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Short communication

Simultaneous determination of inorganic anions, carboxylic and aromatic carboxylic acids by capillary zone electrophoresis with direct UV detection

Jianming Xu[a], ZuLiang Chen[b,*], Jimmy C. Yu[c], C. Tang[d]

[a]*Department of Resource Sciences, Zhejiang University, Hangzhou 310029, China*
[b]*Department of Plant Science, The University of Western Australia, Nedlands, WA 6907, Australia*
[c]*Department of Chemistry, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, China*
[d]*Soil and Plant Nutrition, The University of Western Australia, Nedlands, WA 6097, Australia*

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Abstract

Co-electroosmotic capillary zone electrophoresis (CZE) with direct UV detection was developed for simultaneous determination of inorganic anions, carboxylic and aromatic carboxylic acids. These solutes were separated using a 30 m*M* phosphate buffer containing 1.0 m*M* tetradecyltrimethylammonium bromide (TTAB) and 20% (v/v) acetonitrile at pH of 6.5 and directly detected by UV at 190 nm. Calibration curves were linear in the range 0.01–2.0 m*M*, depending of the solutes. The detection limits ranged from 1.0 to 8.0 μ*M* and the relative standards deviations ($n=5$) in range from 1.9 to 3.6% for the peak area. The proposed method was used to determine inorganic anions and carboxylic and aromatic acids in soil and plant tissue extracts.

Keywords: Soil; Plant materials; Inorganic anions; Carboxylic acids

1. Introduction

There are many chromatographic methods used for the determination of anions, carboxylic and aromatic acids. Ion chromatography (IC) is usually used for the analysis of inorganic anions [1], while ion-exclusion chromatography is frequently employed for the determination of organic and aromatic acids [2,3]. Since the requirements of columns, mobile phase and detectors differ, simultaneous analysis of anions, carboxylic and aromatic acids by HPLC in a single run has proven difficult. In addition, anionic species such as Cl^-, NO_2^- and NO_3^- in environmental and biological samples may interfere with the determination of carboxylic acids by ion-exclusion chromatography. It is difficult to quantify Cl^-, NO_2^-, NO_3^- and oxalic acid because of the poor separation efficiency [4]. Gas chromatography (GC) by trimethylsilylation can be used for the determination of organic acids, which provides excellent resolution and high detection sensitivity [5–7]. However, this method requires a complicated and time-consuming derivatization step. Thus, there is a need for a new analytical method for simultaneous determination of these solutes.

Capillary zone electrophoresis (CZE) is becoming

*Corresponding author. CSIRO Land and Water, PMB2, Glen Osmond, SA5064, Australia.

E-mail address: zuliang.chen@adl.clw.csiro.au (Z. Chen).

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an attractive method for the determination of ionic compounds due to its high separation efficiency, speed and simplicity. Commercial CZE instruments generally employ UV absorption detection systems adapted from HPLC [8]. Since most anions and organic acids have relatively weak absorption in the UV region, these species are frequently measured by CZE using indirect UV detection [9–17]. However, the effect of overloading is a serious problem in CZE by indirect UV detection [10,11]. This effect causes broad, triangular peaks when the effective mobility of the solute differs from that of the co-ion in the buffer. The rule used to minimise this effect is known as the mobility match rule [12], which states that a symmetric and narrow peak will be obtained when effective ionic mobility of the co-ion matches that of the ion of interest. Although a buffer containing multiple co-ions with different mobility can be used to overcome the overloading effect, the system peak would appear at a position between the mobility of the two co-ions. Recently, Soga and Ross [16] reported that simultaneous determination of inorganic anions, organic acids, amino acids and carbohydrates by CZE with indirect UV detection shows the greatest promise. Comparing to indirect UV detection in CZE for separation of organic acids, there are a few studies on CZE separation of anions and organic acids with direct UV detection. Volgger et al. [18,19] separated carboxylic acids using CZE with direct UV detection at 185 nm with a borate–phosphate buffer using hexadimethrine bromide as an electroosmotic flow (EOF) modifier. Barbas et al. [20] have reported the separation of eight carboxylic acids by CZE with direct UV detection at 200 nm using 200 m*M* phosphate buffer at pH 6.0 containing 0.5 m*M* cetyltrimethylammonium bromide (CTAB). This method has been successfully used for the determination of carboxylic acid in root exudates of *Lupinus luteus*. However, some important carboxylic and aromatic acids were not included in their study.

