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Determination of major cations in soil extracts is important for soil research. This study introduced a new buffer system (Buf-I) for capillary-zone electrophoresis (CZE) for the detection of major cations in soil. The Buf-I system achieved baseline separation of major cations with a wider detection range, in less time, and with better reproducibility than the old buffer system (Buf-M). Storage stability and running stability experiments showed that the running current of Buf-I was also more stable and the relative standard deviation (RSD%) was significantly less than those of Buf-M. The recovery (91.13–107.64%) by Buf-I was better than that (78.38–103.87%) by Buf-M. The results by Buf-I method were highly consistent with traditional analysis techniques (with a correlation of 0.9963). Therefore, we conclude that the new Buf-I system is more stable and accurate than the old Buf-M system and is especially suitable to determine major cations in soil extract.

Keywords   CZE determination, major cations, new buffer, soil extracts

Introduction

Determination of major cations [ammonium (NH_4^+), potassium (K^+), calcium (Ca^{2+}), sodium (Na^+), and magnesium (Mg^{2+})] in soil is important for soil research. These cations are important soil nutrients that affect soil productivity, but their accumulation in the soil can result in secondary salinization under conditions of continuous cropping and high rates of fertilizer application, especially in intensive greenhouse production systems (Stigter et al. 1998; Huan et al. 2007; Shi, Yao, and Yan 2009; Sonneveld and Voogt 2009). Furthermore, NH_4^+ in soil is a major source of diffusion pollution through the volatilization of ammonia (NH_3)–nitrogen (N) and the leaching of nitrate (NO_3)-N (Purakayastha and Katyal 1998; Zhao et al. 2010; Olaleye et al. 2008). Therefore, identification of the major cations in soil is important for efficiently providing references for sustainable land use and soil environmental risk assessments.

Compared with traditional analytical methods, the rewards for using capillary electrophoresis (CE) include high separation efficiency, low material and sample consumption, relatively short analysis times, and low investment and operational costs (Timerbaev 2002). The carrier buffer is the key factor in capillary-zone electrophoresis (CZE) analysis. For the detection of major inorganic cations such as NH_4^+, K^+, Ca^{2+}, Na^+, and Mg^{2+} in soil extracts, a carrier buffer system (Buf-M) containing Metol and ascorbic acid has been applied by various researchers (Howald et al. 1995; Göttlein, Hell, and Blasek 1996; Dieffenbach, Göttlein, and Matzner 1997; Göttlein 1998; Wang, Kelly, and Kovar 2007). However, because Metol and ascorbic acid are oxidizable in air, the corresponding buffer systems may be unstable; this may affect the reproducibility and accuracy of results and therefore CZE using Buf-M may not be the best choice for the determination of samples.

Different buffer systems containing imidazole and α-hydroxyisobutyric acid (HIBA) are effective for cation separation. Imidazole is stable and has a similar mobility to these cations as a background agent, and HIBA is a common complexing agent that improves the separation of Na^+ and Mg^{2+} (Beck and Engelhardt 1992; Lin, Lee, and Chen 1993). Francois, Morin, and Dreux (1995) used a carrier buffer containing 10 mM imidazole and 2.5 mM 18-crown-6 ether at a pH of 4.5; Quang and Khaledi (1994) used a buffer containing 6 mM imidazole and 12 mM HIBA; Tangen, Lund, and Frederiksen (1997) used an HIBA/18-crown-6 solution with 25% (v/v) methanol to obtain baseline separation of 70 mg L^{-1} Na^+, 20 mg L^{-1} K^+, 50 mg L^{-1} Mg^{2+}, 30 mg L^{-1} Ca^{2+}, 10 mg L^{-1} strontium (Sr^{2+}), and 10 mg L^{-1} barium (Ba^{2+}).

However, soil compositions are complex, consisting of organic and inorganic components at high or low concentrations depending on soil type and plant growth mode (Strom et al. 2001; Vetterlein and Jahn 2004; Shi, Yao, and Yan 2009; Shen et al. 2007). Whether HIBA and imidazole are adaptable to the determination of soil solutions from various types soil is unclear. In this study, an optimum carrier buffer (Buf-I) mainly containing HIBA and imidazole was used for soil extract determination of major cations (NH_4^+, K^+, Na^+, Mg^{2+}, and Ca^{2+}) in different soil solutions, and its storage and running stability were compared to those of the old buffer (Buf-M).

