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Simultaneous determination of organic and inorganic anions in the sub- $\mu\text{mol/l}$ range in rain water by capillary zone electrophoresis

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Abstract

An electrolyte system using *p*-aminobenzoate was developed to determine anions in individual and size-classified rain drops. In comparison with other electrolyte systems it was found to be the most favourable for the analysis of organic acids with low mobility. Using a cationic additive, inorganic anions with high mobility could also be separated. Limits of detection lower than 50 nmol l^{-1} are attainable when sample stacking for enrichment is used.

1. Introduction

For the examination of scavenging processes in the atmosphere, rain drops can be collected in size classes and short time intervals with a Guttalgor [1,2]. The Guttalgor mainly consists of a dewar vessel filled with liquid nitrogen. Rain drops falling into the liquid nitrogen maintain their spherical shape during the freezing process. Consequently, it is possible to separate individual rain drops of different sizes using sieves of different mesh widths. Because of this special technique, the sample volume for chemical analysis is only about $5 \mu\text{l}$.

To achieve a detailed understanding of the chemistry in rain, it is necessary to determine as many components as possible. However, owing to the low concentrations and the small sample volumes, only the main ionic components in rain

could be determined, e.g., the anions chloride, nitrate, sulphate, formate and acetate [3]. The concentrations range from 10 to $100 \mu\text{mol l}^{-1}$. The determination of dicarboxylic acids at low concentrations ($<1 \mu\text{mol l}^{-1}$) [4] is not yet possible. For inorganic anions and mono- and dicarboxylic acids, several methods of determination have been reported, such as capillary zone electrophoresis (CZE), and ion chromatography [5–8]. Organic acids are mainly determined by GC [9–11]. However, no method fulfils all the necessary requirements: simultaneous determination of organic and inorganic anions; low limits of detection for the dicarboxylic acids; enrichment of the dicarboxylic acids in the presence of relatively high concentrations of inorganic anions; and analysis in small sample volumes ($<5 \mu\text{l}$).

This paper describes the development of a new electrolyte system for indirect UV detection in CZE that satisfies all these requirements.

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2. Experimental

The experiments were performed on a SpectraPhoresis 1000 unit. The length of the untreated fused-silica capillary (I.D. 75 and 50 μm) was 70 cm and 63 cm to the detector window.

The exchange of bromide for hydroxide in a solution of 10 mmol l^{-1} tetradecyltrimethylammonium bromide (TTAB) was performed with the ion exchanger Amberlite IRA-904 (Serva, Heidelberg, Germany).

p-Aminobenzoate (p-AB) electrolyte was prepared from 3 mmol l^{-1} *p*-aminobenzoic acid (Merck) and 4.5 mmol l^{-1} sodium *p*-aminobenzoate (Aldrich), 0.76 mmol l^{-1} barium hydroxide (Merck) and 55 $\mu\text{mol l}^{-1}$ tetradecyltrimethylammonium hydroxide (TTAH) in water purified with a Milli-Q system (Millipore). The acid solution was degassed under vacuum for 30 min in order to remove carbon dioxide, then the pH was adjusted to 9.6 with dilute ammonia solution.

Frozen rain drops were handled in an inert-gas box. With laboratory-made sieves it is possible to transfer the drops quickly into closable receptacles of appropriate size and controlled purity.

3. Results and discussion

3.1. Choice of electrolyte

The most important aspects of the electrolyte for indirect UV detection are its mobility and the limit of detection (LOD) that is attainable. The electrophoretic mobility can be calculated from the equivalent conductance at infinite dilution [12] and the LOD can be calculated by using the equation of Yeung [13].

To compare some frequently used electrolytes, including p-AB, a run with a standard consisting of 1 mmol/l of each dicarboxylic acid was carried out. The parameters that describe the electrolyte system are given in Table 1. Fig. 1 shows as an example three typical electropherograms from electrolyte systems with (A) high mobility (chromate), (B) medium mobility [*p*-hydroxybenzoate (p-HB)] and (C) low mobility [*p*-aminobenzoate (p-AB)]. In the chromate system (A) the peak height of the analytes decreases whereas the peak width increases with decreasing mobility. This system is not suitable for slow analytes.