In a previous paper [4], we reported the ion-exclusion chromatographic separation of carboxylic acids with indirect UV detection using 2,6-pyridinedicarboxylic acid as mobile phase. We found that the anions (Cl^-, NO_2^- and NO_3^-), carboxylic acids (oxalic acid) and aromatic acids could not be resolved because these solutes play important roles in processes at the root–soil interface [21,22]. In this paper, a new CZE method for the simultaneous determination of inorganic anions, carboxylic and aromatic acids with direct UV detection has been developed. The parameters of the electrolyte buffer system, including the pH and organic modifier, were investigated to give a reasonable selectivity and sensitivity. The proposed method was tested for measuring anions, carboxylic and aromatic acids in plant and soil extracts.

2. Experimental

2.1. Chemical and sample preparation

All reagents obtained from Sigma and Aldrich (Sydney, Australia) were of analytical grade and used without further purification. Standards for the anions and organic acids tested were prepared daily from 10 m*M* stock solutions by dilution with deionised water. Electrolyte was prepared by dissolving the appropriate amount of NaH_2PO_4 in deionised water, which also contained appropriate amounts of tetradecyltrimethylammonium bromide (TTAB) and organic solvents. All electrolytes were filtered through disposable Millipore 0.45-μm membrane filters and degassed in an ultrasonic bath prior to use. Electrolyte pH was adjusted with 0.1 *M* NaOH and 0.1 *M* HCl solution. Solutes in powdered plant tissue were extracted twice with 5 ml water (0.1 g/5 ml) held in a water bath at 50°C for 1 h. Soils were mechanically shaken with water at pH 6.8 (1 g/ml) for 8 h as described previously [13]. The extracts were then centrifuged and the supernatants filtered through the Millipore 0.45-μm membrane filter before injecting into the CZE system.

2.2. Instrumentation

All CZE experiments were preformed using a Bio-Rad 30000 capillary electrophoresis system (Bio-Rad, CA, USA) which was controlled with a personal computer running CE-3000 software (Bio-Rad). Separation was carried out on fused-silica capillaries of 75 cm (70.4 cm effective length)×50 μm I.D. The UV detector was set for the rapid scanning of absorbance range between 190 and 230 nm to obtain the optimal wavelength.

2.3. Electrophoretic procedures

Prior to use, a new capillary was pretreated by passing 0.1 *M* NaOH for 20 min, followed by deionised water for 30 min. The capillary was then pre-conditioned based on the following cycles: 0.1 *M* NaOH for 3 min, then deionised water for 5 min and finally run electrolyte buffer for 5 min. The sample was injected in the hydrodynamic mode for 10 s. The capillary was thermostated at 25°C, and a constant voltage of −20 kV. Benzyl alcohol (0.05%, v/v) was used as a neutral marker, and the mobility of the solute was calculated from the equation described in a previously report [8]. The identification of the test solute was based on the migration time and confirmed by spiking the sample of known test solutes.

3. Results and discussion

3.1. Separation of anions, carboxylic and aromatic acids

In CZE, changing the electrolyte pH and the organic solvent in the buffer can control the separation selectivity of organic acids [8,15–17]. In this study, a 30 m*M* phosphate buffer containing 1.0 m*M* TTAB and 10% (v/v) acetonitrile was used to separate the solutes due to the buffer's low UV absorbance. In general, the effective mobility for the test solutes increases, as the electrolyte pH is increases. Clearly, for the separation of carboxylic acids, the electrolyte pH had a significant effect on the resolution. A pH value of 6.5 was used in the subsequence studies. The influence of acetonitrile on the mobility of solute and the EOF was studied by adding acetonitrile to the mobile buffer containing 30 m*M* phosphate at pH 6.5. Considering both separation time and resolution, an acetonitrile content of 20% (v/v) was chosen to add to the buffer electrolyte.

Fig. 1 gives an example of the optimised separation of a standard mixture containing three anions, eight carboxylic and seven aromatic acids, which play important roles at the soil–plant interface [22,23]. The running electrolyte contained 30 m*M* phosphate, 1 m*M* TTAB and 20% acetonitrile at pH 6.5. Anions, carboxylic and aromatic acids can be