Materials and Methods

Instrument of Capillary-Zone Electrophoresis and Agents

All experiments were performed by indirect detection mode of CZE. The equipment was a P/ACE MDQ apparatus (Beckman Instruments, Inc., Fullerton, Calif., USA) equipped with a photodiode array detector (PDA). The fused-silica capillary had an internal diameter of 75 µm and was 60 cm long (effective length 53 cm). Samples were automatically injected with the parameters of 0.3 psi × 5 s. The applied voltage and temperature were 30 kV and 24 °C, respectively. Sample absorbance was monitored at 214 nm for Buf-I and 220 nm for Buf-M (Wang, Kelly, and Kovar 2007) through a 100- × 800-µm aperture. The reagents imidazole, HIBA, 4-methylamino-phenole-sulfate (Metol), ascorbic acid, and 18-crown-6 ether were obtained from Sigma (Sigma, St. Louis, Mo., USA). All other reagents were of guaranteed grade from Nanjing Chemical Plant. Water for calibration samples, buffer, and rinsing solutions was deionized and purified with a Waters Milli-Q water purification system (Millipore, Eschborn, Germany) and sterilized in an autoclave at 120 °C for 20 min.

Table 1
Types and origins of tested soil

Number	Types	Origins	Longitude and latitude
1	Udic Ferralosols	Yingtan, Jiangxi Province	E 116° 55′, N 28° 15′
2	Orthic Anthrosols	Wuxi, Jiangsu Province	E 120° 17′, N 31° 31′
3	Stagnic Anthrosols	Yixing, Jiangsu Province	E 119° 53′, N 31° 19′
4	Udic Isohumosols	Hailun, Heilongjiang Province	E 126° 38′, N 47° 26′
5	Ustic Cambosols	Hebei Province	E 114° 39′, N 38° 10′
6	In situ soil solution in Stagnic Anthrosols with culturing rice (*Oryza sativa* L.)	Yixing, Jiangsu Province	E 119° 53′, N 31° 19′

Soil Extract Samples

Soil samples (1–5) were taken from five different regions of China with unproductive soil types (Table 1). Soil extract solutions for these samples were obtained by shaking soil and water at a 1:2.5 w/w soil/water ratio for 1 h, centrifuging at 5000g for 5 min, and filtering through 0.45-μm membrane filters. Sample 6 was obtained using the micro-suction-cup method on bulk soil used for rice (*Oryza sativa* L.) culturing according to Göttlein, Hell, and Blasak (1996).

Optimization of HIBA and Imidazol Buffer Composition

To optimize the buffer composition for best performance, the resolution degree (Rs) was chosen as the main index. The concentrations of imidazole and HIBA were evaluated from 6 to 16 mM and 4 to 25 mM, respectively. The concentration of 18-crown-6 ether was held constant at 2 mM, and the pH was varied between 3.5 and 4.5. The optimum process to obtain the Buf-I system is described elsewhere (Liu and Shi 2009). To obtain best performance, the optimum buffer, named Buf-I, contained 0% methanol, 10 mM imidazole, 6 mM HIBA, and 2 mM 18-crown-6 ether at pH 3.7 with slightly adjustment on the base of the patent (Liu and Shi 2009).

Buffer Stability Comparisons

The storage stability of Buf-I and Buf-M (5 mM Metol, 1 mM ascorbic acid, and 2 mM 18-crown-6; Wang, Kelly, and Kovar (2007) was examined by remeasuring the standard mixture solution (10 mg L^{-1} NH$_4^+$, K$^+$, Na$^+$, and Mg^{2+}; 100 mg L^{-1} Ca^{2+}) 0, 2, 4, 6, and 8 h after the buffers had been made and kept at room temperature. Prior to standard mixture detection, the capillary rinse procedure was performed as follows: 0.1 M NaOH for 10 min, water for 10 min, and buffer for 10 min; the conditioning sequence was then conducted without sample injection. For successive detection runs of samples of the same

buffer system, the rinse procedure was performed only with the carrier buffer for 2 min. The running stability experiment was carried out by consecutive analysis ($n = 20$) of the tested buffer systems, and the running current and relative standard deviation (RSD) of peak areas were recorded.

