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Portable capillary electrophoresis instrument with contactless conductivity detection for on-site analysis of small volumes of biological fluids

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ABSTRACT

A novel, easy to use and portable capillary electrophoretic instrument for injection of small volumes of biological fluids equipped with contactless conductivity detection was constructed. The instrument is lightweight (<5 kg), all necessary parts including a tablet computer are accommodated in a plastic briefcase with dimensions 20 cm × 33 cm × 17 cm ($w \times l \times h$), allows hydrodynamic injection of small sample volumes and can continuously operate for at least 10 hours. The semi-automated hydrodynamic sample injection is accomplished via a specially designed PMMA interface that is able to repeatedly inject sample aliquots from a sample volume as low as 10 μL, with repeatability of peak areas below 5%. The developed interface and the instrument were optimized for the injection of biological fluids. Practical utility was demonstrated on the determination of formate in blood serum samples from acute methanol intoxication patients and on the analysis of ionic profile (nitrosative stress markers, including nitrite and nitrate) in the exhaled breath condensate from one single exhalation.

1. Introduction

Portable analytical instruments have seen significant development in recent decades and find variety of uses in environmental, forensic and clinical analysis, as well as in food quality control and chemical warfare detection. Portable pH and conductivity meters [1], photometers [2] and flow injection analyzers [3] typically measure only one analyte at the time and have found importance for instance in water quality testing (measurement of pH, chlorine etc. in public pools [4]) or in medical applications (blood glucometers [5] or portable NOx analyzers for asthma control [6]). Portable instruments that can determine multiple species, often based on separation techniques, play a dominant role. Conventional packed column liquid chromatographic techniques, such as HPLC or IC do not transfer easily into portable format due to the high demands on the liquid propelling systems and on the detection modes. On the other hand, gas driven separation systems (GC, IMS) or low pressure liquid separation systems, such as nano-LC or open tubular IC (OTIC) can be easily operated with miniaturized low pressure pumps [7–9] or even by gravity [10].

CE surpasses others analytical techniques in terms of separation efficiency, short analysis time and low consumption of sample, solvents and energy; it is one of the best candidate techniques for miniaturization and portable instrumentation construction. All CE parts can be easily miniaturized also due to the "no-pump" design – the liquid flow in CE is accomplished by applying high voltage over the separation capillary with no moving parts. Recent reviews cover all significant aspects of chip-based [11] and non-chip based [12] portable CE (PCE) systems, the non-chip based CE systems having a slight advantage due to the higher separation efficiency, sensitivity and easier sampling interfacing, but being more bulky and less energy efficient. The first non-chip based PCE instrument was presented by Kappes et al. in 1998 [13]. Several PCE instruments have been developed later including a commercial PCE instrument available on the market since 2001 [14]. The majority of recent research activity in the field of PCE was however devoted to construction of custom-built instruments due to the inherent limitations of the available commercial PCE [15–17]. Development of various, low energy detection schemes was within the scope of the early publications, including potentiometry [13], amperometry [18], and most importantly conductimetry [19], owing to the development of the capacitively coupled contactless conductivity detection (C4D) [20,21]. Selected applications of PCE instruments with electrochemical detection include analysis of anions and cations in water

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samples [13,18,19,22], post-blast residues [23], detection of degradation products of nerve agents in various matrices [24,25] or determination of anions in sewage water [26]. Although electrochemical detection has been mostly used (C4D being by far the most popular) optical methods such as LED based UV absorbance [27,28] and laser induced fluorescence (LIF) [29] have also been tested in PCE.

The improvement of injection systems in PCE has been another area of interest. As first PCE prototypes allowed only electrokinetic (EK) injection [13,18,19] that is less suitable for quantitative analysis, the research has focused on simplification and/or automation of hydrodynamic (HD) injection. HD injection by elevating one capillary end, as practiced in benchtop laboratory-built instruments, is simple, yet yields moderate reproducibility [22] and cannot be so easily applied in PCE due to the instrumental size limitations. Pressure driven injection known from commercial laboratory instruments requires additional mechanical components that render the PCE system complicated. Sample splitting has been very popular in PCE because it can be accomplished with a simple device and theoretically can be well controlled manually. This injection approach, pioneered by Kaljurand's group [24] however showed initially some unacceptably low repeatability due to the difficulty to exactly control the applied pressure and flow rates [30]. The repeatability can be improved by using a flow restriction valve [31], computer controlled semi- or fully-automated pumping of sample with a syringe pump [26] or pressurized air cylinder [32–34] but these solutions are also more technically demanding [26]. One of the obvious disadvantages of sample splitting is that the majority of the sample is wasted and cannot be reused for another injection. Relatively large sample volumes are required, increased further by the sample volume to fill the connecting conduits when samples are exchanged. This is acceptable for applications, where sample size is not restricted. In clinical analysis, the amounts of available biological samples are limited, particularly when using non-invasive or minimally invasive procedures. The analysis from a drop of blood or similar volume of other biological fluid is difficult to achieve with splitting injection schemes.