The mobility of p-HB (Fig. 1B) is expected to be almost as low as that of p-AB. However,

Table 1
Comparison of chosen electrolyte systems for the determination of anions

Parameter	Chromate ^a	Phthalate ^a	Borate/phthalate ^b	p-HB ^a	Salicylate	p-AB	p-AB ^{50 c}
Wavelength (nm)	266	254	200	254	232	264	250
Molar absorptivity, ϵ^d ($\text{l mol}^{-1} \text{cm}^{-1}$)	3900	1900	–25 000	15 100	2500	13 600	7000
Mobility ^e ($\text{cm}^2 \text{V}^{-1} \text{s}^{-1} \cdot 10^{-4}$)	8.8	5.5	5.3/5.5	3.5	3.7	3.1	3.1
Observed mobility ($\text{cm}^2 \text{V}^{-1} \text{s}^{-1} \cdot 10^{-4}$)	8.8	4.5	5.3	5.8	3.7	2.1	2.1
Electrolyte concentration (mmol l^{-1})	5	5	5/1	5	5	7.5	20
Current at 30 kV (μA) ^f	95	25	30	20	10	15	10
Working pH	8.0	5.4	9.0	9.4	9.6	9.6	9.6
LOD ^g ($\mu\text{mol l}^{-1}$)	3.6	4.8	2.1	1.7	3.8	0.6	3.1

^a Waters application note.

^b Thermo Separation Products application note.

^c High-concentration p-AB electrolyte using a capillary of 50 μm I.D.

^d Ref. [12].

^e Calculated from equivalent conductance at infinite dilution.

^f Capillary 70 cm \times 75 μm I.D.

^g Calculated using equation of Yeung [13].

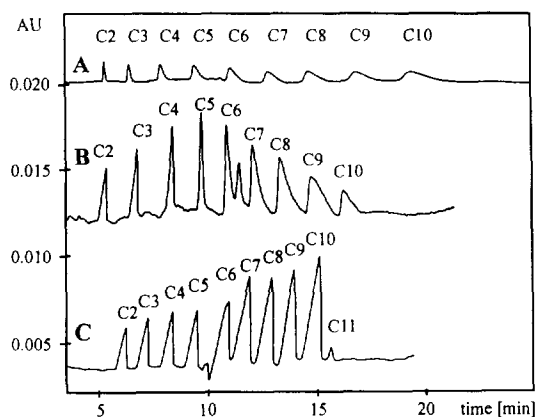


Fig. 1. Comparison of the chosen electrolytes. The run conditions are identical for all electrolyte systems: untreated fused-silica capillaries (70 cm \times 75 μ m I.D.) with detector window at 63 cm; separation voltage, -30 kV; temperature, 25°C; injection, 1 mmol/l of each dicarboxylic acid (C2–C10) for 1 s under vacuum (1.5 p.s.i.; 1 p.s.i. = 6894.76 Pa). (A) 5 mmol l⁻¹ chromate, 55 μ mol l⁻¹ TTAH, pH 8.0; (B) 5 mmol l⁻¹ p-HB, 55 μ mol l⁻¹ TTAH, pH 9.4; (C) 7.5 mmol l⁻¹ p-AB, 55 μ mol l⁻¹ TTAH, pH 9.6. Peaks: C2 = oxalate; C3 = malonate; C4 = succinate; C5 = glutarate; C6 = adipate; C7 = pimelate; C8 = suberate; C9 = azelate; C10 = sebacate; C11 = undecanedioate.

because of the partly dissociated hydroxy group at pH 9.4, the mobility of phthalate is reached. The electropherogram indicates clearly that for analytes with mobility lower than that of the electrolyte, the peak broadening is larger than for analytes which are faster than the electrolyte. The peaks in the electropherograms of both phthalate systems show the same shape as that in the electropherogram obtained with the p-HB system.

The p-AB system (Fig. 1-C) shows increasing peaks up to the slowest analyte owing to the increasing transfer ratio [14], so that even undecanedioic acid (C11), which exists only as a trace contamination in the standard, was detected. Even for the analysis of rain samples this is advantageous because the fast inorganic anions show higher concentrations than slow organic acids. The peaks in the electropherogram of the salicylate system show the same shape as those in the electropherogram of the p-AB system, but

the peak areas in the salicylate system are about five times smaller, as predicted from Yeung's equation, owing to the smaller molar absorptivity (see Table 1). The p-AB system is the optimum system for the determination of the dicarboxylic acids in rain samples, because the LOD decreases with decreasing analyte mobility (Table 2).

