Journal of Chromatography A, 1446 (2016) 158–163

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Dual-channel capillary electrophoresis for simultaneous determination of cations and anions

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ARTICLE INFO

Article history:
Received 27 January 2016
Received in revised form 16 March 2016
Accepted 5 April 2016
Available online 8 April 2016

Keywords:
Dual-channel electrophoresis
Short capillary separation
Inorganic ions
Mineral fertiliser
Piezoelectric micropump

ABSTRACT

An original electrophoresis apparatus for simultaneous rapid determination of cations and anions has been designed and tested. The separation part of the apparatus consists of two identical fused-silica capillaries, each with a length of 10.5 cm and inner diameter of 25 μm. The injection space is formed by the crossing of four channels in a plexiglass cross-piece. The capillaries pass through two opposing channels and their injection ends are located opposite one another at a distance of approx. 0.5 mm in the centre of the crossing point. The exit ends of the capillaries are placed in vessels containing the background electrolyte in which are immersed the electrodes of a high-voltage source. Contactless conductivity detectors with semi-cylindrical electrodes are located 2 cm from the exit ends of the capillaries. The injection part of the apparatus consists of two piezoelectric micro-pumps bringing the solution through another channel in the cross-piece to the injection ends of the capillary. During the injection, the sample is brought through one of them and is injected electrokinetically for a defined time. Then the sample zone is forced out of the injection space by a stream of background electrolyte from the second micro-pump. The timing of the injection process is computer-controlled. Thus the equipment can be considered to constitute electrophoresis in one capillary with injection into its centre. The use of short capillaries and miniature micro-pumps without other mechanical components enabled the construction of the apparatus on a board with dimensions of 20 × 25 cm. The proposed equipment was used to test simultaneous separation of a mixture of cations and anions, NH_4^+, K^+, Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}, Cl^-, NO_3^-, SO_4^{2-}, ClO_3^- and F^-, in BGE with composition 500 mM HAc + 20 mM Tris + 2 mM 18-crown-6 (pH 3.3). Baseline separation of all the components was achieved in time less than 1 min. Quantification of the content of nitrate nitrogen (determined as NO_3^-), ammoniacal nitrogen (determined as NH_4^+), K_2O (determined as K^+) and SO_3 (determined as SO_4^{2-}) was performed on a real-world sample of mineral fertiliser. The determined compositions differed from the declared contents by an amount of 0.5–5.6%; the RSD value expressing the repeatability of the determination was in the range 3.4–7.5%. The LOD values were in the range from 6.9 μM (K^+) to 10.6 μM (NH_4^+).

1. Introduction

The high variability in the experimental arrangement of capillary electrophoresis (CE) permits simultaneous separation of cations and anions during a single analysis, saving time and improving the characterisation of an unknown sample. The basic experimental strategies employed are briefly described below; details and references to the original literature dealing with this subject can be found, e.g., in general reviews [1,2]. Following injection and turning on the separation voltage, the anions move to the anode and the cations to the cathode.

In the experimentally simplest case, where the mobility of the anions is lower than the cationic electroosmotic flow (EOF), the standard CE arrangement with injection of the sample at one end of the capillary can be used; first the cations are detected, followed by a zone of electroneutral substances and finally the anions carried to the detector by faster EOF (EOF-driven separation) [3]. The rate of movement of the ions can be purposefully affected by modifiers permitting EOF to be accelerated, suppressed or reversed and then work in the anionic mode, where cations migrate against the faster EOF [4]. The movement of zones of the separated substances can also be affected by externally applied pressure (pressure-driven separation) – increased pressure at the injection end of the

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http://dx.doi.org/10.1016/j.chroma.2016.04.015
0021-9673/

capillary leads to faster separation [5]; in contrast, increased pressure at the exit end of the capillary retards analyte motion and contributes to the separation of peaks with similar mobility values [6]. In these modes of simultaneous separation of cations and anions, the detector is located close to the exit end of the capillary.

Another possibility lies in simultaneous or step-wise sample injection at both ends of the capillary (dual opposite-end injection) [1]. This method uses several different modes – simultaneous electrokinetic injection (both ends of the capillary are immersed in the vessels with the samples) [7] or sequential injection (the ends of the capillary are immersed in the sample vessels one after another) [8] and sequential hydrodynamic injection [9]. In these modes, the detector is located at a certain point close to the centre of the capillary.

The sample can be injected into the capillary twice, where only one end of the capillary can be used. After the first hydrodynamic injection, the zone of the injected sample is pressure-driven to close to the exit end of the capillary and then the sample is injected again (dual single-end injection) [10]. Similar to the previous case, the detector is located close to the centre of the capillary. This method can be modified so that the sample is added only once and its zone is pressure-driven to the centre of the capillary. In this case, two detectors located close to the two ends of the capillary are used to detect anions and cations.

