Determination of Organic Acids and Phosphate in Soil Aqueous Extracts by Capillary Zone Electrophoresis

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ABSTRACT

Organic acids and phosphate were determined in soil aqueous extracts by capillary zone electrophoresis using indirect UV detection. The electrolyte system was 10 mM sodium benzoate with 0.5 mM tetradecyltrimethyl-ammonium bromide as flow modifier at pH 4.0, pH 4.5, or pH 5.0. This methodology was adequate to determine organic acids and phosphate in soil samples, but the major inorganic anions interfered in the determination. In all the samples of soil extracts, the presence of phosphate was detected. Acetate was found in most of the samples and lactate and formate in some of them.

INTRODUCTION

Low molecular weight organic acids found in the soil are produced by root exudation and organic matter degradation. These acids participate in several processes, the most important of which is the formation of metal complexes that might act as detoxifying agents (Lundström, 1993).

Chromatographic techniques have been used for the determination of these acids in soils (Tani et al., 1993). Based on the work presented here, capillary electrophoresis (CE) provides a good alternative technique. Capillary zone electrophoresis (CZE) is the most common form of capillary electrophoresis used for the analysis of different kinds of samples (Grocott et al., 1994; Romano and Krol, 1993; Dolnik and Dolnikoviá, 1995), but it has not been used to a significant extent for the analysis of soil samples (Garrison et al., 1995; Ahumada et al., 1997). This technology utilizes narrow diameter capillaries and ionic species are separated according to their mobility under the influence of an applied potential, usually 10-30 kV. Detection of anions and short-chained carboxylic acids in CZE is carried out using indirect detection with the addition of a cationic surfactant to the electrolyte. Ions are separated as the electric force drives them at different rate, depending on their charge and size, trough a capillary filled with electrolyte. The most interesting ions are not UV active, the detection principle of indirect photometry is used, where the high background absorbance of the electrolyte system is diminished by the analyte. The appropriate surfactant is added to electrolyte in order to reverse the direction of the electroosmotic flow (EOF) by reversing the charge of the capillary wall. A variety of UV absorbing electrolytes have been used for different applications, including chromate (Wu et al., 1995; Boden et al., 1995), phtalate (Kenney, 1991), benzoate (Romano et al., 1991), p-hydroxybenzoate (Devèvre et al., 1994), pyromellitate (Arellano et al., 1997), borate (Shirao et al., 1994), etc. The purpose of this study was to find and document an adequate methodology for the determination of small amounts of organic acids and phosphate in aqueous extracts of soil.

MATERIALS AND METHODS

Instrumentation

Capillary Ion Analyzer (Waters) with data system using Millenium Software was used. The separations were carried out using a fused-silica capillary (60 cm x 75 µm ID) obtained from Waters. Detection was carried out using indirect photometry at 254 nm. The applied voltage was 20 kV using a negative power supply at a constant temperature of 25°C. Hydrostatic injection was used. The capillary was rinsed with 0.5M NaOH (5 min), water (5 min), and then with separation buffer for 2 min.

