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Alternate injections coupled with flow-gated capillary electrophoresis for rapid and accurate quantitative analysis of urine samples

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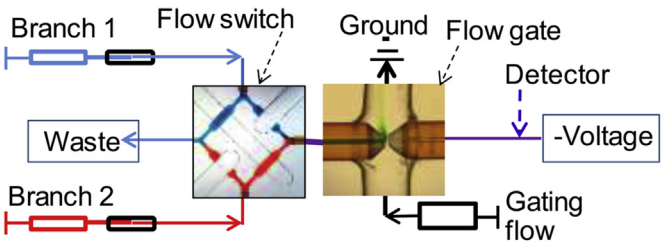
HIGHLIGHTS

- Alternate injections for flow-gated capillary electrophoresis were enabled by using a microfabricated switch.
- The one-point standard addition method was validated for rapid quantitation of biological samples with flow-gated CE.
- Quantitative reproducibility was enhanced by using alternate injections coupled with rapid electrophoretic separations.

GRAPHICAL ABSTRACT



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ABSTRACT

Capillary electrophoresis (CE) is a powerful separation technique with advantages over HPLC in terms of separation efficiency, speed, and cost. However, CE suffers in poor reproducibility in quantitative chemical analysis, which is one of major drawbacks preventing its widespread use in routine analytical laboratories. Here we report a novel strategy to enhance the quantitative capability of flow-gated CE. The platform integrated dual flow branches to respectively supply a sample and its standard additions that were then alternately injected into a single capillary for rapid separations (typically 20–90 s). A microfabricated switch was used to enable the alternate injections. It was assumed that the analytical system maintained constant conditions during neighboring injections that served as external self-standards for quantitation. This strategy was expected to reduce uncertainties caused by the fluctuation in capillary conditions and the drift of detection systems. Experimental results demonstrated that the dual-branch flow-gated CE coupled with alternate injections significantly improved the reproducibility with respect to peak height ratios under deliberate variations in injection volumes, separation voltages, optical focusing, and laser power; and thus the interday precision was ensured. To demonstrate its applicability, cyanide and amino acids in human urine were quantified rapidly with the one-point standard addition method after fluorogenic derivatization with naphthalene-2,3-dicarboxaldehyde (NDA), and the measurement accuracy was validated by determining the recovery of standard cyanide added to a urinary matrix. This strategy would be valuable to enable the quantitative capability of flow-gated CE in the measurements of a broad range of analytes, especially those lacking suited internal standards.

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Abbreviations: BGE, background electrolyte; EOF, electroosmotic flow; HP-β-CD, hydroxypropyl-beta-cyclodextrin; IS, internal standard; LIF, laser-induced fluorescence; NDA, naphthalene-2,3-dicarboxaldehyde; PDMS, polydimethylsiloxane; PEA, Phosphoryethanolamine; SAM, standard addition method; SDS, sodium dodecyl sulfate.

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1. Introduction

Capillary electrophoresis (CE) is a powerful separation technique over HPLC in terms of separation efficiency, analytical speed, and operational cost [1–3]. Since its renaissance attributed to Jorgenson and Lukacs in the early 1980s [4,5], CE-related instrumentation and technologies have been considerably advanced [6]. However, the real-world application of CE is still limited in routine analytical laboratories. This unpopularity is mainly due to two major drawbacks: detection sensitivity and reproducibility [7]. The detection sensitivity has been significantly improved by using sensitive detectors such as laser-induced fluorescence (LIF) and mass spectrometry (MS) [8–10]; and nano- or pico-molar limits of detection (LODs) have been achieved via appropriate detection configuration and/or various sample preconcentration techniques [11–13]. However, the reproducibility of CE is still a concern although many efforts have been made to overcome this barrier [14]. This obstinateness is mainly attributed to the inconsistent injection and unstable migration of analytes [15].

Typically, hydrodynamic or electrokinetic injection is employed to introduce a sample plug into a separation capillary. To compensate for the potential variation of injection volumes or amounts, an internal standard (IS) should be selected and used in quantitative analysis with CE [16]. The IS method works better in the hydrodynamic injection than the electrokinetic due to its associated injection bias of analytes with different mobilities. In addition, an appropriate IS is often hard to find for specific analytes; a single IS may not be simultaneously suited for multiple analytes; and the LIF detection has higher requirements for the IS due to the non-uniform photo-bleaching effect on different fluorophores [17]. In the electrokinetic injection, it is essential to maintain a stable electroosmotic flow (EOF) to ensure reproducible injections. However, almost always, the EOF fails to be stable on an inter-day basis due to various factors including sample matrices, background electrolytes (BGE pH, composition, and concentration), temperature, and non-specific adsorption on capillary walls [15]. As a routine procedure, frequent capillary re-conditioning is performed in conventional CE to refresh inner capillary walls, but this procedure cannot ensure the capillary recovery. Therefore, how to enhance the quantitative reproducibility of CE becomes a challenging but crucial task for further research and development.

