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Direct electrokinetic injection of inorganic cations from whole fruits and vegetables for capillary electrophoresis analysis

Umme Kalsoom[a,b], Rosanne M. Guijt[c], Mary C. Boyce[b], Ashley T. Townsend[d], Rob Haselberg[e], Michael C. Breadmore[a,*]

[a] Australian Centre for Research on Separation Science (ACROSS), School of Physical Sciences Chemistry, University of Tasmania, Private Bag 75, Hobart, Tasmania, 7001, Australia
[b] Centre for Ecosystem Management, School of Natural Sciences, Edith Cowan University, Perth, Western Australia, 6027, Australia
[c] School of Medicine and Australian Centre for Research on Separation Science (ACROSS), Private Bag 26, Hobart, Tasmania, 7001, Australia
[d] Central Science Laboratory, University of Tasmania, Private Bag 74, Hobart, Tasmania, 7001, Australia
[e] VU University Amsterdam, Division of BioAnalytical Chemistry, AIMMS research group BioMolecular Analysis, De Boelelaan 1083, 1081 HV, Amsterdam, The Netherlands

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ABSTRACT

A novel approach for the direct injection from plant tissues without any sample pre-treatment has been developed by simply placing a small piece of the tissue into a capillary electrophoresis vial followed by application of a voltage for electrokinetic injection. Separations of sodium, potassium, calcium and magnesium were achieved using a BGE comprising 10 mM imidazole and 2.5 mM 18-crown-6-ether at pH 4.5. The addition of 2% (m/v) hydroxypropylmethyl cellulose to the separation buffer allowed for precise and accurate electrokinetic injection of ions from the plant material by halting the movement of tissue fluid into the capillary. This method provides both qualitative and quantitative data of inorganic cations, with quantitation in zucchini, mushroom and apple samples in agreement with Sector Field Inductively Coupled Plasma Mass Spectrometric analysis ($r^2 = 0.98$, $n = 9$). This method provides a new way for rapid, quantitative analysis by eliminating sample preparation procedures, and has great potential for a range of applications in plant science and food chemistry.

1. Introduction

Minerals and vitamins are essential nutrients required for a healthy functioning body [1,2] with vegetables and fruits being an important source [3]. The relative abundance of minerals in different foods vary significantly [4] and is of interest to a health conscious public [5,6]. Furthermore, seasonal variations [7,8] and growing [9] and storage conditions can impact the nutrient levels and hence quality of a food [10]. Therefore, efficient methods for the analysis of minerals in food are necessary.

Determination of inorganic mineral cations, such as Ca^{2+}, Na^{+}, Mg^{2+} and K^{+} in fruits and vegetables is typically achieved by atomic spectroscopic methods [7] including inductively coupled plasma-mass spectrometry (ICP-MS) [11], but alternatives such as ion chromatography [12] and capillary electrophoresis (CE) [13] have also been reported. In all cases sample preparation is required and typically involves drying and pulverizing the sample followed by acid digestion and dilution [2–4,14,15]. Fukushi et al. reported an electrophoresis method for free calcium in vegetable that was slightly simpler, but still required boiling pulverized vegetable for 15–20 min, cooling, filtering and making to volume prior to analysis [16]. Sample preparation is not only time consuming and labor intensive but also provides opportunity for sample contamination and analyte loss. A simpler method for direct analysis of plant tissue is highly desirable.

Methods for direct analysis of tissues of biological or clinical interest have emerged over the last decade [17–20]. For example, the direct determination of drugs in tissue samples have been achieved using mass spectrometry (MS) in combination with matrix-assisted laser desorption/ionization (MALDI)-MS [14]. MS methods are typically limited to providing qualitative information of the analytes. Quantitative information in direct analysis of a bulk sample was obtained by MS in combination with internal extractive electrospray ionization. The capillary tip was placed inside the sample and a solvent was introduced into the sample matrix to extract the analytes at high voltage (±4.5 kV) for direct injection

* Corresponding author. Tel.: +61 3 6226 2154.
E-mail address: mcb@utas.edu.au (M.C. Breadmore).

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into the MS [20]. The signal intensities were highly dependent on the position of the ESI capillary in the sample with slight changes in capillary position resulting in differences in the injected sample volume, compromising repeatability. The approach also required samples to be precisely cut to ensure a uniform size and shape to achieve reproducible results, which combined with the solvent required for the extraction of analytes from the sample matrix, complicates the method.

Analytical separation techniques offer the possibility of separating target analytes based on their physicochemical properties, avoiding the reliance on the resolving power of the mass spectrometer. CE is known for its ability to perform rapid separations with very small sample volumes, and there are two reports in which analytes have been directly injected from tissue samples. For example, Oguri *et al.* reported the direct sampling from rat's brain using CE in combination with laser induced fluorescence (LIF) for the analysis of taurine [21]. Electrokinetic injection was performed by piercing a rat's brain with the capillary and allowed for the determination of both intra- and extra-cellular taurine, an advantage in comparison with microdialysis only extra-cellular taurine can be sampled. However, this approach only provided qualitative information, as it was not possible to control the amount of sample injected. Also, sampling was achieved only from the surface to minimize the accidental release of taurine from damaged tissues. The use of a tapered capillary was suggested as a way to minimize damage and for sampling deep inside the brain. This approach was subsequently employed by Wang et al. who etched the capillary to a sharp point using HF and used this to detect the anticancer drug doxorubicin in human liver tissue [22]. For direct sampling from thin slices of liver tissues, a negative pressure of −7.6 kPa for 2s was applied. However, etching is a hazardous process and the resulting fragile capillary is likely to break when directly sampling from more solid samples such as many plant tissues. This method also required tissues to be cut into very thin slices (5 μm) to prevent large injections, thus making it technically demanding and unsuitable for analysis of intact plant tissues. In addition to this, electrokinetic injection of intracellular content of single cells using CE in combination with laser induced fluorescence (LIF) has also been demonstrated [23,24] illustrating the potential of CE to provide information on biological systems.