3.2. Concentration of electrolyte

High electrolyte concentrations result in a high enrichment factor and a large number of plates. The Joule heating and, for a high molar absorptivity, the linear range of the detector limited the concentration of the electrolyte. With a concentration of 7.5 mmol l⁻¹ of p-AB in a capillary of 75 μ m I.D., the maximum signal-to-noise ratio and the minimum LOD are obtained. This system is used to determine anions at trace levels in rain samples with small volumes (Fig. 2). Applying a capillary of 100 μ m I.D., the concentration of the electrolyte had to be decreased

Table 2
LODs for anions

Anion	LOD* (nmol l ⁻¹)
Chloride	40
Sulphate	30
Nitrite	40
Nitrate	40
Formate	30
Acetate	20
Propionate	20
Methanesulphonate	30
Oxalate	30
Malonate	30
Maleate	20
Succinate	20
Glutarate	20
Adipate	20
Pimelate	10
Suberate	10
Azelate	10
Sebacate	10

* Evaluated from calibration graph [15].

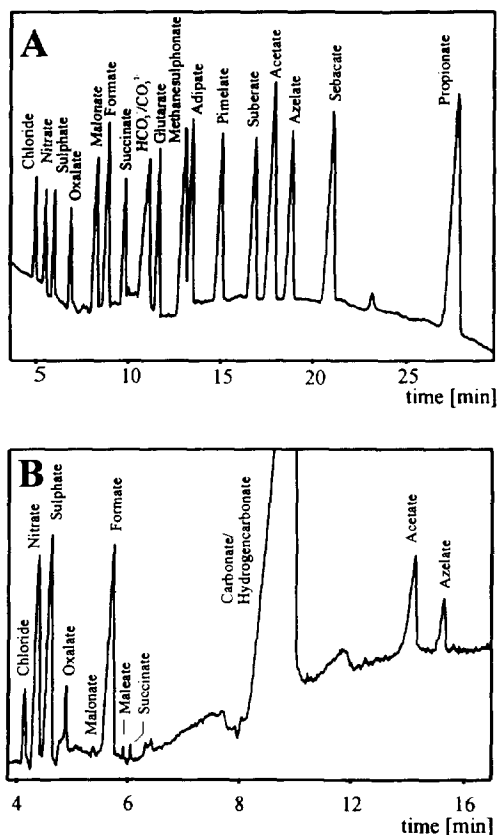


Fig. 2. Electropherograms of (A) a standard ($10\text{--}25\ \mu\text{mol l}^{-1}$) and (B) a rain water sample. $7.5\ \text{mmol l}^{-1}$ p-AB, $0.76\ \text{mmol l}^{-1}$ $\text{Ba}(\text{OH})_2$, $55\ \mu\text{mol l}^{-1}$ TTAH (pH 9.4). Untreated fused-silica capillaries ($70\ \text{cm} \times 75\ \mu\text{m}$ I.D.) with detector window at $63\ \text{cm}$. Separation voltage, $-30\ \text{kV}$; temperature, 25°C ; hydrodynamic sample injection for $30\ \text{s}$ under vacuum ($1.5\ \text{p.s.i.}$).

to prevent thermal effects. In this case the resolution decreased. Using a capillary of $50\ \mu\text{m}$ I.D. the efficiency of the separation was improved but the LOD increased. This trend can be enhanced by increasing the electrolyte concentration. Fig. 3 shows the electropherogram of a standard consisting of 27 anions performed with the p-AB⁵⁰ system (p-AB $20\ \text{mmol l}^{-1}$, capillary I.D. $50\ \mu\text{m}$, $\lambda = 250\ \text{nm}$). That this system gives a two to three times better resolution and a five times higher LOD compared with the p-AB system with the lowest LOD (7.5