Comparison of the Buf-I Method of CZE to Inductively Coupled Plasma and Flow Stream Analysis

The performance of Buf-I was quantified using indices including linearity, correlation coefficients, detection range, and recovery percentage. Indices were recorded for consecutive runs using Buf-I and Buf-M (if needed), except for recovery, which was evaluated using running sequences of the soil samples, the standard mixture, and the soil-sample-added standard mixture. To demonstrate the applicability of the Buf-I method to soil cation determination, the soil extracts of the five different types soil (1–5) from China were evaluated. To evaluate its accuracy, the same samples were analyzed by inductively coupled plasma (ICP) for K^+, Na^+, Mg^{2+}, and Ca^{2+} (McGrath and Cunliffe 1985) and by flow stream analysis (using an instrument from Skalar, Breda, the Netherlands) for NH_4^+.

Data Analysis

Data were analyzed with Beckman 32 Karat v. 8.0 (Beckman Coulter, Brea, Calif.) and SPSS systems (SPSS, Chicago, Ill.). The average relative peak area was considered the average of those at the different storage times (2, 4, 6, 8 h) relative to that at 0 h.

Results

Determination of Cations by CZE using the Buf-I System

To evaluate the separation effect of Buf-I, random soil sample 3 was repeatedly analyzed by both the Buf-I system and the Buf-M system in 4-h intervals. The separation of the five major cations in the soil sample is shown in Figure 1. Both buffer systems achieved baseline separation of all cations, but the order of the Ca and Na peaks differed between the two systems. The reproducibility of peaks also differed significantly; that is, the migration times and peaks of two Buf-I separations performed 4 h apart could be superimposed, both those of the Buf-M separations could not. Also, the determination time of the Buf-I system was shorter than that of the Buf-M system (Figure 1). However, the peak area and peak height by Buf-I are all less than that by Buf-M system.

Storage Stability of the Buf-I and Buf-M Systems

To evaluate the storage stability of Buf-I and Buf-M, determination of a known standard mixture was performed using both buffer systems 0, 2, 4, 6, and 8 h after the buffers were made. The running current of Buf-I was constant at 22.51 ± 0.07 mA, with only a slight increase over time, whereas that of Buf-M decreased with storage time, from 20.01 to 19.17 mA (Figure 2). The RSDs of all peaks at different storage times were calculated according to the area of the separation peaks. The RSD value for Buf-I varied from 3.11% to 7.77% and was significantly less than that for Buf-M, which varied from 9.05% to 13.18% (Table 2). To elucidate the changing trends of separation at different storage times, the average relative peak area at 2, 4, 6, and 8 h was considered (Figure 3). The average
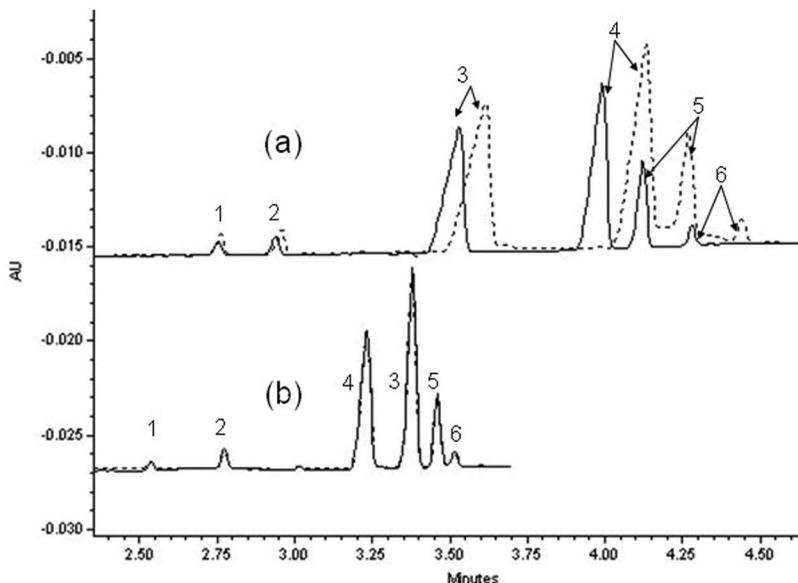
Figure 1. Comparison of electropherograms of soil sample 3 with 4-h intervals by CZE with buffer of Buf-M (a) and Buf-I (b). The solid line is the first separation and the dotted line is the second separation after 4 h. Peak NH_4^+, K^+, Na^+, Ca^{2+}, and Mg^{2+} are in that order in all figures; the component concentrations of soil sample are provided in Table 5. The y axis shows absorbance values at 220 nm (a) and 215 nm (b).
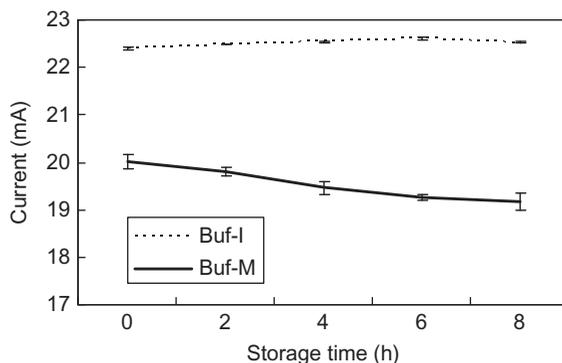
Figure 2. Comparison of running current by Buf-M and Buf-I with different storage times (0, 2, 4, 6, and 8 h).

