage power supply (0–30 kV with a reversible polarity output) is used (FUG, Rosenheim). Indirect fluorescence detection was carried out with two Spectroflow 980 fluorescence detectors (ABI, Weiterstadt) which were modified for on-column detection.

**Chemicals.** Cerium(III) sulphate, 99.99%, Alfa (Karlsruhe, Germany); 2,5-dihydroxybenzoic acid, 99%, Aldrich (Steinheim, Germany); all other reagents were of p.a. quality, Merck (Darmstadt, Germany). All solutions were prepared using water purified by a Milli-Q system (Millipore, Eschborn, Germany) and were degassed under vacuum. Additionally, the electrolyte was filtered through a 0.22  $\mu\text{m}$  membrane filter type GV (Millipore).

## Results and discussion

For the simultaneous determination of inorganic cations and anions with the instrumental configuration described above it is necessary to use an electrolyte system with two detector-active components for indirect detection. A two-component electrolyte system is more complex than a one-component system and requires a more difficult choice of electrolyte substances and optimisation.

Therefore, it is necessary to avoid interferences as to the detection and the separation caused by the two electrolyte components. A major requirement for the two detector active components is that the excitation wavelengths are significantly distinguished. We used a fluorescent two-component electrolyte system which contains cerium(III) for the cation and 2,5-dihydroxybenzoic acid for the anion analysis. Both electrolyte components were successfully used for the separate determination of cations and anions in our previous work [5]. The excitation wavelength for cerium(III) is 251 nm and a 345 nm cut-off filter was used to eliminate scattering light during fluorescence measurement. The fluorescence maximum for cerium(III) was observed at 360 nm and will not be disturbed by the excitation maximum of 2,5-dihydroxybenzoic acid, which lies at 314 nm. The fluorescence of 2,5-dihydroxybenzoic acid was measured above 390 nm.

Figure 2 shows two electropherograms of different inorganic cations and anions. For injection, the sample vial in the middle of the capillary is lifted 10 cm higher than both capillary outlets for 30 s. The electropherogram demonstrates that the instrumental configuration and the two-component electrolyte system used are suitable for the simultaneous determination of cations and anions.

The development of a system with indirect photometric detection for practical applications is under investigation.

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## Simple fluorophotometric determination of cobalt(II) with p-hydroxy-2-anilinopyridine and hydrogen peroxide\*

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**Summary.** A simple and sensitive determination of cobalt(II) is based on its fluorescence reaction with p-hydroxy-2-anilinopyridine and hydrogen peroxide. The relative standard deviation is 2.5% ( $n = 5$ ). An application to pharmaceutical preparations is recommended.

\* The application of diarylamine compounds in analytical chemistry. IV.-Part III see ref. [1]

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## Introduction

Hitherto, numerous spectrophotometric assays of trace amounts of metal ions such as vanadium and cobalt using various kinetic and catalytic reaction systems [2–7] have been reported and used together in the study of environmental pollution. We now observed that the fluorescence reaction of p-hydroxy-2-anilinopyridine (HAP, its preparation is very easy and its methanol solution is very stable in comparison with previous diarylamine derivatives [8, 9]) with hydrogen peroxide as an oxidizing agent and cobalt(II) as a kinetic action-metal ion was sharp and instantaneous. The fluorescence reaction was applied to the spectrofluorophotometric determination of cobalt(II).

## Experimental

**Apparatus and reagents.** Fluorescence measurements were performed on a Shimadzu fluorometer, model RF 540 or RF 5000 equipped with Ushio 150 W Xenon lamp and 10  $\times$  10 mm quartz cells. A Hitachi-Horiba model F-7AD pH-meter, equipped with glass and calomel electrodes, was used for all pH-measurements.

All materials and reagents were of analytical-reagent grade unless specified otherwise. De-ionized water was used throughout. A stock solution ( $1.0 \times 10^{-2}$  mol/l) of cobalt(II) was pre-