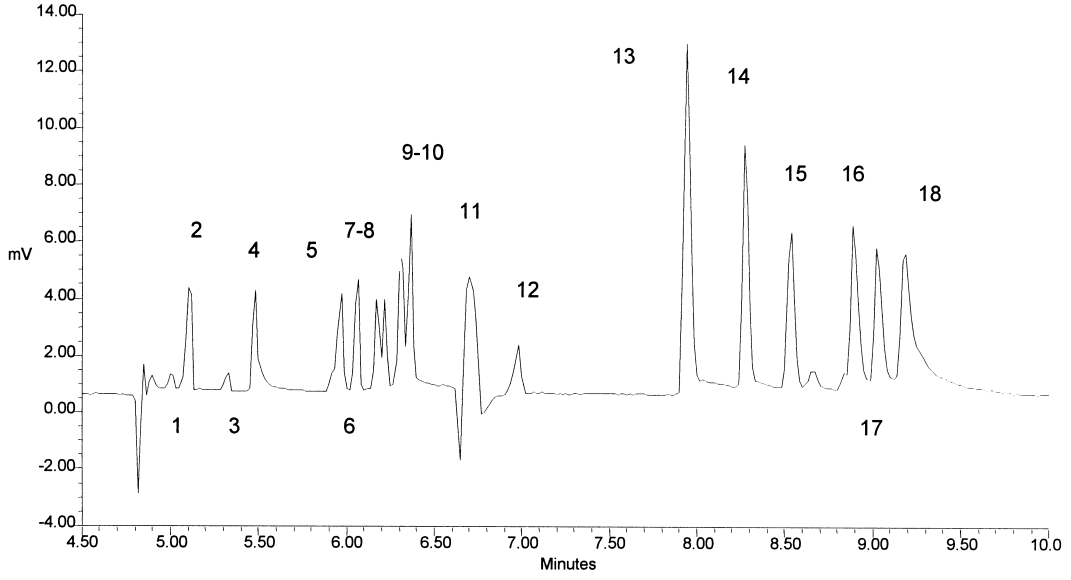

Fig. 1. A typical electropherogram obtained using optimised conditions. Peaks: (1) NO_2^-; (2) NO_3^-; (3) SO_4^{2-}; (4) oxalic acid; (5) fumaric acid; (6) tartaric acid; (7) malonic acid; (8) malic acid; (9) citric acid; (10) maleic acid; (11) phthalic acid; (12) acetic acid; (13) benzoic acid; (14) salicylic acid; (15) *p*-hydroxybenzoic acid; (16) coumaric acid; (17) ferulic acid; (18) sinapinic acid. Electrolyte, 30 m*M* sodium+1.0 mm TTAB+20% (v/v) acetonitrile at pH 6.5. Concentration for each solute: 0.10 m*M*. Applied potential, −20 kV; hydrostatic injection: 10 s, UV detection at 190 nm. Capillary temperature, 25°C.

separated with adequate resolution and detected at 190 nm. The migration time for the solutes increased as its effective mobility decreased. However, no relationship was observed between migration order and pK_a among mono-, di- and tricarboxylic acids, and aromatic acids. The reproducibility, linearity and detention limits for the solute using the proposed method are listed in Table 1. The relative standard deviations ($n=5$) of the method for the solutes were acceptably low at between 1.9 and 3.6% for the peak area. The linearity of the method was evaluated by injecting standards with concentrations ranging from 0.1 to 5 m*M*. The calibration plots for all test solutes were linear in the concentration range of 0.01–2.0 m*M* (depend on the solute) with correlation coefficients of better than 0.9993. Detection limits ($S/N=3$) ranging from 1.0 to 8.0 μ*M* were achieved.

3.2. Analysis of plant tissue and soil extracts

The proposed method was used to determine the concentration of inorganic anions and organic acids in soil and plant extracts. Fig. 2a shows a typical electrophoregram obtained from the soil extract. Clearly, there is no interference from the matrix with direct sample injection. Anions, carboxylic acids, and aromatic acids were identified by their migration time and by spiking known standards. The concentrations of SO_4^{2-}, oxalic, acetic and benzoic acid in the soil extract were 150, 9, 210 and 10 μ*M*. The proposed method was also used for the determination of carboxylic and phenolic acids in plant extracts as shown in Fig. 2b. The carboxylic and phenolic acids were identified in plant tissue extracts, and reasonable resolution between each solute was obtained. The concentrations of tartaric, malic, citric, benzoic, *p*-hydroxybenzoic, and coumaric acids in plant tissue extract were 15, 165, 30, 70 and 55 μ*M*, respectively. Satisfactory reproducibility for the peak areas of the solutes were obtained with 2.0–4.9% RSD ($n=5$). Recoveries of 91.5–102.5% soil and plant extracts spiked 0.1 m*M* were obtained. The proposed CZE method demonstrated that simultaneous determination of anions, carboxylic and aromatic acids in a single run is now possible. The results have shown less interference from sample matrices than