relative peak area for the Buf-I system remained constant at 97.5–101.3%; in contrast, that for the Buf-M system increased with storage time from 114.9 to 128%.

Running Stability Comparison of the Buf-I and Buf-M Systems

The running stability of the two buffer systems was examined by consecutive runs ($n = 20$) of both systems. Figure 4 shows the running current of each separation. Showing the same constancy as storage stability, the running current of Buf-I remained at

Table 2
Comparison of relative standard deviation of peak area by
Buf-I and Buf-M with different storage times

	RSD% storage time	
Cations	Buf-M	Buf-I
Ammonium (B)	12.09	6.03
Potassium (B)	13.18	7.77
Calcium (B)	10.83	3.11
Sodium (B)	9.05	4.62
Magnesium (B)	9.10	3.79

Note. The B in the table means significant difference at
$P < 0.05$ between two buffer systems.
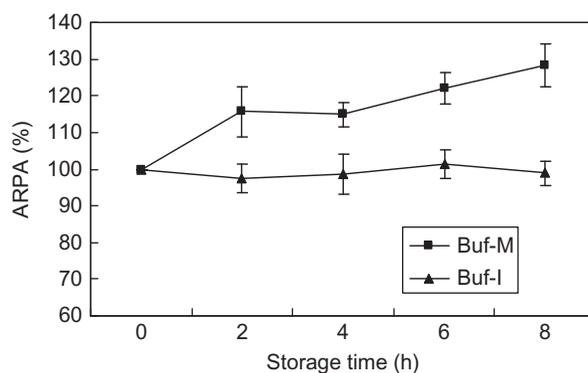
Figure 3. Comparison of average relative peak areas (ARPA) by Buf-I and Buf-M with different storage times to that at 0 h.
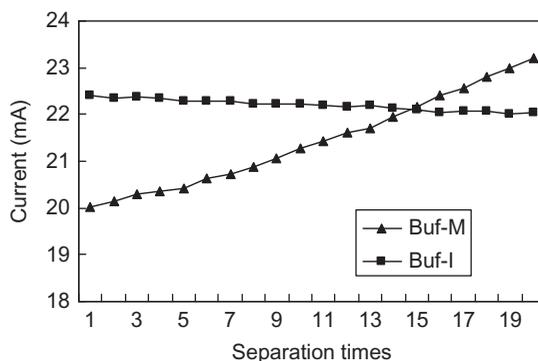
Figure 4. Comparison of running current of the consecutive separation by Buf-M and Buf-I.