Reagents and Samples

All the standard solutions were prepared with reagents from Sigma (St. Louis, MO) and deionized water obtained from a Milli-Q system (Millipore, Bedford, MA). The electrolyte solution was 10 mM sodium benzoate with 0.5 mM tetradecyltrimetylammonium bromide as a flow modifier at pH 4.0, pH 4.5, or pH 5.0 (Romano et al., 1991). The samples and the electrolyte were filtered with a 0.45 µm pore membrane filter.
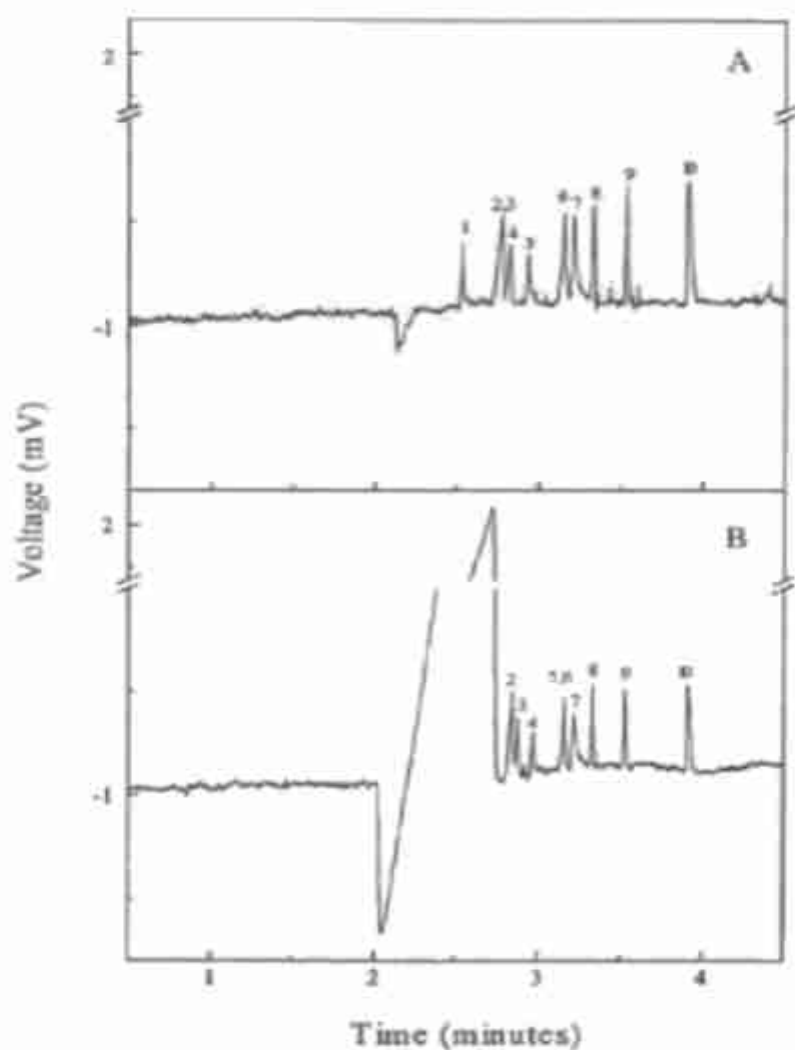
FIGURE 1. Electropherogram of 9 organic acids and phosphate without inorganic anions (A) and with inorganic anions (B), using benzoate at pH 4.5. 1) oxalate, 2) formate, 3) tartrate, 4) piruvate, 5) citrate, 6) malate, 7) phosphate, 8) lactate, 9) succinate, 10) acetate.
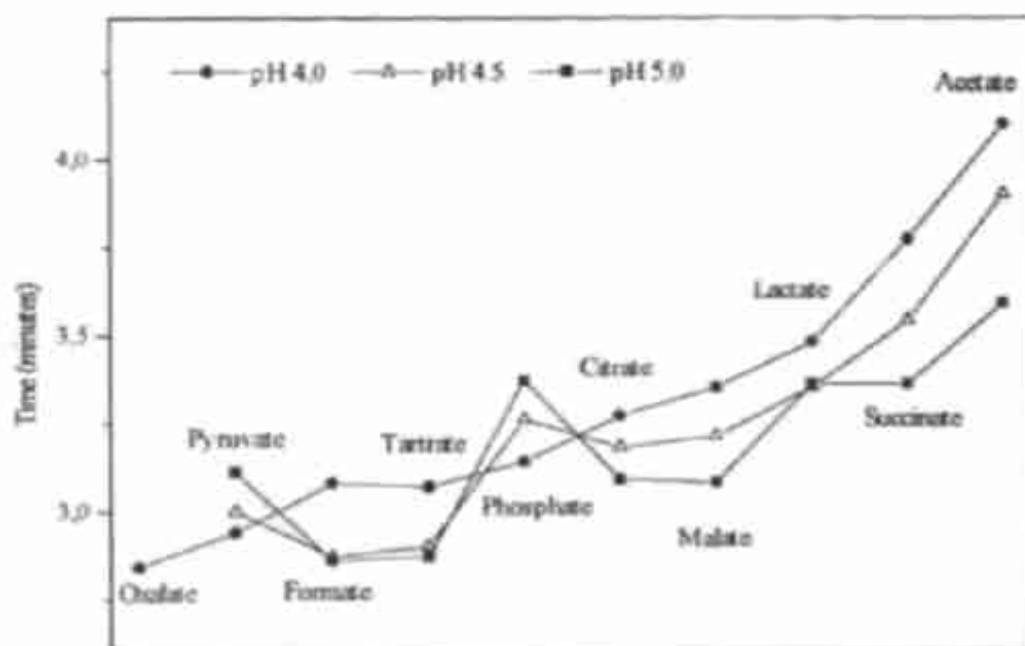
FIGURE 2. Migration times of organic acids and phosphate at different pH.

