

Simultaneous Determination of Inorganic Anions and Organic Acids by Capillary Electrophoresis*

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Received 09.10.2000

A rapid, easy, and reproducible method for the simultaneous determination of inorganic anions and organic acids is reported. The method is based on the separation of anions in a capillary coated with a cationic polymer, polyethyleneimine (PEI). Four inorganic anions (chloride, sulfate, iodate and phosphate) and eight organic acids (oxalate, tartarate, malate, succinate, citrate, acetate, lactate, and propionate) were separated and quantified within 7 min by using 2,6-pyridinedicarboxylic acid (PDC) as the background electrolyte. The minimum detection limits are 0.67-2.87 $\mu\text{g}/\text{mL}$ for inorganic ions and 0.38-2.84 $\mu\text{g}/\text{mL}$ for organic acids. Reproducibilities in migration times (RSD%) for both inorganic and organic anions were 0.27-1.05. The method was applied to the analysis of white wine.

Introduction

The analysis of small organic and inorganic anions is of interest in a wide range of industries, and clinical and forensic science^{1,2}. Capillary electrophoresis (CE) has recently become a complementary analytical technique to chromatographic methods. Separation speed, superior separation efficiency, and direct injection of samples without labor-intensive sample preparation are the major advantages of the CE method for the analysis of real samples. Since most inorganic and organic anions do not absorb ultraviolet (UV) light, detection is done indirectly by the addition of UV absorbing ion having the same charge as the sample ions.

The electrophoretic mobilities of small inorganic anions and small organic acids are of the same magnitude as, but of the opposite sign to, the electroosmotic flow (EOF), resulting in excessive migration times of the anions. Separation can be performed by reducing or reversing EOF with the addition of an EOF modifier to the buffer. The use of commercial products based on a polyvalent or hydrophobic cations³⁻⁷, cationic surfactants^{8,9}, or cationic polymers¹⁰ has been reported for this purpose. The addition of electroosmotic modifier to the separation electrolyte can cause selectivity changes for anions due to ion-pairing or ion-exchange interactions of the analytes with EOF modifiers and loss of efficiency.

The aim of the present study is to demonstrate the simultaneous separation of inorganic anions and organic acids in a capillary coated with a cationic polymer, polyethyleneimine (PEI), without using an

*This paper has been presented at MBCAC III (3rd Mediterranean Basin Conference on Analytical Chemistry) 4-9 June, 2000 Antalya-Turkey

electroosmotic modifier in the buffer. For this purpose, a non-covalent coating procedure developed by Erim et al. was used¹¹. The surface of the PEI-coated capillary bears a positive charge over a wide pH range and this causes the reversal of EOF. The anodic EOF generated by PEI coating increases the separation speed of small anions. In this work, the separation experiments reported recently in the PEI-coated capillaries^{12–15} are extended to the simultaneous separation of inorganic and organic anions. The method was applied to the analysis of white wine.

Experimental

Chemicals

PEI (molecular mass range $6.10^5 - 1.10^6$) was purchased from Fluka (Buchs, Switzerland). Sodium salts of inorganic anions, organic acids and 2,6-pyridinedicarboxylic acid (PDC) were obtained from Merck (Darmstadt, Germany). The standard solutions of inorganic and organic anions were prepared from their sodium salts or free acids. All reagents were of analytical grade. Wine was from the local market. All solutions were prepared with deionized water purified in an Elgacan C114 filtration system.

PDC was used as the background electrolyte solution. The pH was adjusted to 5.5 with 1M NaOH.

Apparatus

Separations were performed with a commercial CE injection system (Prince Technologies BV, Emmen, Netherlands) in combination with an on-column variable wavelength UV Visible detector (Lambda 1000, Bishoff, Leonberg, Germany). The wavelength was set at 217nm. Sample injection was carried out with pressure (4.10^{-3} MPa, 6 s.) at the cathodic side. The analysis voltage was -28kV. The fused silica capillaries used for separation experiments were 68.5cm \times 75 μ m I.D. fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA). The distance to the detection window was 54.5cm. The measurements were performed at 25°C.

Automated capillary rinsing, sample introduction, and execution of the electrophoretic runs were controlled by a personal computer. Data processing was carried out with the Caesar software program (Roman Scientific,1995).

