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Capillary Electrophoresis—a Fast and Universal Tool in Soil Analysis

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Introduction

Until now in soil analysis different time consuming techniques and expensive methods [ion chromatography (IC) and high performance liquid chromatography (HPLC)] have been used for most different species of molecules (inorganic ions, carbohydrates). Capillary electrophoresis (CE) represents a low cost and time-saving alternative to the commonly used techniques for the analysis of small molecules and ions^[1,2].

The availability of narrow bore fused silica capillaries and the recent introduction of commercially available CE units have once again brought electrophoresis techniques to the attention of analytical chemists. Although the main input for developing electrophoretic methods has come from the biological applications, CE offers, in connection with UV detection and its high resolution ($N > 1\,000\,000$), the possibility to separate almost any type of compound. When high voltage is applied between the ends of the capillary tube filled with an electrolyte solution, the surface of the inner wall of the tube is negatively charged. If a sample is injected into the tube from the anionic end (cationic mode), all compounds of the sample are transported to the cathode at a uniform velocity, anionic compounds are pulled back by electrostatic force (electrophoresis). In addition, the electroosmotic flow (EOF) in uncoated fused-silica capillaries is significantly greater than the electrophoretic mobility of the ions. Consequently, an EOF modifier that reverses the direction of the flow is necessary in anionic mode.

Inorganic ion analysis, however, has received relatively little attention. This is due to the existence of other sensitive techniques (ion chromatography) and the lack of a direct detection method. The latter problem has been solved by introducing the indirect UV absorption

Abstract: Fast analysis of different species of molecules in soils is investigated by capillary electrophoresis (CE). Several CE techniques for the analysis of inorganic ions and carbohydrates have been tested. With regard to the intents of pedologists and the usually large number of soil analyses a bundle of CE systems is proposed, capable of effecting time-saving soil analyses. Adapted electrolyte systems recently published and new separation systems are described. Examples of the application of these methods to two different soil samples are presented.

method^[3–8]. Weston *et al.*^[5,6] investigated the factors that affect the separation of metal cations and they optimized the detection sensitivity of indirect photometric detection. Foret *et al.*^[3] utilized indirect UV detection to demonstrate an efficient separation of fourteen lanthanide cations within 5 min with the aid of hydroxyisobutyric acid (HIBA) as a complexing agent. But no electrolyte system was found that was capable of clearly separating all the inorganic cations pedologists were interested in: NH_4 , Ca, Mg, Mn, Na, Al, K, Fe(III). In this study, we investigated the use of creatinine as a UV-absorbing background electrolyte with 18-crown-6 as the complexing agent. This agent can selectively modulate the mobility of the cations (NH_4 , K), and with creatinine as the co-ion even aluminium is sharply detectable. This electrolyte allows a clear separation of the usually interesting eight inorganic cations.

Jones *et al.*^[2,9–11] found the background electrolyte chromate to be the most useful for highly mobile inorganic anions. Incorporated in this electrolyte there is an additive that reverses the direction of EOF in the capillary, so that the EOF augments the mobility of the analytes. We adapted this electrolyte system for a clear separation of chloride, sulfate and nitrate.

The interest in soil analysis of carbohydrates is restricted to glucose, mannose, arabinose and xylose. Due to the low absorbency of these monosaccharides, indirect UV detection^[12,13] or pre-column derivatisation^[14–19] are recommended. Oefner *et al.*^[13] proposed sorbic acid as both carrier electrolyte anion and chromophore with indirect UV detection, but glucose and arabinose could not be separated. Recently, in-column derivatisation of reducing monosaccharides^[19] has been demonstrated. Regarding simplicity, reproducibility and sensitivity, we adapted a pre-column derivatisation with 4-aminobenzonitrile and separation by micellar electrokinetic capillary chromatography (MECC) with a carrier containing borate and sodium dodecylsulfate (SDS)^[2,20]. This method makes sensitive, direct UV detection possible and allows fast selectivity for the separation of oligo- and monosaccharides. The large peak caused by the excess concentration of the pre-column reagent is well separated from all analytes of interest.