For quantitative analysis of complex samples, the standard addition method (SAM) is preferred to the standard calibration curve since the SAM can compensate for rotational matrix effects [18]. The multiple-point SAM was usually considered superior to the one-point SAM with regard to the measurement accuracy. However, the multiple-point SAM assumes that the zero response corresponds to a zero concentration of an analyte, but it often fails to be true [18]. Moreover, the response linearity in the concentration range of standard additions should be verified, but it becomes impractical and time-consuming due to the complexity of matrices [18,19]. Alternatively, the one-point SAM has been advocated and should be seriously considered [18,19]. Theoretical and experimental results have established that the one-point SAM can be precise and accurate in quantitation when appropriate experimental conditions are used [19,20]. Specifically in CE, sample matrices affect sample injection and analytes' migration besides conventional matrix effects, which may cause inaccuracy of measurements when using the multiple SAM. In addition, the multiple-point SAM lacks analytical throughput since multiple points at least three replicates each are required to quantify a single sample. On the other hand, the one-point SAM reduces the number of standard additions thus facilitating the analytical throughput. Moreover, flow-gated CE using a short capillary and a high separation voltage [21–24]or microchip CE is able to perform rapid separations which further improve the analytical throughput.

Flow-gated CE mimics the configuration of a single-cross microchip for CE in which the flow-gated injection is frequently used for effective flow control and sample injection [25,26]. Compared with microchip CE, flow-gated CE has numerous advantages. First, fresh BGE is supplied via a syringe, which reduces the pH variation due to electrolysis. Second, a silica capillary with various inside diameters may produce better separation efficiency thanks to its feasible surface property and the circular channels. Third, a silica capillary is more compatible with various detectors for sensitive detection. It is convenient to couple with a MS detector, and to use a sheath flow cuvette or an oil-immersion objective for LIF. Fourth, the separation capillary, if getting clogged or broken, can be easily replaced with a new one at a low cost. Especially when a polydimethylsiloxane (PDMS) flow gate is used, assembling a new capillary to the flow gate becomes more convenient [25,27].

In this paper, we report a novel strategy for rapid quantitation of biological samples by using the one-point SAM on a flow-gated CE system coupled with alternate injections of a sample and its standard additions. A PDMS micro-switch was fabricated and integrated with the flow-gated CE system to enable alternate injections. This method assumed that the detection system was stable during two neighboring separations in a short time. As a proof of concept, cyanide and amino acids in human urine were determined on the platform by using the one-point SAM.

2. Experimental

2.1. Chemicals and reagents

Sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$), phosphoryethanolamine (PEA), taurine, and all amino acids were purchased from Sigma (St. Louis, MO). Potassium cyanide, dimethylformamide (DMF), sodium dodecyl sulfate (SDS) and tetrasodium salt of ethylenediaminetetraacetic acid (EDTA) were purchased from Fisher Scientific (Chicago, IL). Naphthalene-2,3-dicarboxaldehyde (NDA) was from Invitrogen (Eugene, OR). Hydroxypropyl-beta-cyclodextrin (HP-β-CD) was purchased from ACROS (New Jersey, USA). Fused silica capillaries were ordered from Polymicro Technologies (Phoenix, AZ). Silicon wafers at 3 inches in diameter were ordered from University Wafer, Inc. (South Boston, MA).

The NDA stock solution at 10.0 mM was prepared in DMF, and the working NDA was prepared by diluting the stock solution to 2.0 mM or 5.0 mM with deionized (DI) water on a daily basis. The stock borate buffer (pH 9.2) was prepared by dissolving tetraborate (100.0 mM) in DI water. The stock solution of PEA (400.0 mM) was prepared in 100 mM tetraborate, and the working PEA was diluted to 80.0 mM unless otherwise stated. EDTA at 100.0 mM was prepared in DI water without pH adjustment. Cyanide derivatization was performed by off-line mixing of NDA (2.0 mM in DMF/water at 50/50 by volume), PEA (80 mM in 80 mM tetraborate pH 9.2 plus 10 mM EDTA), and cyanide sample at 1:1:1 volume ratio. Dual sample flows were supplied via micro-syringes powered by a Hamilton syringe pump at a flow rate of 600 nL/min.

2.2. Fabrication of micro-switch

The design of the micro-switch is shown in Fig. 1c which was fabricated with PDMS by following the reported procedure with appropriate modification [28]. Briefly, the master mold of the fluidic layer was prepared on the surface of a silicon wafer by using standard photolithography. First, SU-8 photoresist features at 60 μm in thickness and in width were fabricated on a silicon wafer to accommodate capillaries at 150 μm in OD and 40 μm in ID for

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