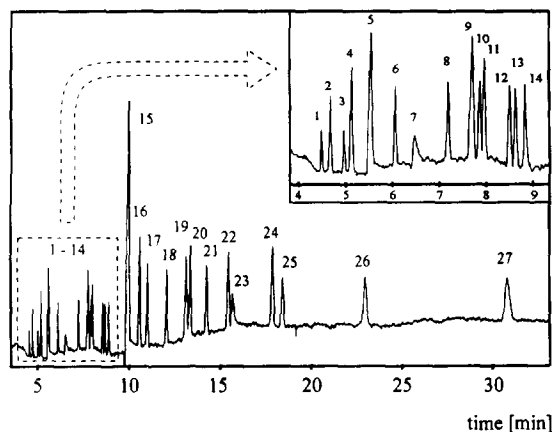


Fig. 3. Electropherogram of a standard with 27 anions ($25\text{--}50\ \mu\text{mol l}^{-1}$). $20\ \text{mmol l}^{-1}$ p-AB, $0.4\ \text{mmol l}^{-1}$ $\text{Ba}(\text{OH})_2$, $70\ \mu\text{mol l}^{-1}$ TTAH (pH 9.6). Untreated fused-silica capillaries ($70\ \text{cm} \times 50\ \mu\text{m}$ I.D.) with detector window at $63\ \text{cm}$. Separation voltage, $-30\ \text{kV}$; temperature 25°C ; hydrodynamic sample injection for $3\ \text{s}$ under vacuum ($1.5\ \text{p.s.i.}$). Peaks: 1 = bromide; 2 = chloride; 3 = nitrite; 4 = nitrate; 5 = sulphate; 6 = oxalate; 7 = sulphite; 8 = malonate; 9 = formate; 10 = fumarate; 11 = maleate; 12 = succinate; 13 = malate; 14 = tartrate; 15 = carbonate; 16 = methanesulphonate; 17 = adipate; 18 = pimelate; 19 = acetate; 20 = suberate; 21 = azelate; 22 = sebacate; 23 = citronate; 24 = propionate; 25 = lactate; 26 = benzoate; 27 = mandelate.

mmol l^{-1} p-AB, capillary I.D. $75\ \mu\text{m}$, $\lambda = 264\ \text{nm}$).

3.3. Cationic additives

The separation of nitrate and sulphate was not possible without the addition of cations (Fig. 4), which decreased the mobility of sulphate by forming ion pairs. In addition, the mobility of dicarboxylic acids was also affected, especially for oxalate. It can be seen from Table 3 that barium is an optimum cationic additive, based on the solubility product of the different salts. Because of the good solubility of barium hydroxide it is possible to work at a high pH, which increases the dissociation of the organic acids and gives a low LOD. In Fig. 4B the effect of barium ions on the separation is shown. Sulphate is separated from nitrate and, further, the

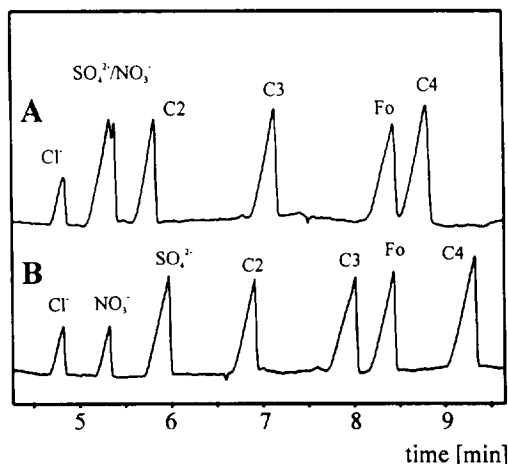


Fig. 4. Effect of a cationic additive on the separation. (A) Without additive; (B) with 0.76 mmol l^{-1} barium. Peaks: C2 = oxalate; C3 = malonate; C4 = succinate; Fo = formate.

mobility of the dicarboxylic acids decreases. The other cations listed in Table 3 influenced the mobility of the dicarboxylic acids more than that of sulphate.

3.4. Electroosmotic flow (EOF) modifier

At pH 9.6 the EOF is so fast that analytes which are slower than oxalate cannot reach the detection window at the anode side. A concentration of $55 \mu\text{mol/l}$ of TTAH in the electrolyte is sufficient to lower the EOF. Because the EOF is not reversed, sample stacking is possible. The use of TTAB would cause a positive system peak in the position where the bromide peak is expected. This interferes at

higher chloride concentrations. By exchanging bromide in TTAB for the hydroxide ion this interference is eliminated.

3.5. Sample injection and enrichment

Electrokinetic sample injection discriminates ions with low mobilities. This means for the analysis of rain samples that the higher concentration inorganic acids with high mobilities are enriched compared with the dicarboxylic acids, which are only partly dissociated in rain samples. This becomes obvious when the peak of phosphate (No. 11) is compared with that of methanesulphonate (No. 13) in Fig. 5. Both peaks have nearly the same size with hydrodynamic sample injection whereas the phosphate peak does not even reach half of the size of the