The soil samples for this study were collected from ten sampling sites of an Inceptisol soil irrigated with untreated industrial and domestic wastewater. The soil aqueous extracts were prepared by mixing soil and water in a ratio of 1:0.5, after 12 hours in contact, the soil was separated by centrifuging and the supernatant was filtered through a 0.45 μm cellulose nitrate membrane filter. The filtrate was stored at -30°C.

TABLE 1. Evaluation of quantification method (mg L⁻¹).

Method	Phosphate		Acetate	
	Added	Found	Added	Found
External standard without inorganic matrix	6.0	5.2	5.0	5.3
External standard with inorganic matrix	6.0	6.0	5.0	5.1
Standard addition	6.0	3.3	5.0	3.6
Internal standard	6.0	6.1	5.0	4.9

RESULTS AND DISCUSSION

The influence of chloride, sulphate and nitrate, the major inorganic anions of the soil matrix, on the determination of organic acids and, in addition to phosphate was evaluated by using the electrolyte at pH 4.5. A standard of 10 analytes with and without incorporating the inorganic matrix was utilized. Figure 1A shows the standard without the presence of inorganic anions, where only formate and tartrate are observed to comigrate. In the presence of inorganic anions, as shown in Figure 1B, formate and tartrate emerge after the peak due to the inorganic matrix and are resolved. Under these conditions, formate and tartrate do not comigrate, but oxalate is not detected.

Electrolyte pH is also an important factor in the determination of these analytes, since a pH variation can cause the separation of comigrating analytes. Thus, formate and tartrate comigrated at pH 4.0, but were separated at 4.5. At pH 5.0 citrate and malate as well as lactate and succinate comigrated, whereas at pH 4.0 or 4.5 they did not (Figure 2). It was also found that oxalate can only be detected at pH 4.0 in presence of inorganic matrix.

By simulating a soil extract with known amounts of phosphate and acetate, the quantification method described here was evaluated. The following methods were tested: external standard with and without inorganic matrix, standard addition, and internal standard. The best quantification method turned out to be external standard reproducing the soil inorganic matrix (Table 1). Thus this method was utilized to carry out the precision and recovery studies in the analysis of some organic anions using an aqueous soil extract without organic acid detected. Known amounts of standards of the following analytes were added to this soil extract: oxalate, tartrate, citrate, and acetate. They were quantified and the results are shown in Table 2. Good repeatability was obtained, with relative standard deviations (R.S.D.) less than 6%. In relation to recovery of added analyte, this was 97% acetate, and 102-105% of the other analytes.

The detection limits of some analytes such as oxalate, formate, tartrate, citrate, lactate, phosphate, and acetate were calculated through calibrations curves following Miller and Miller (1993). The calibration curves were made considering an inorganic matrix similar to that of the samples. The detection limits are between 0.1 and 1.5 mg L^{-1} for the different analytes (Table 3). These analyte detection limits are related to the concentration of inorganic anions in the sample, thus if the concentration of the latter is not very high, smaller analyte concentrations could be detected, especially those near the inorganic anion peak

In all the samples of soil extracts, the presence of phosphate and some organic acids was detected. Acetate was found in most of the samples; lactate and formate were detected in some (Figure 3). Analyte identity was confirmed by adding standard analyte solution and noting the corresponding increase in the peak area. Analyte concentrations found in 10 soil samples are shown in Table 4. Precision of the results is acceptable, with R.S.D. less that 9% in most of the cases. Only

TABLE 2. Determination of precision and % recovery (n=5) in the analysis of phosphate and some organic acids incorporated to soil aqueous extract.

Analyte	Amount added (mg L^{-1})	Amount found (mg L^{-1})	R.S.D.* (%)	Recovery (%)
Oxalate	8.3	8.6	5.3	104
Tartrate	7.7	8.1	2.8	105
Citrate	7.7	7.9	1.4	102
Acetate	7.7	7.4	2.8	97

*R.S.D.=relative standard deviation.