Table 1
Linearity, detection limit and reproducibility

Solute	Linearity (m*M*)	Detection limit (μ*M*)	Reproducibility (peak area, RSD, %, $n=5$)
NO_2^-	0.05–1.5	3.0	2.8
NO_3^-	0.01–1.0	2.0	2.1
SO_4^{2-}	0.01–1.5	8.0	3.2
Oxalic acid	0.05–1.0	5.0	1.9
Fumaric acid	0.05–1.0	1.0	2.3
Tartaric acid	0.01–1.0	1.0	2.1
Malonic acid	0.01–1.0	1.0	1.9
Malic acid	0.01–1.0	2.0	2.8
Citric acid	0.01–1.0	1.0	3.1
Maleic acid	0.01–1.0	1.0	2.8
Phthalic acid	0.01–2.0	3.0	3.6
Acetic acid	0.01–1.0	2.0	3.2
Benzoic acid	0.01–2.0	2.5	2.9
Salicylic acid	0.01–2.0	3.0	3.2
p-Hydroxybenzoic acid	0.01–2.0	4.0	2.9
p-Coumaric acid	0.01–2.0	4.0	2.8
Ferulic acid	0.01–2.0	4.0	2.7
Sinapinic acid	0.01–2.0	7.0	3.1

Conditions as in Fig. 1; detection limit: $S/N=3$.