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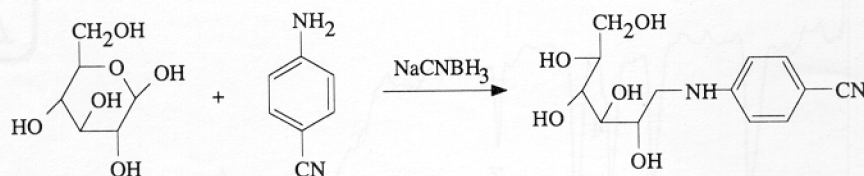


Figure 1. Reaction scheme for the reductive amination of glucose with 4-aminobenzonitrile and sodium cyanoborohydride.

2. Experimental

2.1. Chemicals

Reference solutions were prepared from chromatographic or analytical reagent grade chemicals (Fluka, Buchs, Switzerland) in deionized water for CE. Running electrolyte solution for analysis of cations was prepared by dissolving appropriate amounts of creatinine, acetic acid and 18-crown-6 in doubly-distilled water and similarly for anions of sodium chromate and tetradecyl-dimethylammonium bromide, respectively. For the analysis of carbohydrates the borate running buffer for MECC was purchased (Fluka).

2.2. Apparatus

Analyses were performed on a Prince (Lauerlabs, Emmen, Netherlands), the programmable injector, combined with a 206 PHD UVIS-detector (Linear Instruments, Reno, USA), which were equipped with a 75 cm fused silica capillary of I.D. 50 μm and a 100 cm fused silica capillary of I.D. 75 μm , respectively. Detection was carried out by on-column measurement of UV absorption at different wavelengths at 15 cm from the cathode. Pherograms were recorded on an IBM-compatible i486-33 with the UVIS-206 Computer Software for MS[®]-Windows[™] (Linear Instruments, Reno, USA). In the case of indirect UV

detection the pherograms were inverted by a self-made computer program written in TurboPascal for MS[®]-Windows[™]. All pherograms were integrated with the JCL 6000 Chromatography Data System (Jones Chromatography LTD, Hengoed, UK).

2.3. Capillary Conditioning

Each new fused silica capillary was flushed with 1 M NaOH for 1 h followed by 0.1 M NaOH for 10 min and by running buffer for 5 min. Between runs, the capillary was flushed with running buffer for 2 min. Finally, the system was pre-run for 10 min in order to obtain a stable background signal. The capillary was stored in 0.1 M NaOH overnight.

2.4. Sequential Extraction Methods of Organic Matter from Soils^[21-26]

The air-dried soil samples were shaken in *n*-pentane for 72 h. The undissolved part was extracted with a mixture of chloroform, methanol and water (1:2:0.8) for 24 h. The two phases were separated on adding chloroform and water (1:1:0.9). The residue was again shaken intensively with 25% N NH₃ under argon for

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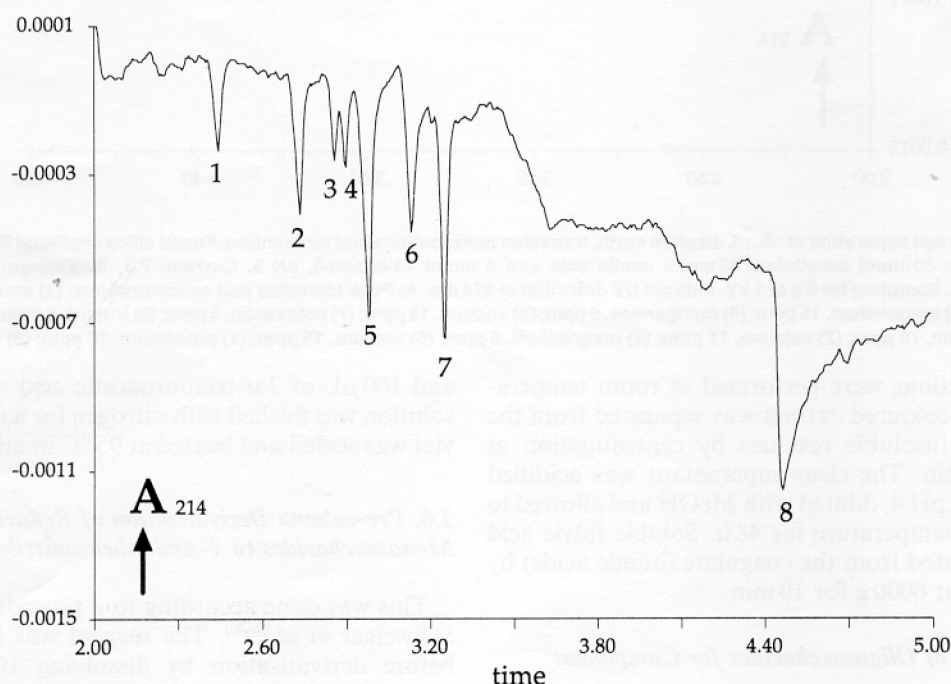