22.20 ± 0.12 mA, with a small decline as separation time increased. In contrast, the running current of Buf-M increased from 20.01 to 23.20 mA as running time increased. The RSD of the peak area of consecutive runs of the two buffer systems is shown in Table 3; that for the Buf-I system (1.7–5.53%) was significantly less than that for Buf-M (5.30–10.33%).

Table 3
Comparison of relative standard deviations of peak areas by
consecutive separations (n = 10) of Buf-I and Buf-M

	RSD% replication	
Cations	Buf-M	Buf-I
Ammonium (B)	8.61	4.40
Potassium (B)	10.33	5.53
Sodium (B)	7.30	1.88
Magnesium (B)	5.36	1.90
Calcium (B)	5.30	1.70

Note. The B in the table means significant difference at $P < 0.05$ between
two buffer systems.

Table 4
Linearity, recovery (%), and RSD (%) for major cations by Buf-I system
and Buf-M system of CZE

Cations	Slope	Y intercept	Correlation coefficient (r^2)	Detection range ($mg \cdot L^{-1}$)		Recovery (%)	
				M	I	M	I
Ammonium	0.0045	−0.6006	0.9988	1–40	1–50	103.87	107.64
Potassium	0.0072	−0.0318	0.9980	1–40	1–50	96.15	100.23
Sodium	0.003	−0.9059	0.9997	1–200	1–400	83.46	95.09
Magnesium	0.0031	−0.7488	0.9986	1–40	1–50	80.93	91.61
Calcium	0.0015	−0.1466	0.9994	1–40	1–50	78.38	91.13

Note. Recovery was calculated from the data of sample and sample added standard solution that
were determined at five-run intervals without renovating the carrier buffer.

Quantification

Table 4 lists the quantification parameters, including linearity, correlation coefficients, detection range, and recovery percentage of the Buf-I system, and some parameters of the Buf-M system for comparison. Using the Buf-I system, all cations exhibited coefficients of linearity (r^2) greater than 0.998. In addition, its detection range (50 mg L^{-1} NH_4^+, K^+, Na^+, Mg^{2+}, and 400 mg L^{-1} Ca^{2+}) was greater than that of Buf-M, and its recovery ratios (91.13–107.64%) were better than those of Buf-M (78.38–103.87%).

Application of the Buf-I Method to Different Soil Extracts

To validate the applicability of the Buf-I method of CZE to the detection of major cations in a variety of soils, five soil extract samples from different regions of China were analyzed by the Buf-I method of CZE, as well as by traditional methods using ICP–atomic emission spectroscopy (AES) for K^+, Na^+, Mg^{2+}, and Ca^{2+} and flow stream analysis for NH_4^+. As shown in Table 5, the determination results using Buf-I were consistent with those of traditional analysis, with a correlation of 0.9963 and determination values that were not significantly different in most cases.

Table 5

Separation application of Buf-I to five different types of soil from China (mg L^{-1}; n = 3)

Soil	Ammonium		Potassium		Calcium		Sodium		Magnesium	
	ICP/FSA	CZE-Buf-I	ICP/FSA	CZE-Buf-I	ICP/FSA	CZE-Buf-I	ICP/FSA	CZE-Buf-I	ICP/FSA	CZE-Buf-I
1	2.09	2.21	3.58	4.52	6.75	7.78	6.0	5.38	1.09	1.26
2	2.34	2.29	11.5	10.3	66.6	60.1	22.2	23.0	11.1	10.7
3	2.01	1.69	6.23	5.71	28.8	25.1	32.7	28.9	5.25	5.0
4	3.04	2.71	4.8	3.94	46.3	39.9	18.1	19.3	6.42	6.49
5	1.93	0.72	3.67	2.78	63.6	61.9	54.4	55.4	9.41	8.94
6	7.05	6.69	6.49	5.87	292	300	30.4	29.4	31.6	30.9
	P:0.802 > P:0.05		P:0.820 > P:0.05		P:0.908 > P:0.05		P:0.823 > P:0.05		P:0.966 > P:0.05	

Note. ICP/FSA means traditional detection methods; K^+, Na^+, Mg^{2+}, and Ca^{2+} are detected by the inductively coupled plasma (ICP); NH_4^+ is detected by flow stream analyzer, Skalar (FSA). CZE-Buf-I means new Buf-I system method by capillary-zone electrophoresis. The results are means of three replicates (n = 3).