We have therefore developed a PCE system with C4D that allows repeated injections of microliter volumes of biological fluids. In this contribution we show the applicability of the developed PCE instrument in analysis of 10 μL of blood serum sample for quick (<2 min) methanol poisoning assessment, based on the separation method previously developed with a benchtop instrument in our laboratory [35]. We further demonstrate the utility of the developed PCE instrument in analysis of exhaled breath condensate collected from a single exhalation (volume 10–20 μL) using an on-line coupled sampling device. The developed PCE instrument was shown to be applicable in on-site clinical analyses of low volumes of selected biological fluids.

2. Materials and methods

2.1. Instrumentation

The portable CE instrument was built for maximum simplicity and easy operation so that it could be handled by medical personnel during emergency clinical analysis and monitoring. The whole instrument, including the 10 in. tablet computer was accommodated in a waterproof plastic case (Pro's Kit Model TC-267, Prokit's Industries Co., Ltd, Taiwan), with dimensions 22.9 cm (w) × 33 cm (l) × 17 cm (h). The weight of the instrument was less than 5 kg (case 1.3 kg, batteries 1.4 kg, instrumental panel and electronics 0.7 kg, high voltage (HV) power supply 0.2 kg, detector 0.2 kg, data acquisition system 0.2 kg, pinch valve and accessories 0.1 kg, tablet computer 0.6 kg) so that it could be easily transported to the sampling site, for instance in the medical emergency, directly to the patient. All electronic parts (except the tablet computer) were powered by two rechargeable Li-ion 14.4 V batteries (capacity 8.8 Ah each). The batteries were connected to an in-house built voltage regulator unit able to provide a stable 12 V output for the electronic parts of the instrument including HV power supply, injector, detector and data acquisition. The batteries gave sufficient power for continuous work for at least 10 h. Typical electrophoretic run in our case takes approximately 4 min, including ca. 2 min for sample injection and flushing between the analyses, so at least 150 analyses can be performed before the batteries have to be recharged.

The separations were driven by a small size (3.8 cm (w) × 9.5 cm (l) × 2.5 cm (h)) HV power supply (EMCO DX250R, Sutter Creek, CA, USA) able to deliver separation voltage from 4 kV to 25 kV. In the current configuration, only positive HV supply was used for determination of anions, however, a second, negative HV power supply was at hand and could be easily replaced in case of analysis of cations. A custom made C4D detector (Version 5.06, ADMET s.r.o., Prague, Czech Republic, operating at a frequency of 1.8432 MHz) was used for sensitive detection. The advantage of this detector is that it operates from a single 12 V source and includes all excitation, rectification and signal conversion electronics built on a 3.2 cm (w) × 10 cm (l) electronic board. The data from the detector were acquired through a LAN connector of the data acquisition system (ORCA 2800 24-bit A/D converter, ECOM spol s.r.o., Prague, Czech Republic) via UTP cable to Micro Port USB 2.0 to Fast Ethernet Adapter and recorded with ECOMAC software (ver. 0.254, ECOM spol s.r.o., Prague, Czech Republic) working under Windows 8 OS. For operator safety, the instrument can be operated with the lid closed, thus avoiding accidental contact with the high voltage when separation is running. In such case, the tablet computer is removed from the lid and placed next to the instrument. The injection and buffer flushing is accomplished with the lid open.

The separation capillary was a polyimide coated fused-silica (FS) capillary of 50 μm ID, 360 μm OD, 31 cm total length, 22 cm effective length (Microquartz GmbH, München, Germany). The capillary ends were inserted in two interfaces machined from PMMA block.

2.2. Chemicals

All chemicals were of reagent grade and deionized (DI) water (Purite, Neptune, Watrex, Prague, Czech Republic) was used for stock solution preparation and dilutions. Background electrolyte 1 (BGE 1, pH 6) for analysis of anions consisted of 20 mM 2-(N-morpholino)ethanesulfonic acid (MES)/L-Histidine (HIS), 2 mM 18-crown-6 (18-c-6) and was prepared daily by diluting from 200 mM stock solutions of MES, HIS and 100 mM 18-c-6 (all Sigma–Aldrich). Cetyltrimethylammonium bromide (CTAB, Sigma–Aldrich) was prepared as 10 mM stock solution in 5% acetonitrile and was added to the BGE 1 to yield the final concentration of 30 μM. Background electrolyte 2 (BGE 2, pH 4.6) for fast screening of formate consisted of 15 mM glutamic acid (GLU) and 10 mM HIS and was prepared daily by diluting 200 mM stock solution of HIS and 50 mM stock solution of GLU (all Sigma–Aldrich) in DI water, with addition of CTAB yielding the final concentration of 30 μM.

Standards were prepared from sodium chloride, potassium nitrite, sodium nitrate, sodium sulfate, sodium thiocyanate, lithium lactate, propionic acid, butyric acid, formic acid (all Sigma–Aldrich), and acetic acid (Lach-Ner, Neratovice, Czech Republic) as 10 mM stock solutions and diluted to the required concentration with DI water.