Figure 2. Peak identities, concentrations and areas: (1) ammonium, 16 ppm, 108; (2) calcium, 20 ppm, 204; (3) magnesium, 6.5 ppm, 108; (4) manganese, 17 ppm, 103; (5) sodium, 16 ppm, 418; (6) aluminium, 11 ppm, 244; (7) potassium, 13.5 ppm, 322; (8) iron(III), 28 ppm, 1155.

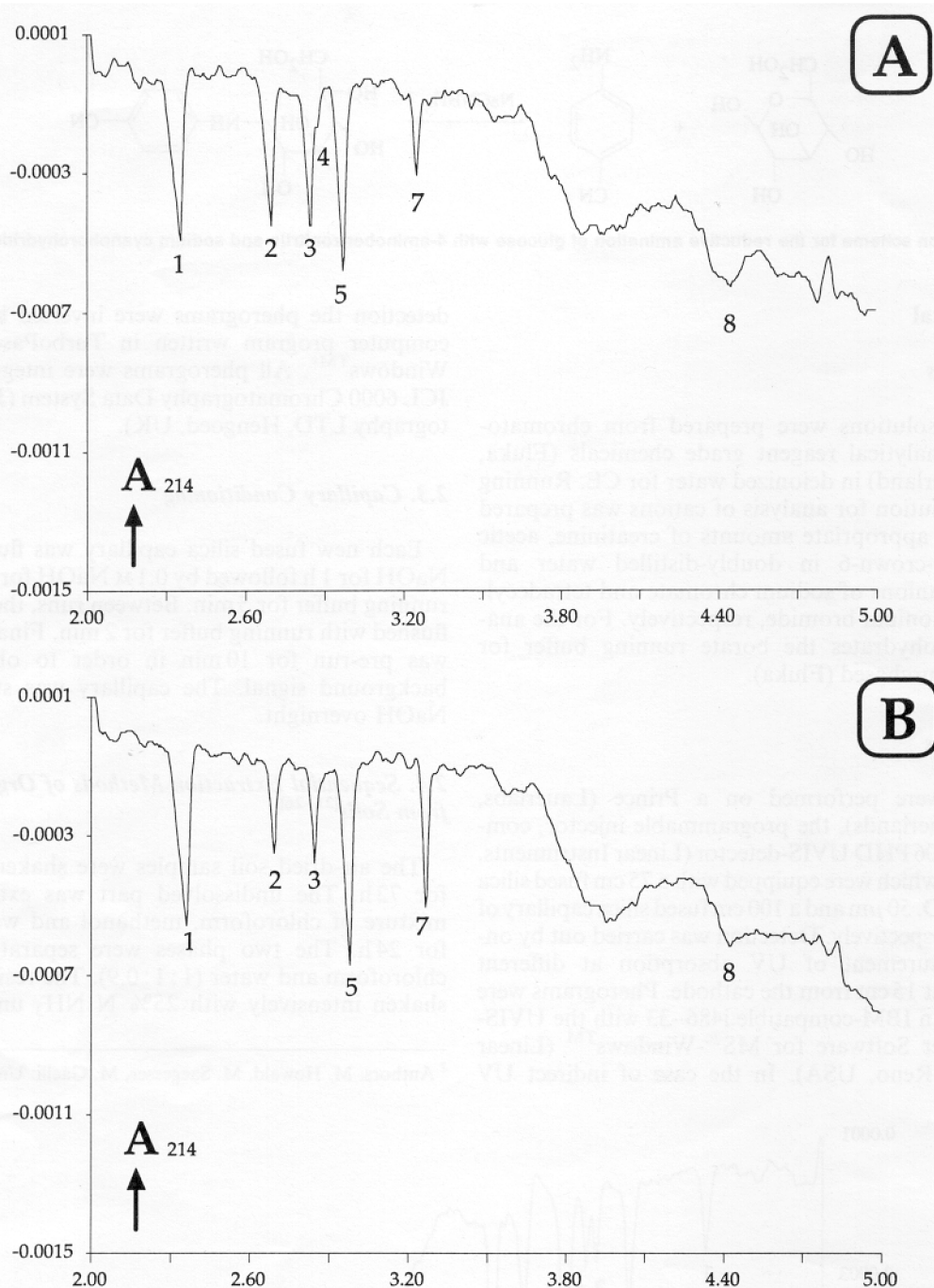


Figure 3. Simultaneous separation of alkali, alkaline earth, transition metal cations and ammonium. Fused silica capillary: 75 × 60 cm, 50 μ m I.D. Carrier electrolyte: 30 mmol creatinine, 30 mmol acetic acid and 4 mmol 18-crown-6, pH 3. Current: 7.3 μ A. Voltage: +25 kV. Separation temperature: 35 °C. Sampling for 6 s at 1 kV. Indirect UV detection at 214 nm. A: Peak identities and concentrations: (1) ammonium, 67 ppm; (2) calcium, 28 ppm; (3) magnesium, 15 ppm; (4) manganese, 6 ppm; (5) sodium, 13 ppm; (7) potassium, 5 ppm; (8) iron(III), 6 ppm. B (same conditions as A): (1) ammonium, 70 ppm; (2) calcium, 11 ppm; (3) magnesium, 6 ppm; (5) sodium, 15 ppm; (7) potassium, 10 ppm; (8) iron(III), 4 ppm.

24 h. All extractions were performed at room temperature. The dark coloured extract was separated from the corresponding insoluble residues by centrifugation at 6000 g for 15 min. The clear supernatant was acidified with 5 M HCl to pH 4, diluted with MeOH and allowed to stand at room temperature for 48 h. Soluble fulvic acid was then separated from the coagulate (humic acids) by centrifugation at 6000 g for 10 min.

2.5. Hydrolysis of Oligosaccharides for Component Analysis^[14,17]

2 mL of each water sample were taken in a capillary vial

and 100 μ L of 2 M trifluoroacetic acid were added. The solution was flushed with nitrogen for a few minutes. The vial was sealed and heated at 95 °C in an oven for 12 h.

2.6. Pre-column Derivatisation of Reducing Monosaccharides to 4-Aminobenzonitrile Derivatives

This was done according to a procedure published by Schweiger *et al.*^[2,20]. The reagent was freshly prepared before derivatisation by dissolving 10 mg of sodium cyanoborohydride in 1 ml methanol containing 4-aminobenzonitrile and acetic acid to a common concentration of 10% (w/v). 1 mL of the mixture of reducing

