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Improving precision of manual hydrodynamic injection in capillary electrophoresis with contactless conductivity detection

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ABSTRACT

Reproducible injection in capillary electrophoresis has been difficult to achieve with manual injection techniques using simple injection devices, such as gravity injection (siphoning) or hydrodynamic sample splitting. We demonstrate that the injection reproducibility can be improved using very simple means. With hydrodynamic sample splitter, a passive micro-metering valve can be inserted in-line to regulate the sample flow rate through the splitter interface. A significant improvement of both reproducibility and repeatability was achieved. The reproducibility of RSD of the peak areas improved from 25.4% to 4.4%, while the repeatability was below 4.1% when micro-metering valve was used. Additional simple correction that can be used to further improve the variability of injected sample volumes in any hydrodynamic injection mode in CE with conductivity detection was proposed and verified. The measured EOF peak can serve as a simple indicator of the injected volume and can be effectively used for additional correction. By a linear function between the injection volume and the peak area of the EOF, the RSD values of peak areas for both manual gravity injection and hydrodynamic sample splitter were further improved below 2% RSD. The linearity of the calibration curve was also significantly improved. The proposed correction works even with slight differences in matrix composition, as demonstrated on the analysis aqueous soil extract of model mixture of five nerve agent degradation products.

1. Introduction

Capillary electrophoresis has enjoyed relatively high popularity in last decades due to its superior performance in regard to efficiency, separation times, selectivity, and minimal sample and electrolyte consumption. It has however not, to a large extent, replaced the liquid chromatographic techniques used for routine analysis in analytical laboratories, owing to several shortcomings that currently limit CE, especially in quantitative analysis. For instance, only gel electrophoretic methods are listed in the Official Methods of Analysis handbook's latest edition [1]. Among the most cited drawbacks of CE appear low sensitivity, matrix dependence and low reproducibility. Perhaps the low reproducibility is one of the major problems that CE is being faced with and has been subject to several recent reviews [2,3]. The factors that can have an effect on the reproducibility of CE separation are numerous and include for instance poor sampling precision, changes in EOF induced by temperature and viscosity changes, adsorption of sample compounds onto the capillaries, various inherent sampling and detection biases, improper buffering of the electrolytes, decomposition due to electrolysis etc. Consequently there is no simple and/or generally applicable solution to improve the poor reproducibility of CE. Several approaches were however suggested to improve the reproducibility of the peak areas of the analytes in CE that are obtained through integration of the measured peaks in the electropherograms. The peak area is a representation of the analyte concentration, and its accurate assessment is important for quantitative analysis. Peak area in CE, however, is prone to change significantly between runs. For instance if EOF changes due to the sample component adsorption, the peak areas will change as well, as they depend greatly on the analyte migration velocity through the detector. Dividing the peak areas by their respective migration times [4] is commonly applied and can to some extent eliminate the peak area variation. This correction however accounts only for the changes due to the differences in migration velocities. More often, internal standardization is practiced [5–7], as this can also account for the errors in injection volumes. However it may not be universally applicable in all cases, as it may be difficult to find a suitable IS, especially for complex sample matrices.

In a well-controlled CE method, e.g. when repeatable migration times are obtained, poor peak area precision most often relates to the poor repeatability of the sample injection. This applies especially for the manual injection techniques. Manual injection is

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susceptible to the variations induced by the operator, because it is more difficult to control all the procedures exactly in the same way manually as can do the automated instruments. The repeatability issues have been most pronounced in the home-built field portable CE instruments that are otherwise one of the strongest CE assets. The development of portable CE instruments has seen a remarkable surge in the last decade, as more than ten articles on newly developed portable CE instruments appeared [8–17] including a recent comprehensive review [18]. A quick overview of the published work reveals that the sampling precision is not very good, typically in the range of 10–15% RSD of peak areas. The sampling devices used in existing portable CE instruments make use of either manual electrokinetic [8–13,16] or hydrodynamic injections [12,13,15–17]. Electrokinetic injection, has been shown to suffer from multiple biases [19,20] and is thus difficult to apply for quantitative analysis. Manual hydrodynamic injection, such as siphoning injection, though suitable for quantitation, is not robust enough, nor sufficiently reproducible to allow operation under difficult conditions. Some of the portable CE instruments include a simple sampling carousel, similar to those present in the commercial instruments that is operated manually, however the general performance of these instruments still calls for improvement. Automated injection devices have been difficult to implement in the home-built portable instruments because they add to an increased complexity. Thus, the development of a simple injection method with high precision for portable CE instruments is probably one of the most important tasks to be resolved. The injection should preferably be done without any capillary movement and ideally the two capillary ends should be in fixed position during the sample injection and analysis. The attempts to simplify the design of manual samplers in portable CE instrument were shown by Kaljurand's group [17,21]. The tedious manual hydrodynamic or EK injection was replaced by a flow splitting device(s) of various geometries. However even with this simplified injection, based on flow splitting, the reproducibility of peak areas was no better than 9% RSD.