A different strategy in principle is used in simultaneous separation of cations and anions where two capillaries are employed – anions are separated in one capillary and cations in the other. This method was first described by Bachmann et al. [11]. Two identical capillaries had one end (the injection end) immersed in a common vessel containing the sample for sample injection or containing the background electrolyte (BGE) for the separation. The second ends of the capillaries were immersed in vessels containing the BGE and the high-voltage electrodes. The sample was injected hydrodynamically by lifting the common vessels with the sample and the detectors were located close to the exit end of the capillaries. Indirect fluorescence detection requiring demanding optimisation of the BGE containing two fluorescent reagents was required for reliable detection of both types of ions. Consequently, much more advantageous contactless conductivity detection (C^4D) was used for later dual-channel CE systems [12–14]. Movable C^4D can be used with advantage for exact positioning at different places along the capillary [15].

Most subsequently published dual-channel CE apparatuses are based on the same design principle. The injection ends of the capillaries are located in a common injection space from which the sample is injected simultaneously into both capillaries. The use of a high voltage (HV) in the injection spaces of both capillaries constitutes a certain safety and functional problem in dual-channel CE [16–18]. Consequently, two HV sources with a common grounding electrode located in the injection space of the two capillaries are generally used. The exit ends of the capillaries are immersed in separate vessels containing the BGE, in which are located the high-voltage electrodes with the polarity required for separation of the anions and cations. C^4D is located close to the exit ends of the capillaries. In most cases, separation of the anions and cations was performed in the same BGE. Capillaries with length common in classical CE, 55–70 cm, were used.

The individual systems basically differ only in the means of injecting the sample into the capillaries. The sample solution is fed into the injection space by a piston pump [16] and injected into the capillaries electrokinetically or using compressed air [18] or nitrogen [17] with hydrodynamic injection. Exchange of solutions in the injection space (sample during injection, BGE in separation and rinsing) is operated by a system of switching valves controlled by a computer program.

The apparatus described by Huang et al. [19] differs somewhat from the given scheme. The sample is hydrodynamically injected into the separation capillaries in various ways – for separation of cations by lifting the injection end, for separation of anions using a flow-injection interface. The cations and anions were separated in various BGE's.

This work describes the assembly and testing of a dual-channel CE apparatus with standard design, i.e. with the injection end of the capillaries in a common injection space. The basic difference compared to the above-described apparatuses lies in the use of short capillaries with a length of 10 cm; the sample or BGE solution is delivered to the injection space by two piezoelectric micro-pumps without any other mechanical components. This injection apparatus was originally developed and tested in detail for single-channel CE [20].

When piezoelectric micro-pumps are used (in which the pumped medium is in contact only with polyphenylsulfone plastic), the high-voltage electric field does not come into contact with any external electrically conductive component (similarly as in work [11]), and thus it is safe to use only one HV source with a grounding electrode in the terminal vessel with the exit end of one capillary and with a high-voltage electrode in the second terminal vessel (in addition, lower separation voltage can be used in separations in short capillaries). This leads to further simplification of the apparatus.

The entire functional part of the apparatus with short separation capillaries and small piezoelectric micro-pumps are set up on a board with dimensions of 25 × 20 cm – the apparatus can thus be considered to be a "Dual-channel CE on a board". The apparatus was tested by separation and determination of a mixture of inorganic ions in laboratory samples and applied to a real-world sample of artificial fertiliser.

2. Experimental

2.1. Description of the apparatus

Fig. 1A depicts a scheme of the apparatus. This is based on a plexiglass block (1) with dimensions of 30 × 30 × 10 mm. Channels with a diameter of 1.55 mm are bored into the block in the shape of a cross; the crossing point is an injection space. At a distance of cca 30 mm, the injection ends of the separation capillaries (2a, 2b) are tightly fitted into PTFE tubes 1/16" O.D., 0.3 mm I.D. so that their ends extend cca 0.5 mm out of the tube (the PTFE tubes were heated to cca 300 °C to facilitate insertion of the capillaries). Then the PTFE tubes with the capillaries were tightly inserted into two opposing openings in the plexiglass block so that the ends of the capillaries in the injection space are about 0.5 mm apart. The C^4D (3a, 3b) are fitted at the other end and their exit ends are fitted through seals into the terminal vessels (4a, 4b) containing the HV source electrodes.

PTFE tubes 1/16" O.D., 0.5 mm I.D. are fitted into the remaining two opposing openings in the plexiglass block for feeding BGE and draining the solution from the injection space (8). Another opening with diameter 1.55 mm perpendicular to the opening for feed of the BGE has its opening cca 1 mm under the separation space. In it is tightly inserted and sealed a PTFE tube 1/16" O.D., 0.5 mm I.D. for sample solution input.

The sample is pumped into the injection space by a piezoelectric micro-pump (5) and BGE is drawn from reservoir (7) by a piezoelectric micro-pump (6). A three-way valve (9) is connected between the pump (6) and the injection space, enabling direct injection of the solution into the injection space, e.g. a NaOH solution in activation of the capillaries. The capillaries are rinsed with the BGE