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Stacking in a continuous sample flow interface in capillary electrophoresis

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ABSTRACT

Using a tee connector in a commercial capillary electrophoresis instrument, the effect of field amplified sample injection from both flowing and static sample volumes was investigated. It is shown that under identical conditions (40 min electrokinetic injection at 5 kV from a sample volume of 295 μL) the limit of detection using the continuous sample flow interface is 4 times lower than from a static vial. The relationship between different flow rates and injection voltages on the injected sample amount was also investigated using a 2D axisymmetric simulation (COMSOL 4.3b) and verified experimentally, confirming conditions under which there is near-quantitative injection of the sample target ions. Using electrokinetic injection at 30 kV and a flow rate of 558 nL/s the same enhancement from an even smaller volume of 184 μL could be achieved in 5.5 min than could be achieved from 295 μL and a 40 min injection. This sensitivity enhancement factor corresponded to four orders of magnitude improvement compared to a hydrodynamic injection. This is the first report showing that a continuous sample flow interface combined with stacking methods under conditions approaching quantitative injection from the entire sample volume has the potential to be more sensitive than a static system.

1. Introduction

The flexibility and versatility of CE are two of its greatest strengths while the low concentration sensitivity is one of its most significant limitations [1]. In order to improve CE concentration sensitivity, a number of on-line sample preconcentration strategies have been developed [2–13]. Most of these strategies are based on a chemical discontinuity that is located inside the capillary around which analytes are concentrated. This concept is commonly called 'stacking' or 'sweeping' and a range of different discontinuities can be created including conductivity (field amplified sample injection [14–21], field amplified sample stacking [20,22] and isotachophoretic stacking [12,23–26]), pH (dynamic pH junction [27]) and micellar effects [28,29] (sweeping [30,31] and micelle collapse [32,33]).

All of these stacking methods can be used with sample injected either hydrodynamically or electrokinetically. Sensitivity enhancements with hydrodynamic injection are limited by the volume of the capillary – it is impossible to inject more than one capillary volume. Therefore much greater sensitivity enhancements can be achieved with electrokinetic injection as it is not limited by the volume of the capillary. When electrokinetic injection is performed under field amplified conditions, then there are further gains – sensitivity enhancements of 10^4–10^6 have been demonstrated, although with these enhancements sample depletion is observed which requires that a new portion of sample from which no injection has been performed should be used for each injection [34,35].

Hirokawa et al. explored the issue of depletion further by examining the relationship between electrode position and the amount of sample injected [36]. They found that only analytes in an effective potential field, which is essentially the volume of sample between the electrode and the capillary tip, could be introduced into the capillary while analytes outside the field are not injected. This suggests a localized injection zone in which the ions are depleted. The analyte ions from outside this region only enter the field region by diffusion. Therefore long injection times may be required to inject a large fraction of ions from the total sample volume. This led them to develop circular electrodes placed at the very top of the sample such that a 3D electric field is created that covers almost the entire sample volume [37]. This greatly improved the transport of ions to the capillary tip, providing near quantitative injection of all the sample ions into the capillary.

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An alternative approach that may overcome the issue of localized depletion and increase the mass of sample ions that are electrokinetically injected is to use a flowing sample stream. Kuban et al. [38] who developed a flow-through channel and Fang et al. [39] who developed a flow-through reservoir separately reported the first flowing interfaces for CE. Different interface designs have since been used, e.g., an H-channel structure [40], a modified flow through chamber interface [41], various interfaces using tubular electrodes [42–45], and an interface using an on-column polymer–embedded graphite inlet electrode [46]. Using these types of interfaces, there have been three reports on the examination of field amplified sample injection (FASI). Kuldvee et al. [47] demonstrated that a short FASI (10 s at 8 kV) from a flowing sample stream afforded a 100-fold increase in sensitivity when compared to FASI from a static sample (2.5 s at 18 kV). It should be noted, however, that this static sample was in the custom-interface and the actual volume that was sampled was considerably smaller than is typically used with a conventional CE. The static sample was also considerably smaller than the volume sampled while flowing. Also, the electrokinetic injection time could not be extended due to the hydrodynamic introduction of sample matrix which reduced the field strength at the capillary tip during injection. Liu et al. [48] used FASI (15 s at 7.5 kV) in combination with sweeping micellar electrokinetic chromatography (MEKC) and obtained a sensitivity enhancement of 64–86 compared to non stacking conditions. The system was not systematically studied. Kuban et al. [49] were able to perform a longer FASI (6 min at 2 kV) by eliminating the pressure-induced flow through the capillary, and this resulted in a 2000-fold enhancement compared to a typical hydrodynamic injection. They used a homebuilt CE system as well as a homemade interface and no comparison was made to FASI in a static sample to establish whether there was any improvement from having a flowing sample during injection.