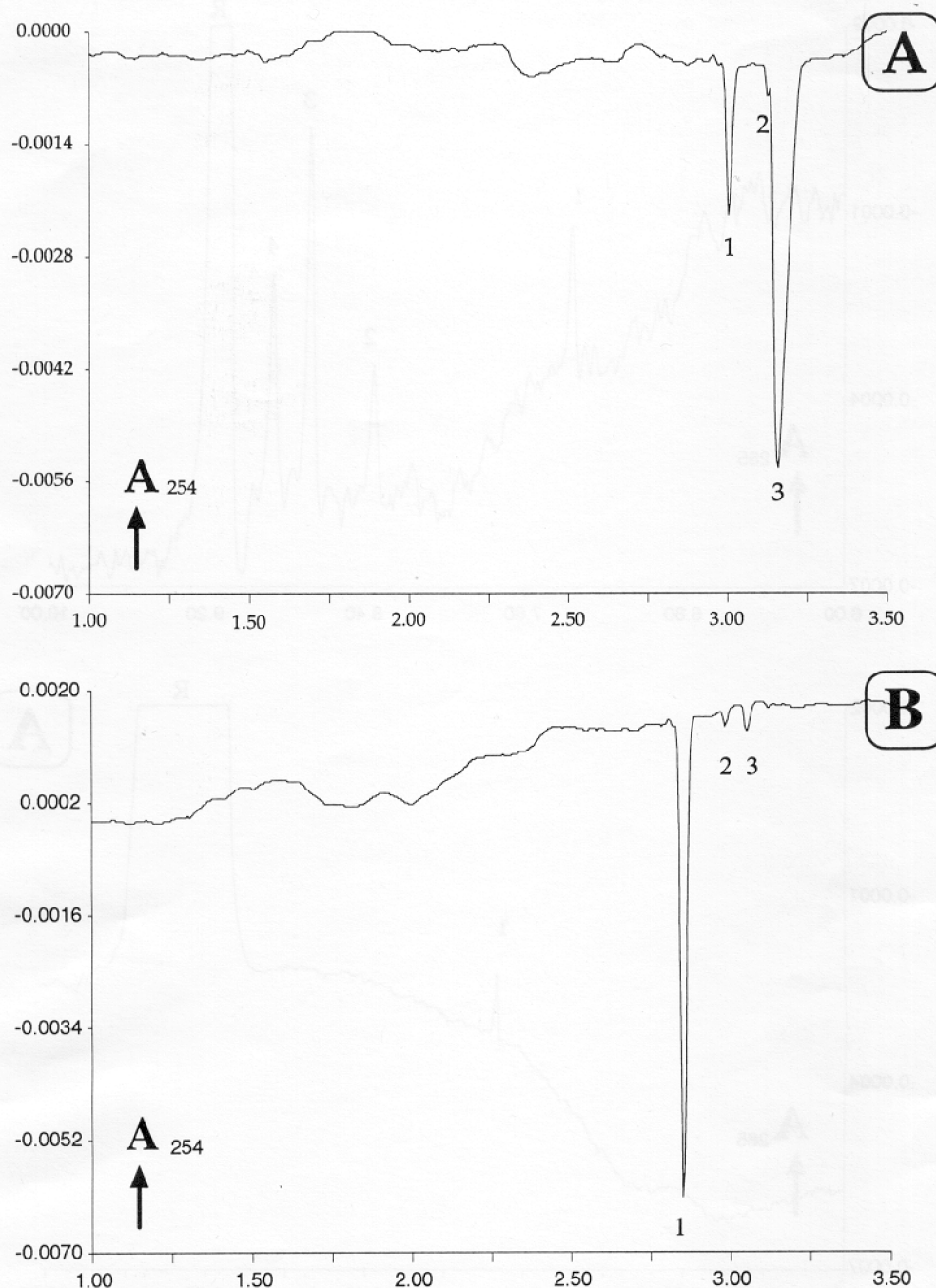


Figure 4. Two electropherograms of the three important anions in soil analysis. Fused silica capillary: 75×60 cm, $50 \mu\text{m}$ I.D. Carrier electrolyte: 5 mM sodium chromate, 0.5 mmol tetradecyldimethylammonium bromide, pH 7.0. Current: -12 mA. Voltage: -22 kV. Separation temperature: 35°C . Sampling for 6 s at -1 kV. Indirect UV detection at 254 nm. A: Peak identities and concentrations: (1) chloride, 3 ppm; (2) sulfate, 0.4 ppm; (3) nitrate, 34 ppm. B (same conditions as A): (1) chloride, 13 ppm; (2) sulfate, 0.2 ppm; (3) nitrate, 1 ppm.

monosaccharides was taken in a capillary vial and 1 mL of the reagent solution was added. The rate of the selected precolumn derivatisation is determined by relatively slow reaction kinetics. To reach a maximum yield, the mixture was maintained at 95°C in an oven for 1 h. The reaction mixture was directly injected into the capillary system (see Figure 1).