Discussion

Capillary electrophoresis is a highly efficient tool for determination of cations. Göttlein, Hell, and Blasek (1996) first detected major cations in soil using the Buf-M system of CZE. However, the components of Buf-M are unstable and thus not suitable for the determination of batches of samples. In this study, a new carrier buffer system containing mainly HIBA and imidazole (Buf-I) was applied to soil extract determination by CZE. The separation effect, stability, and applicability of Buf-I were evaluated relative to the old buffer system (Buf-M).

First, Buf-I achieved baseline separation of all major cations with better reproducibility and less separation time. The different orders of the peaks of Ca and Na in the two buffer systems (Figure 1) may be due to the different complexing effects of HIBA and Metol on cations; this affects mobility, especially of Ca and Mg (Lin, Lee, and Chen 1993; Pacakova, Caufal, and Stulik 1999). Furthermore, the two buffer systems have different electrophoresis conditions; the running current of Buf-I was greater than 22 mA and more than that of Buf-M, which was about 20 mA (Figure 2). A higher running current is associated with faster mobility of separation components, which results in earlier appearance and detection of component peaks (Timerbaev 2002). The separation time difference observed in this study is consistent with the current effect theory. Considering that the peak area and peak height by Buf-I are all less than that by Buf-M system, the detection lower limit by Buf-I may greater than that by Buf-M; however, more injection time could make up this with better reproducibility (Schaeper and Sepaniak 2000).

Second, the Buf-I system was more stable. The RSD reflecting storage stability of Buf-I (3.11–7.77%) was better than that of Buf-M (9.0–13.18%) (Table 2); the corresponding average relative peak area for Buf-I (97.5–101.3%) at 2, 4, 6, and 8 h was more constant than that for Buf-M (114.9–128%); and the RSD reflecting running stability of Buf-I (1.7–5.53%) was better than that of Buf-M (5.30–10.33%) (Table 3). The main reason for the better relative stability of Buf-I is likely that the components of Buf-M are unstable, which may reduce the stability of the running current. The Buf-I system exhibited a more stable separation current than the Buf-M system under all conditions (Figures 2 and 4). Because running current is the key to capillary electrophoresis and reflects both running status and buffer character, changes in running current indicate variable separation effects or even an inability to achieve separation (Rohliek and Deyl 1989; Esaka et al. 1993). The migration time of components is highly correlated (Colyer and Oldham 1995) with current, and changes in migration time decrease quantitative reproducibility by peak area (Altria 1993). The increased stability and reduced deviation of the separation effect of the new Buf-I system relative to those of the old system at different storage times as well as consecutive running times may have been due to the constant current of the Buf-I system.

Third, the quantification index of Buf-I was satisfactory. The recovery by Buf-I (91.13–107.64%) was greater than that by Buf-M (78.38–103.87%) (Figure 4), indicating that the Buf-I method is more accurate than the Buf-M method, especially for batch determination. The wider detection range of Buf-I indicates that this system is extensively applicable, convenient, efficient, and suitable for batch detection.

Fourth, application of the Buf-I method to different soil extract samples produced results that were highly consistent with ICP and flow stream analysis (with a correlation of 0.9963). The determination values produced by the new Buf-I system of CZE did not differ significantly from traditional methods in most cases and

thus can be extensively applied to the determination of major cations in soil extract samples.

Conclusion

This study introduced a new carrier buffer mainly containing HIBA and imidazol for the simultaneous detection of major cations in soil extracts by CZE. Compared to the old buffer system that mainly contained Metol, the new system is more stable, accurate, and efficient. The new Buf-I system of CZE could be extensively used for the batch detection of soil sample.

Acknowledgments

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