Coating procedure

Separation of inorganic anions and acids was performed with PEI-coated capillaries. High molecular mass PEI is adsorbed irreversibly on the silica surface and the resultant non-covalently coated capillary is stable for a long period even under harsh conditions such as flushing the capillary with strong basic and acidic solutions. The coating procedure, reproducibility, and long-term stability experiments for this coating were reported before¹¹. In brief, the coating procedure involves the following:

The fused silica capillary was first etched by flushing the capillary with a solution of 1 M sodium hydroxide for 30 min at 1.10^{-1} MPa and with water for 15 min at the same pressure. Then the capillary was flushed with a solution of 10% PEI in water at $1.5.10^{-1}$ MPa for 10 min and the PEI solution was left in the capillary for one hour. Next, the polymer solution was pressed out of the capillary with air at $1.5.10^{-1}$ MPa. Finally the capillary was rinsed with water and the running buffer for 15 min.

Results and Discussion

Separation of anions in the coated capillary

The coated PEI layer on the silica surface has a positive charge due to protonation of the imine groups, thus resulting in the reversal of EOF toward the anode. In this case, electrophoretic mobilities of anions and EOF are in the same direction, which enables the fast separation of anions.

The choice of background electrolyte is extremely important for the success of indirect detection. Sensitivity in indirect UV detection is governed by the molar absorptivity of the carrier electrolyte and by its mobility. The zones of non-absorbing ionic species are revealed by changes in light absorption due to charge displacement of the absorbing co-ion. The displacement of the background ion by the migrating sample ion will occur only if the sample ion has the same electrophoretic mobility as the background ion. The best sensitivities can be obtained in low-concentration background electrolytes containing a co-ion with high absorption at a given detection wavelength. Furthermore, the mobility of the BGE should be similar to that of the analytes of interest for there to be good peak shapes. A range of anionic UV absorbing species has been employed to provide the background UV signal for indirect detection in CE, including chromate^{16–20}, pyromellitate²¹, benzoate^{22,23}, aminobenzoate²⁴, 2,6-pyridine dicarboxylate⁹, naphthalenesulphonate⁷ and phthalate^{4,8,23}.

PDC was chosen as chromophore ion in this study. Here, pH is the most important parameter to supply the match of electrophoretic mobilities between analytes, chromophore ions, and buffer ions. An optimization should be made on pH and concentrations, regulating the EOF of the coating, the dissociation of PDC, and the dissociation of organic acids. Ref. 11 reports the effect of pH on the EOF for the PEI coating used here. A pH value of 5.5 was selected as optimal for the separation of four inorganic anions and eight organic acids.

The effective mobilities of analytes calculated according to the following equation are given in Table 1. The electrophoretic mobility of PDC is between those of the inorganic ions and organic acids.

Table 1. Mobility, LODs and RSD% in migration times for inorganic anions and organic acids.

| | Mobility ($10^{-4}\text{cm}^2\text{V}^{-1}\text{s}^{-1}$) | LODs ($\mu\text{g}/\text{mL}$) | RSD% (n=6) (migration time) |
|------------|--|-------------------------------------|--------------------------------|
| Chloride | 6.79 | 0.67 | 0.27 |
| Sulfate | 6.32 | 1.25 | 0.29 |
| Oxalate | 5.88 | 1.14 | 0.37 |
| Tartarate | 4.81 | 0.77 | 0.48 |
| Malate | 4.52 | 0.53 | 0.62 |
| Succinate | 4.05 | 0.53 | 0.98 |
| Iodate | 3.46 | 2.87 | 0.79 |
| Citrate | 3.26 | 2.84 | 0.71 |
| Acetate | 3.13 | 0.41 | 0.91 |
| Lactate | 2.87 | 0.38 | 0.92 |
| Phosphate | 2.69 | 0.50 | 0.96 |
| Propionate | 2.57 | 1.10 | 1.05 |
| PDC | 3.33 | | |

$$\mu_e = 1L/V(t_i - t_{EOF})(\text{cm}^2\text{V}^{-1}\text{s}^{-1}) \quad (1)$$

where L and l are total and effective lengths of the capillary, respectively. V is the applied voltage, t_i is the migration time of the anion, and t_{EOF} is the migration time of the neutral marker (acetone).

Figure 1 shows the optimum separation electropherogram. As seen from the electropherogram, the separation is very fast, all anions migrating within 7 min.