TABLE 3. Limits of detection (LOD).

Analyte	LOD (mg L^{-1})
Oxalate	1.5
Formate	0.2
Tartrate	1.3
Citrate	1.1
Lactate	0.3
Phosphate	0.4
Acetate	0.1

TABLE 4. Concentrations of organic acid and phosphate in the soil extract sample (n=5).

Soil	Formate ppm	Formate R.S.D.* %	Phosphate ppm	Phosphate R.S.D.* %	Lactate ppm	Lactate R.S.D.* %	Acetate ppm	Acetate R.S.D.* %
1	n.d.	-	6.9	4.5	n.d.	-	1.2	8.4
2	n.d.	-	6.8	2.9	n.d.	-	1.4	8.7
3	n.d	-	5.1	5.1	n.d.	-	0.4	4.8
4	0.9	20	6.4	2.0	1.5	7.5	0.7	6.8
5	n.d.	-	6.1	3.6	n.d.	-	n.d.	-
6	n.d.	-	4.0	3.0	n.d.	-	1.5	5.1
7	n.d.	-	4.3	3.4	n.d.	-	2.0	1.6
8	n.d.	-	3.7	2.4	n.d.	-	1.7	3.1
9	n.d.	-	4.2	2.8	7.1.	6.9	4.8	8.9
10	1.8.	6.4	33.5	1.2	n.d.	-	2.8	4.3

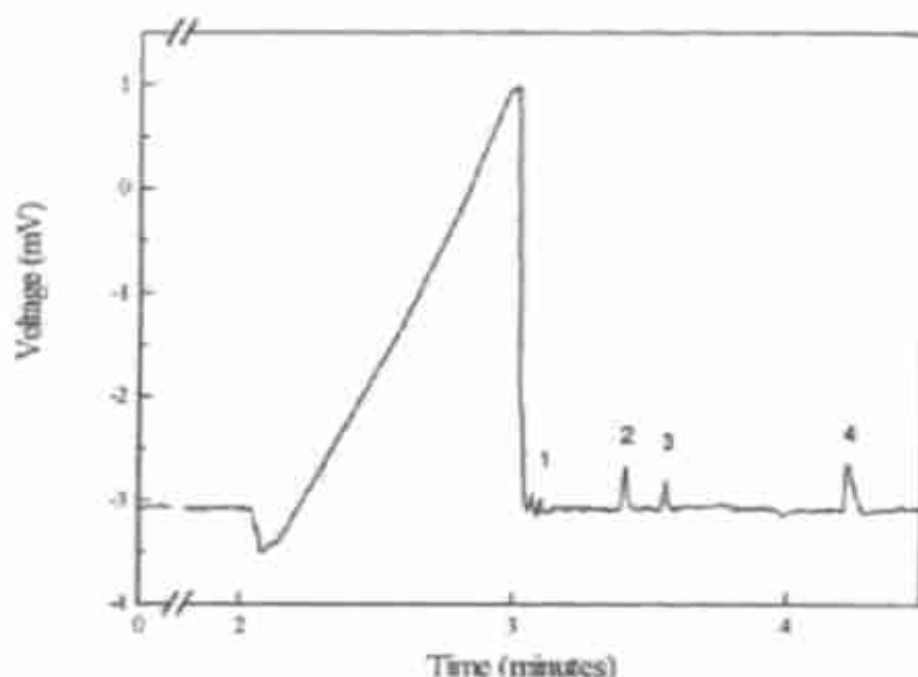*R.S.D.=relative standard deviation, n.d.=not detected.

FIGURE 3.   Electropherogram of a soil sample using benzoate at pH 4.5.   1) Unknown, 2) phosphate, 3) lactate, 4) acetate.

soil 4 showed a high R.S.D. of 20% for formate, which may be due to the small concentration of formate found and to the vicinity of the inorganic acid peak.

CONCLUSIONS

The methodology using benzoate as a support electrolyte is adequate to determine organic acids and phosphate in soil samples. The inorganic matrix of each sample must be considered in preparing the quantification standards. Utilization of the electrolyte at different pH is essential to solve the comigration problems. Acetate was detected in most of the soil samples; formate and lactate, in some soil samples.

ACKNOWLEDGMENTS

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