3. Results and Discussion

Two soil samples from the Valle Onsernone, Ticino, Switzerland were chosen to demonstrate the sensitivity,

rapidity and capability of the CE technique for the detection, separation and determination of small ions and molecules. Soil A represents an agriculturally used and fertilized, grass-covered area. Soil B has lain fallow for about ninety years and is totally overgrown by trees. The soils were extracted in the manner described above, all the cations, anions and reducing carbohydrates being dissolved in the methanol/water phase of the second extraction step.

The concentrations of each molecule were correlated with the measured peak area. Figure 2 shows as an example the reference electropherogram for cations with the concentrations and peak areas.

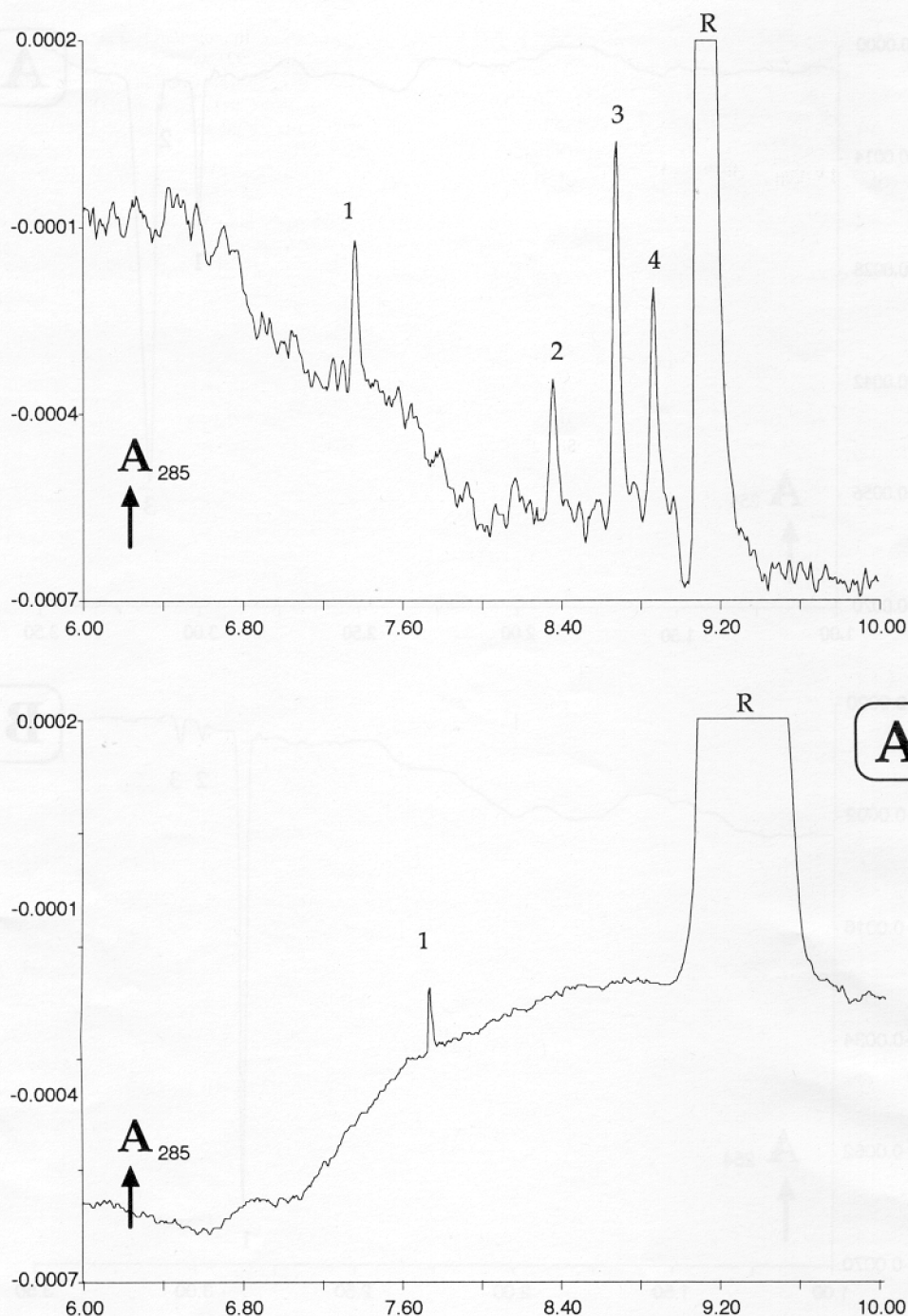


Figure 5. Micellar electrokinetic chromatography of 4-aminobenzonitrile derivatives of hydrolysed monosaccharides. Fused silica capillary: 75×60 cm, $75 \mu\text{m}$ I.D. Carrier electrolyte: 20 mM borate with 20 mmol SDS, pH 7.0. Current: $158 \mu\text{A}$. Voltage: +22 kV. Separation temperature: 35°C . Sampling for 1 s at 10 mbar. Direct UV detection at 285 nm. Peak identities and concentrations: (1) glucose 8 ppm; (2) mannose 11 ppm; arabinose 19 ppm; (3), xylose 14 ppm, (R) 4-aminobenzonitrile. A: (1) glucose 2 ppm.

3.1. Capillary Zone Electrophoresis of Inorganic Cations

Different soils can be classified according to their content and concentration of inorganic ions. Besides alkali and alkaline earth cations, pedologists are additionally interested in ammonium, aluminium and iron(III). Creatinine as the co-ion offers the possibility of detecting the eight interesting cations simultaneously. Since creatinine also acts as a highly absorbing background, the running buffer becomes very simple. Additionally, the complexing agent 18-crown-6 is needed to separate potassium from ammonium. Varia-

tion of the concentration of this agent makes it possible to control the shift of the potassium peak. We chose a concentration which shifted the potassium peak to a point behind the first group of cations. This electrolyte allows a clear, sensitive separation of the eight cations within 4.5 min, Figure 3.