In this paper, our aim was to develop a simple and robust method for the direct injection of ions from plant tissue, improving analytical simplicity by eliminating the requirement for sample treatment and hence reducing the risk of contamination. Direct analysis only requiring tissue to be cut and placed in a CE vial, when implemented in a more portable platform and extended to other analytes, may form the basis for rapid on-site analysis of food products to inform agricultural production and nutrition as well as food safety.

2. Experimental

2.1. Chemicals

Imidazole, 18-crown-6 ether, sodium chloride, potassium chloride, hydoxypropyl methyl cellulose (HPMC) (viscosity 3500–5600 cP, 2% in H_2O, 20 °C), sodium hydroxide, acetic acid and nitric acid were all purchased from Sigma Aldrich (Sydney, Australia). Calcium chloride dihydrate was from Univar (New South Wales, Australia). Magnesium chloride hexahydrate was from BDH Laboratory Supplies (Poole, England).

2.2. Instrumentation

A Hewlett Packard 3D CE (Waldbron, Germany) instrument equipped with a diode array UV absorbance detector and Agilent Chemstation software Rev. A. 08.03 (847) was used. The instrument was connected to the building nitrogen supply to provide up to 6 bar of pressure using the external adaptor provided with the instrument.

The cassette temperature set at 30 °C. Untreated fused silica capillaries (Polymicro, Phoenix, AZ, USA) with an internal diameter of 50 μm and outer diameter of 350 μm were used for separation unless otherwise stated. Initially, the length of capillary and separation voltage was kept at 100 cm (91.5 cm to the detector) and +20 kV. However, due to the increased back pressure from the high viscosity buffer, the capillary was shortened to 50 cm (41.5 cm to the detector) for the repeatability experiments and separation was carried at +8 kV. The capillary length was further reduced to 40 cm (31.5 cm to the detector) for the separation of cations in other fruits and vegetables at +5.5 kV).

2.3. CE analysis

A new capillary was conditioned sequentially with 0.1 N NaOH, deionized water and BGE for 15 min each at 5 bar. Once in use, the capillary was flushed daily with deioninzed water and then BGE for 10 min each at 5 bar at the start of the day. At the end of each day, the capillary was flushed with Milli Q at 5 bar for 10 min and stored in MilliQ water. The capillary was flushed with BGE for 2 min prior to each run.

For separation the BGE was 10 mM imidazole, 2.5 mM 18-crown-6-ether, 2% (w/v) HPMC at pH 4.5, adjusted with acetic acid, was used prepared daily from a 10X stock solution. The BGE was replaced after every 5 runs. For detection, the maximum absorption wavelength (214 nm) of imidazole was used.

2.4. Standards and sample solutions

Standard solutions of K^+ (350 mg/mL), Ca^{2+} (100 mg/mL) and Mg^{2+} (100 mg/mL) were prepared in water from KCl, $CaCl_2{\cdot}2H_2O$ and $MgCl_2{\cdot}6H_2O$ salts.

For preparation of the zucchini gel, 50.0 mL of hot water was blended with 50.0 g of zucchini (2 min or until zucchini formed a paste with water). To this hot mixture 5.0 g of gelatin was added and mixed with a magnetic stirrer for approximately 1 min. The mixture was degassed by sonication (10 min), poured into plastic moulds and allowed to solidify for 1 h in a refrigerator (3 °C). The gelatin slices were then cut into approximately 5 mm cubes for direct injection and CE analysis.

For quantitation of cations in whole fruit, a series of external calibration standards were prepared using a fruit or vegetable matrix. 10 mL of the paste prepared as above was spiked with the appropriate range of standards. For spiking, a rough estimate of cation concentration in the given fruit and vegetable was considered and standards falling in that range were prepared. Spiked standards of zucchini were prepared as follows; K^+ (0–14, mg/mL), Mg^{2+} and Ca^{2+} (0–0.900 mg/mL). For apple, standards in the range of 0–1.5 mg/mL, 0–0.2 mg/mL, and 0–0.075 mg/mL for K^+, Ca^{2+} and Mg^{2+}, respectively were prepared, for four different varieties of apples: fuji, pink lady, red delicious, and royal gala. For mushroom, the standards were in the range of 0–9, mg/mL, 0–0.04 mg/mL, and 0–0.3 mg/mL for K^+, Ca^{2+} and Mg^{2+}, respectively. The mushroom matrix was prepared by blending 50 g of mushroom with 100 mL of Milli Q water. The spiked standards of each fruit or vegetable were used to construct a calibration curve and from that curve the concentration of analytes in that particular fruit or vegetable was determined.