Simultaneous analysis of the major metal cations and ammonium by CZE

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DISCLAIMER
Before using this or any other analytical method it is imperative that you check that it works with your samples. The bare minimum is to test accuracy and precision.
- Test accuracy by creating a standard curve by serial dilution of a sample and/or via spike and recovery tests. Both tests will show if the analysis is affected by the sample matrix.
- Test precision by repeated analysis of the same sample. It's best to do separate precision tests for the analytical method (replicate analyses of the same extract) and for the entire extraction and analysis procedure (extract the same sample several times and carry each extract through the analysis procedure). These tests will show you where poor precision is creeping into your analysis.

Remember that your results are qualitative if you rely on a standard curve with a purified analyte.

Introduction
Separation of cations by capillary electrophoresis was first demonstrated some 20 years ago and in recent times determination of cations by CE has become widespread (Macka and Haddad 1997; Yang et al. 1995). CE requires only very small sample volumes and permits the simultaneous detection of different elements and thus is potentially faster than existing methods (e.g. IC). There are numerous CE methods suitable for the determination of metal cations and ammonium, but few have been tested on biological samples. Bazzanella et al. (1998) developed a CE method suitable for analysis of vacuolor sap extracted from wheat, and this is a modification of that method.

1.1 Detection
Most cations lack chromophores and do not give a UV-Vis response. One elegant approach is to use indirect UV detection with a background electrolyte such as imidazole with a high UV absorbance. The background electrolyte provides a high background UV signal, when analytes pass the detector they reduce UV absorbance, thus analytes are detected as "negative peaks" against a high background. Indirect UV is thus a "universal" detection method – it detects anything. The primary drawback of this is that it increases the possibility of interference from similarly charged species.

1.2 Separation
In CZE (capillary zone electrophoresis) analytes migrate according to their mass/charge ratio. Hence a prerequisite of CZE is that analytes are charged. Any species that is a cation at pH 4.2 will thus migrate toward the cathode. Potassium and ammonium have similar electrophoretic mobility in an imidazole electrolyte, separation of one from the other is possible by adding the crown ionophore 18-crown-6, which forms complexes with potassium, increasing it's size and slowing down its migration.

2 Preparation of samples

Samples generally require little pre-treatment before analysis. Samples such as phloem and xylem sap only require dilution prior to analysis. Analytes can be extracted from leaves with hot water,

methanol/chloroform/water(MCW), or aqueous ethanol. MCW extracts will be cleaner than hot water extracts and should present fewer problems for analysis. Proteins are present in hot water extracts (but not MCW), and these may be a problem for analysis since they bond to the inside of the capillary and consistently increase migration time. Protein-rich samples can be either precipitated with an organic solvent, TCA or ultrafiltered prior to analysis.

References

Bazzanella A, Lochmann H, Mainka A, Bächmann K (1997) Determination of inorganic anions, carboxylic acids and amino acids in plant matrices by capillary zone electrophoresis. Chromatographia 45: 59-62

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Materials and methods

BGE: 10 mM imidazole, 2 mM 18-crown-6

Background electrolyte (BGE) should be prepared fresh whenever you wish to analyse a batch of samples. BGE probably lasts for a couple of weeks if tightly capped.

To make 250 mL of 10 mM imidazole*, 2 mM 18-crown-6*:

1. Clean electrode and calibrate pH meter using **fresh** pH standards (ca. pH 3 and 5).
2. To a 250 mL erlenmeyer flask add:
   a magnetic stirrer bar;
   0.1702 g of imidazole;
   0.132 g of 18-crown-6;
   approx 230 mL of double DI water.
3. Place on a magnetic stirrer and heat to dissolve
4. Adjust pH to 4.2 with 1 or 0.1 M HCl
5. Make up to 250 mL with double DI water
6. Store in tightly capped container

*Imidazole is toxic and corrosive
*18-crown-6 is toxic and it lives in the fridge

CE setup

Capillary: bare fused silica 75 m i.d. x 50 cm effective length
Cartridge and carousel temp: 20°C
Polarity: positive to negative
Run voltage:25 kV
Run time: 5 minutes
Detector: 200 nm, 10 nm bandwidth, 8 Hz, indirect detection
Sample injection: 5 sec * 0.5 psi (standard, but can be changed if necessary)
Purge cycles (2): 0.25 M HCl (1 min), BGE (2 min)

It is important that the capillary is rinsed with HCl and BGE prior to each run so as to ensure good reproducibility (see Figs 1b,c). Rinsing with NaOH causes a huge loss of resolution (see Fig 1a) and poor reproducibility. If the capillary is rinsed only with BGE reproducibility is poor, with large increases in MT between runs.

Continual electrophoresis affects the electrophoretic properties of the BGE – this leads to small increases in MT with every run. This increase stays well within acceptable limits if the BGE vials are replaced every 6-10 runs.

Table 1. Linearity and sensitivity of the method for analysis of the major metal cations and ammonium. The linearity of detector response (peak area) was assessed with standards over the range 0.01 to 1 mM. Detection limit was determined at a signal-to-noise ratio of 3.

Cation	Linearity (r^2)	Detection limit (μM)
NH_4^+	0.998	10.0
K^+	0.999	7.5
Na^+	0.999	0.3
Ca^{2+}	0.999	0.4
Mg^{2+}	0.999	0.3

Table 2. Reproducibility of migration time (MT) and peak area (Area) of cations in a standard solution and a sample. Reproducibility was determined by repeated injection ($n = 6$) of a mixture of the 5 cations at 0.1 mM each (Standard), or a 5-fold diluted hot-water extract from leaves of *E. globulus* (Sample). %RSD, percentage relative standard deviation.

Cation	Standard		Sample	
	MT (%RSD)	Area (%RSD)	MT (%RSD)	Area (%RSD)
NH_4^+	0.099	4.9	0.17	12.5
K^+	0.11	5.7	0.18	3.0
Na^+	0.15	5.1	0.19	6.2
Ca^{2+}	0.13	1.7	0.20	3.5
Mg^{2+}	0.09	5.4	0.20	2.4

Figure 1. Electropherograms of cations in a) standard mixture of 0.1 mM cations, b) first injection of a standard mixture, c) 10th injection of the same standard mixture, and d) hot-water extract from *E. globulus* diluted 5-fold with water. In electropherogram a) the capillary was rinsed with 0.25 M NaOH and BGE prior to injection. In b), c) and d) the capillary was rinsed with 0.25 M HCl and BGE prior to injection. CE conditions: capillary, bare fused silica, 57 cm total length, 50 cm effective length x 75 µm i.d.; BGE, 10 mM imidazole, 2.0 mM 18-crown-6, pH 4.2; applied voltage, 25 kV; temperature 20˚C. Detection was by indirect UV at 200 nm with a reference at 350 nm. Peaks have been inverted for display. Peaks are: 1, ammonium; 2, potassium; 3, sodium; 4, calcium; 5, magnesium