Surprisingly, not much effort has been devoted to improvement of the data evaluation techniques with respect to precision of sample injection in CE. In a sole report from 2006, Erny and Cifuentes [22] have shown that the hydrodynamically injected sample plug length can be estimated by measuring the electrophoresis current. It was shown that the current increases suddenly when the injected water plug exits the separation capillary. The measured dip can be used for correcting the injection imprecision. This approach is however only suitable for relatively large injected volume of samples having sufficiently different conductivity from that of the BGE. If this condition is not fulfilled the current monitoring approach may not work.

In here, we propose several ways to improve the performance of the manual hydrodynamic injection techniques. We show that with hydrodynamic sample splitter, an inclusion of a simple micrometering valve helps regulate the injection reproducibility to an acceptable level. We also show that with conductivity detection, the measured EOF peak correlates well with the injected volume and can be used for correcting the injection imprecision with simple manual HD injection. By a linear function between the injection volume and the peak area of the EOF (EOF peak area can be simply integrated from the registered electropherogram) we show that further improvement in injection precision can be achieved. The RSD values of peak areas for both manual gravity injection and hydrodynamic splitter device can be improved to below 2% RSD. The calibration linearity also improves in the same way. We demonstrate the applicability of this approach on determination of a model solution of five degradation products of chemical warfare agents, that are commonly analysed by portable CE instruments.

2. Experimental

2.1. Materials and methods

2.1.1. Electrophoretic system

A purpose-built CE instrument with either manual siphoning injection or hydrodynamic sample splitter was employed for all electrophoretic runs. The separation voltage was provided by a high voltage power supply unit (Spellman CZE2000R Start Spellman, Pulborough, UK) that was operated at a potential of −18 kV applied at the detection side of the separation capillary. The separation capillaries used were fused-silica (FS) capillaries (75 μm I.D., 375 μm O.D., 45 cm total length, 35 cm effective length, Microquartz, GmbH, Munich, Germany). The separation capillaries were preconditioned with 0.1 M NaOH for 30 min, deionized water for 10 min and with respective background electrolyte (BGE) solution for 10 min. Between two successive injections, the capillary was flushed with BGE solution for 1 min. All CE experiments were performed at ambient temperature.

2.1.2. Injection

Injection of standard solutions and real samples was carried out either hydrodynamically or using an in-house built sample splitter injector. In a hydrodynamic (HD) injection mode one capillary end with the sample vial was elevated to a fixed height of 15 cm for a specific time interval (typically 10 s, manually timed) and injection was carried out by siphoning effect. The hydrodynamic sample splitter consisted of a splitter interface machined in a piece of polyimide block. The schematic of the splitter injector is shown in Fig. 1. The splitter includes a 3 cm long horizontal flow through channel of 1 mm I.D. to which two vertical channels of the same diameter are connected. A separation capillary and a grounding Pt electrode were tight fitted into a PTFE tubing (350 μm I.D., 1/16″ O.D.), inserted in the two vertical channels and secured with 1/16″ flangeless fittings (Upchurch Scientific, Oak Harbor, WA, USA). One side of the horizontal channel of the splitter interface was connected with the injection syringe via a micro-metering valve (P446, Upchurch Scientific), while the other side included a 10 cm long, 250 μm I.D. PTFE tubing directed to waste. The splitting ratio was adjusted by choosing the length and I.D. of this waste tubing. A fixed volume of sample (500 μL) was delivered by a 1 mL disposable plastic syringe and injected manually by forcing the sample to flow by the splitting point in the splitter interface. The sample injection was followed by the BGE injection to clean the interface from the remaining sample before the application of high voltage.

2.1.3. Detection system

A high voltage capacitively coupled contactless conductivity detector (C4D) was used. It was described in a recent publication [23]. It consists of a detector cell, an external ac voltage source for excitation and an external detector circuitry for processing the cell current. The excitation voltage was provided by a circuitry based on a MAX038 oscillator (Maxim Integrated Products, Sunnyvale, CA, USA). The oscillator operated at various frequencies between 100 and 400 kHz and a voltage booster using a high voltage operational amplifier (PA91, Apex Microtechnology, Tucson, AZ, USA) produced an output of maximum 360 V_{pp} (peak-to-peak). The detector was operated at 200 kHz and 300 V_{pp} in all experiments. Data were collected using in-house written software and a 20 bit sigma-delta data acquisition card (Lawson Labs Inc., Malvern, PA, USA).

2.2. Chemicals

2.2.1. Reagents, standards, electrolytes

All chemicals were of reagent grade and deionized (DI) water (MilliQ Water System, Millipore, Molsheim, France) was