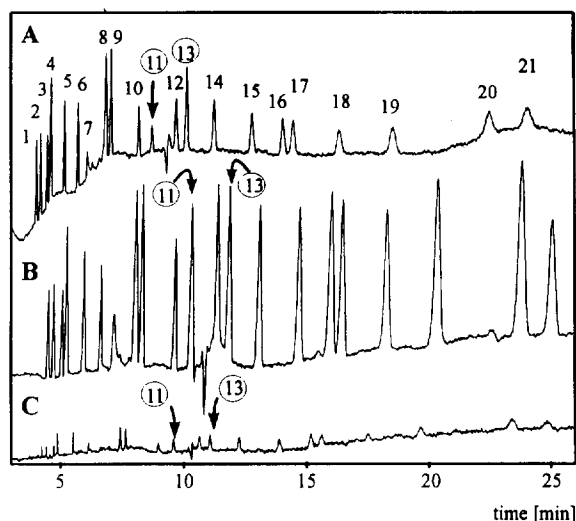


Fig. 5. Comparison of injection methods. (A) Electrokinetic sample injection, 5 s, 10 kV; (B) Hydrodynamic sample injection (sample stacking), 30 s, vacuum (1.5 p.s.i.); (C) Hydrodynamic sample injection, 1 s, vacuum (1.5 p.s.i.). Conditions: untreated fused-silica capillaries ($70 \text{ cm} \times 50 \mu\text{m}$ I.D.) with detector window at 63 cm; separation voltage, -30 kV ; temperature, 25°C . Peaks: 1 = bromide; 2 = chloride; 3 = nitrite; 4 = nitrate; 5 = sulphate; 6 = oxalate; 7 = sulphite; 8 = malonate; 9 = formate; 10 = succinate; 11 = phosphate; 12 = glutarate; 13 = methanesulphonate; 14 = adipate; 15 = pimelate; 16 = acetate; 17 = suberate; 18 = azelate; 19 = sebacate; 20 = propionate; 21 = lactate.

Table 3
Comparison of the $\text{p}K_{\text{L}}$ values (negative logarithm of the solubility product) of some cationic additives

Cation	Sulphate	Oxalate	Hydroxide
Calcium	4.21	8.75	8.33
Strontium	6.12	7.17	3.38
Barium	9.97	6.82	2.37
Lead	7.80	10.56	14.38

methanesulphonate peak with electrokinetic sample injection. The dissociation equilibrium of phosphoric acid in the sample (pH 4.5) differs from that in the electrolyte (pH 9.6), whereas methanesulphonic acid as a strong acid is completely dissociated in both the sample and the electrolyte. Therefore, the determination of weak acids in real samples is difficult and requires a lot of work and time. Electrokinetic sample injection is unfavourable for the determination of dicarboxylic acids in rain samples with small volumes.

With sample stacking no effects of discrimination arise and therefore the quantification is just as simple as with normal hydrodynamic sample injection. However, the migration time shift becomes stronger because of EOF variations caused by wall effects in the sample zone. The normally used correction of the peak area by migration time is sufficient for evaluation.

The high content of carbon dioxide in rain samples causes an interfering carbonate peak, but its migration time can be influenced by changing the pH of the electrolyte.

With 10% filling of the capillary, which corresponds to a sample volume of about 300 nl, the LOD given in Table 2 can be reached. With a higher sample volume the baseline noise becomes worse.

3.6. Results of the analysis of size-classified rain samples

As Fig. 6 demonstrates, the dicarboxylic acids show a different concentration–radius depen-

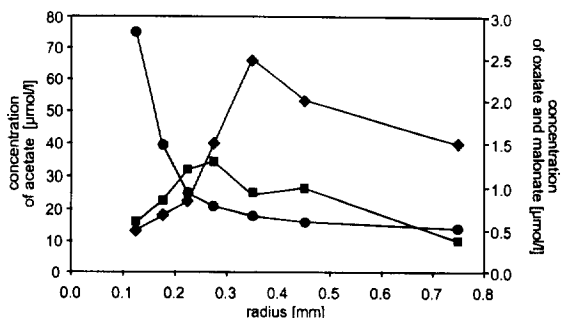


Fig. 6. Concentration of organic acids vs. droplet radius in rain samples. ● = Acetate; ■ = oxalate; ◆ = malonate.

dence than the monocarboxylic acids because of different scavenging processes, which will be studied in further investigations.

4. Conclusions

The p-AB electrolyte system is suitable for determining simultaneously inorganic and the organic acids in small-volume rain samples. The separation of the inorganic anions is facilitated by the addition of barium ions. Using sample stacking as an on-line enrichment technique, low-concentration dicarboxylic acids can also be detected. With an injection of 300 nl the limits of detection are between 30 nmol l^{-1} for oxalate and 10 nmol/l for sebacinate. Because the EOF is decreased with TTAH, the running time is less than 30 min. In contrast to conventional electrolyte systems, the limit of detection decreases with decreasing mobility using the p-AB system.

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