In the paper which follows, we describe a continuous sample flow interface that was constructed using a commercially available Tee connector integrated into a commercial CE to allow direct comparison of the benefit of performing FASI on a flowing sample. The hydrodynamic introduction of sample was minimized by adjusting the liquid levels in the buffer and waste vials allowing injection times of up to 40 min. FASI with sweeping followed by micellar electrokinetic chromatography (FASI-sweep-MEKC) was used to compare sample injection from a static system and a flowing stream. We demonstrate that by continuously flushing the sample through the interface, the efficiency of FASI is increased, providing enhanced sensitivity. Simulations along with experimental studies were used to study the influence of injection voltage and flow rate, and to establish the conditions in which there is near quantitative injection of the selected analytes. Significant enhancement in the proportion of sample ions that are injected when injecting from a flowing sample stream is demonstrated and this work is the only to compare electrokinetic injection of the same sample volume, under the same conditions with the only difference being whether the sample stream was flowing or static.

2. Experimental

2.1. Reagents

All reagents (sodium dodecyl sulphate (SDS), phosphoric acid, sodium hydroxide, HPLC grade acetonitrile) were purchased from Sigma–Aldrich (St. Louis, MO). Stock solutions of 1 M phosphoric acid, 200 mM SDS and 4 M sodium hydroxide were prepared. 1 M phosphoric acid was prepared by mixing an appropriate amount of purified water with phosphoric acid. Other solutions were prepared by dilution of the stock solutions with water. All solutions were filtered through a 0.45 μm filter from MicroScience (Co Durham, UK) prior to use. The background electrolyte (BGE) was 200 mM phosphoric acid with 20% (v/v) acetonitrile, and the sweeping solution was 100 mM phosphoric acid, 100 mM SDS with 20% (v/v) acetonitrile. The $^{s}_{w}$pH (pH measured in acetonitrile/water with electrodes calibrated in water)[50] values of these solutions were adjusted to 2 with 4 M NaOH after the addition of acetonitrile and before final dilution in a volumetric flask. The sample diluent was 0.5 mM phosphoric acid or 200 mM phosphoric acid with 20% (v/v) acetonitrile, $^{s}_{w}$pH 2, for the FASI-sweeping-MEKC and the capillary zone electrophoresis (CZE) experiments, respectively. Alprenolol hydrochloride and Propanolol hydrochloride were purchased from Sigma–Aldrich (St. Louis, MO) and were prepared in water (1000 mg/L).

2.2. Instrumentation

Water was purified using a Milli-Q system from Millipore (Bedford, MA). The $^{s}_{w}$pH was measured using an Activon Model 210 pH meter (New South Wales, Australia). All capillary electrophoresis experiments were conducted on an Agilent 3D-CE instrument (Waldbronn, Germany) equipped with a diode array detector and a fused silica capillary (25 μm and 365 μm inner and outer diameters, respectively) from Polymicro Technologies (Phoenix, AZ). The total length was 50 cm with 20 cm from the inlet end to the detector. Capillary temperature was controlled at 20 °C. The lift offset, which determines the distance between the capillary entrance and the tip of the cylindrical electrode which surrounds the capillary, was set to 4 mm.

2.3. Continuous sample flow interface

The construction of the continuous sample flow interface was similar to that described by Blanco et al. [42]. A schematic of the flowing sample interface can be seen in Fig. 1A. Briefly, a Tee connector (P-727, Upchurch Scientific) with a 500 μm thru-hole was used to connect the capillary and stainless steel electrode (200 mm of stainless steel tubing, U-145, Upchurch Scientific), which served as the anode during injection and as the cathode during separation. The stainless steel electrode was aligned opposing the sample inlet tube (508 μm inner diameter and 794 μm outer diameter) which consisted of a 22 cm piece of polyether ether ketone (PEEK) tubing (1569, Upchurch Scientific). For the waste outlet, a piece of rubber tubing (1250 μm × 500 μm × 30 cm) was connected to the electrode in the interface. The sample inlet tube was connected to a 30 cm piece of capillary (25 μm ID, 365 μm OD from Polymicro Technologies (Phoenix, AZ)) using a connector (P-643, Upchurch Scientific) which was placed in the inlet vial that contained either the sample solution or the micellar solution. All capillaries and the electrode in the interface were connected using the supplied fittings and ferrules. The entrance of the separation capillary was aligned so that it was 365 μm away from the opposing wall of the Tee connector. The continuous sample flow interface axis between the inlet capillary and the electrode was positioned vertically inside the capillary cassette. The separation capillary coupled to the Tee connector was horizontal. To more accurately regulate the external pressure of the CE instrument, a manual pressure regulator (Norgren, R37G-3GK-FRN) was installed in the external pressure line. This allowed adjustments of pressures from 0.1 to 6 bar ± 5%, which are below the capabilities of the Agilent CE to be applied at the inlet of the capillary coupled to the sample inlet tube. In order to switch quickly between the full external pressure (6 bar) and the lower external pressure from the manual regulator, the first open–close valve (Onomi, 1/4) was installed parallel to a series connection of the regulator and a second open–close valve.