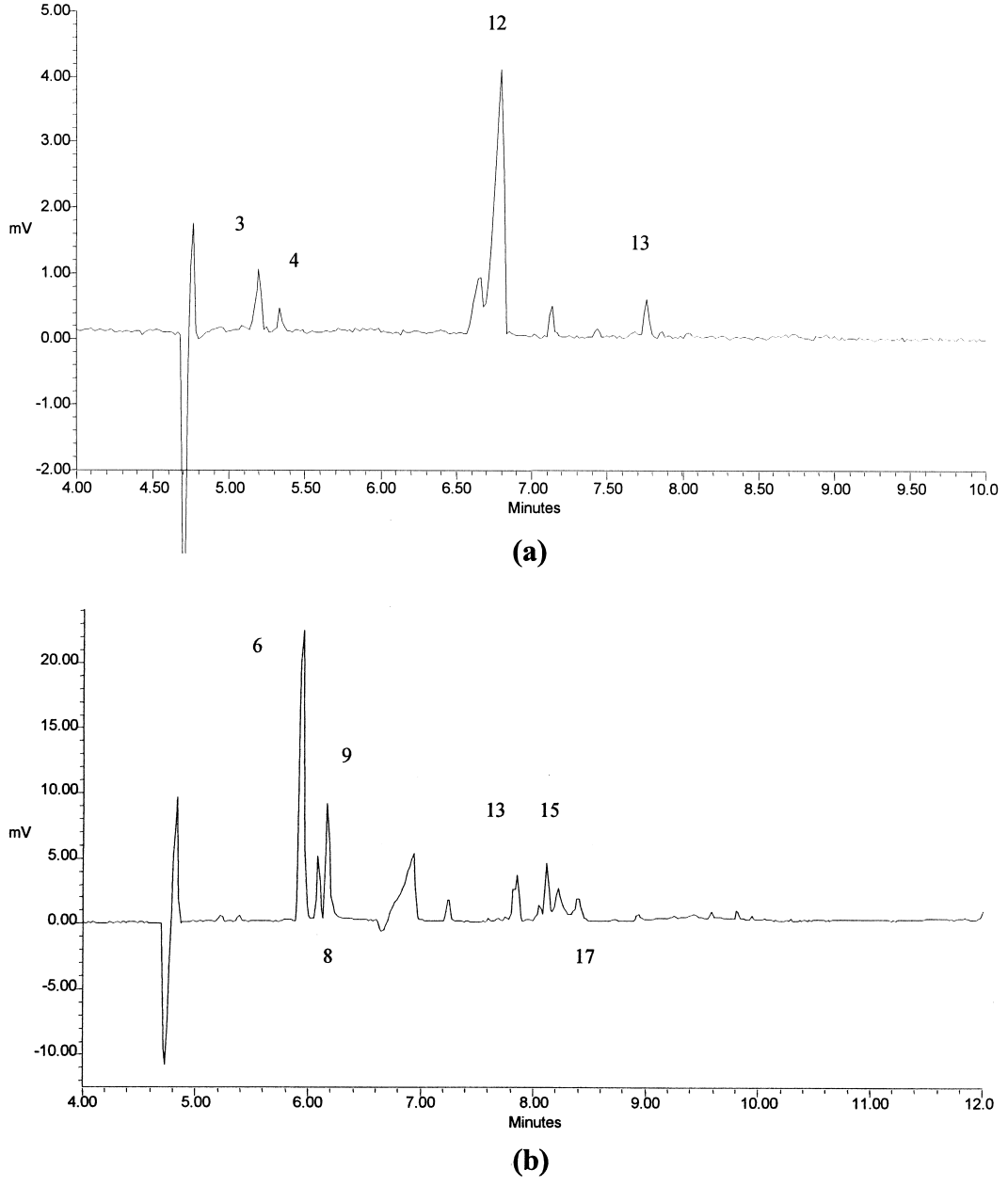

Fig. 2. Soil and plant tissue samples analysed by the proposed method. (a) Soil extract, (b) plant extracts. Conditions as in Fig. 1.

ion exclusion since the use of CZE offers higher selectivity. Therefore, solutes such as Cl^-, NO_2^- and NO_3^- or oxalic, citric and malic acids were resolved, although co-elution occurred in ion-exclusion chromatography [1–4].

4. Conclusions

A CZE method for the simultaneous determination of anions, carboxylic and aromatic acids was developed with direct UV detection at 190 nm, where

the running buffer contains 30 m*M* phosphate, 1 m*M* TTAB, and 20% (v/v) acetonitrile at pH 6.5. Compared to other separation methods, these solutes can be simultaneously analysed without derivatization, and reasonable resolution can be achieved without matrix interference. In addition, the proposed method also offers good reproducibility of the peak area and simplicity in terms of sample preparation. The method has been applied to demonstrate the simultaneous determination of anions, carboxylic and aromatic acids in plant and soil extracts.

Acknowledgements

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References

[1] Z.L. Chen, M.A. Adams, Chromatographia 49 (1999) 496.
[2] Z.L. Chen, M.A. Adams, Acta Anal. Chim. Acta 386 (1999) 249.
[3] Z.L. Chen, M.A. Adams, J. Liq. Chromatogr. Rel. Technol. 21 (1998) 2435.
[4] Z.L. Chen, C. Tang, J. Xu, J. Chromatogr. A 859 (1999) 173.
[5] M.A. Adams, Z. Chen, P. Landman, T.D. Colmer, Anal. Biochem. 264 (1999) 77.
[6] Z.L. Chen, P. Landman, T. Colmer, M.A. Adams, Anal. Biochem. 259 (1998) 203.
[7] A.M. Szmigielska, K.C.J. Van Rees, G. Cieslinski, P.M. Huang, D.R. Knott, J. Agric. Food Chem. 43 (1995) 956.
[8] J.P. Landers, in: Handbook of Capillary Electrophoresis, 2nd edition, CRC Press, Boca Raton, FL, 1997.
[9] W.K. Klampfl, W. Buchberger, P.R. Haddad, J. Chromatogr. A 881 (2000) 357.
[10] H. Poppe, Anal. Chem. 64 (1992) 1908.
[11] X. Xu, W.Th. Kok, H. Poppe, J. Chromatogr. A 742 (1996) 211.
[12] F. Foret, S. Fanali, L. Ossicini, P. Bocek, J. Chromatogr. 470 (1989) 299.
[13] Z.L. Chen, C. Tang, J.C. Yu, J. High Resolut. Chromatogr. 22 (1999) 379.
[14] T. Wang, R.A. Hartwick, J. Chromatogr. 589 (1992) 307.
[15] T. Soga, G.A. Ross, J. Chromatgr. A 767 (1997) 223.
[16] T. Soga, G.A. Ross, J. Chromatogr. A 837 (1999) 231.
[17] C.H. Wu, Y.S. Lo, Y.H. Lee, T.I. Lin, J. Chromatogr. A 716 (1995) 291.
[18] D. Volgger, A.J. Zemann, G.K. Bonn, J. High Resolut. Chromatogr. 21 (1998) 3.
[19] D. Volgger, A.J. Zemann, G.K. Bonn, M.J. Antal, J. Chromatogr. A 758 (1997) 263.
[20] C. Barbas, J.A.L. García, F.J.G. Manero, Phytochem. Anal. 10 (1999) 55.
[21] S.M. Masselter, A.J. Zemann, Anal. Chem. 67 (1995) 1047.
[22] M. Mench, E. Martin, Plant Soil 132 (1991) 187.
[23] W. Petersen, M. Bottger, Plant Soil 132 (1991) 159.