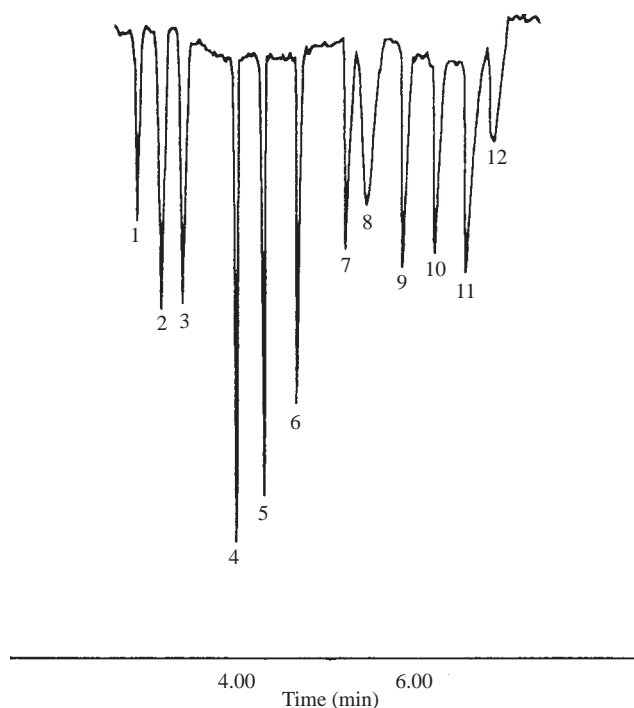


Figure 1. Electropherogram of a standard mixture of four inorganic anions and eight organic acids. Peaks: 1= chloride, 2 = sulfate, 3 = oxalate, 4 = tartarate, 5 = malate, 6 = succinate, 7 = iodate, 8 = citrate, 9 = acetate, 10 = lactate, 11 = phosphate, 12 = propionate. Background electrolyte: 3mM PDC at pH 5.5. Injection 4×10^{-4} MPa, 6s. Concentrations of ions are 0.2 mM for chloride, sulfate, oxalate, iodate, and citrate, 0.1mM for others.

Quantitation

For the separation of four inorganic anions and eight organic acids, 3 mM PDC at pH 5.5 was chosen as the background electrolyte. The separation was performed under these conditions. The minimum limits of detections (LOD) are 0.38-2.87 $\mu\text{g}/\text{mL}$ for a signal-to-noise ratio of 3. The LODs and relative standard deviations (RSDs) in migration times for six successive injections are given in Table 1.

Application

The method was applied to the analysis of white wine. Wine was diluted 1:40 with deionized water and injected directly. Figure 2 shows the electropherogram of white wine.

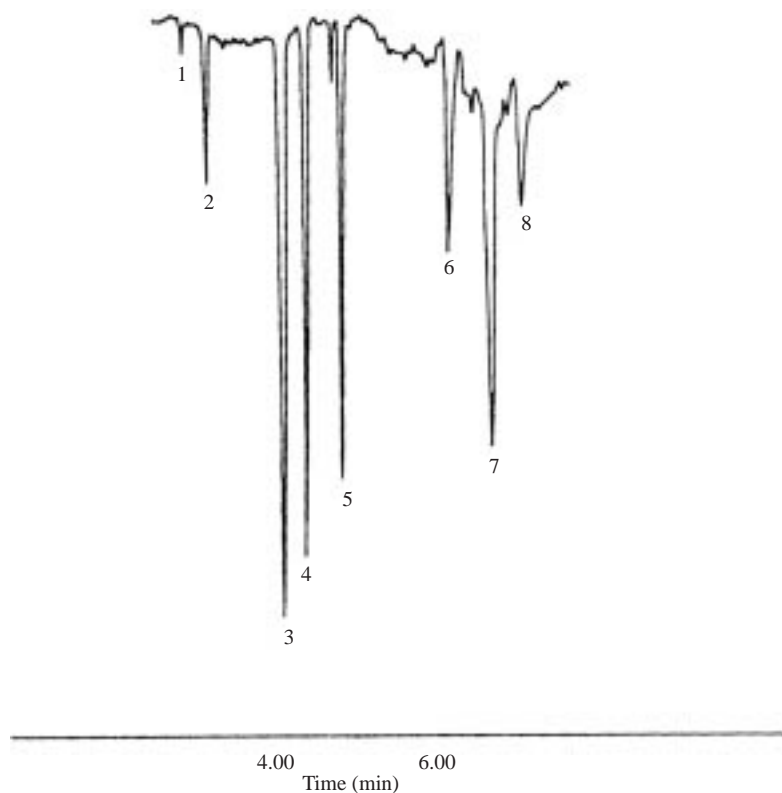


Figure 2. Electropherogram of white wine. Peaks: 1= chloride, 2 = sulfate, 3 = tartarate, 4 = malate, 5 = succinate, 6 = acetate, 7 = lactate, 8 = phosphate.

Conclusion

The use of the PEI-coated capillary reverses EOF and offers a suitable alternative to the use of cationic surfactants for the simultaneous separation of inorganic anions and organic acids. The method is extremely fast and LODs are sufficiently low for the determination of anions in food samples.

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