3.2. Capillary Zone Electrophoresis of Inorganic Anions

For the three anions of interest, the often used and tested electrolyte containing chromate as a background

was selected. In the case of the anionic mode used for the separation of anionic molecules, an additive is necessary in order to obtain a reverse EOF that pushes the anions to the anionic end of the capillary. With tetradecyldimethylammonium bromide as the EOF modifier, all three anions were clearly separated and detected within 3 min, Figure 4.

3.3. Micellar Electrokinetic Capillary Chromatography of Monosaccharides

Component analysis of monosaccharides offers pedologists a further approach to the classification of a soil. Therefore, the carbohydrate material has first to be hydrolysed. The analysis of the four monosaccharides demanded (arabinose, glucose, mannose, xylose) led, among a large number of separation systems with different derivatisation methods, to reductive amination with 4-aminobenzonitrile. The derivatives are separated by the distribution of the aqueous electrophoretic and the micellar phase. This method allows an efficient separation of the four derivatized molecules within 9 min and simultaneously an excellent sensitivity and sharp direct UV detection at 285 nm. Due to the fact that in the two different soils only glucose was detected, the reference electropherogram is presented in addition, Figure 5.

4. Conclusion

CE of soil components fulfils the postulated major advantages: the analyses are made in a very short time and, the different techniques developed in the course of the experiment are highly sensitive. Moreover, the samples can be handled very easily: they may be directly injected into the tube, even after pre-column derivatisation. Regarding the intents of the pedologists and the normally large number of soil samples, CE offers a very efficient and simple method of analysis.

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