Droplet-Based Sample Injection for Chip-Based Analytical Separations

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The advent of capillary and chip-based electrophoresis has opened up new possibilities for high-throughput analysis of a diversity of biological systems. The high-resolution separations typical of chip-based electrophoresis are in large part defined by the ability to introduce small sample volumes into the separation channel. This is not a trivial process and standard electrokinetic and hydrodynamic methods are far from ideal. Herein, we review recent studies that integrate droplet-based and continuous flow microfluidics to provide an alternative injection mechanism that is simple to implement and enables reproducible transport of defined sample volumes without bias.

Electrophoresis remains one of the most powerful tools in separation science. In recent years capillary and chip-based electrophoresis (CE/MCE) formats have been developed and refined to provide rapid, high-throughput and automated analysis of a broad range of targets, including nucleic acids, proteins and peptides, small molecules, enzymes and cells. [1-3] Indeed, benefits including reduced sample and reagent volumes, facile automation and integration with other analytical techniques (such as chromatography and mass spectrometry), improved efficiency and high analytical throughput are inherent to both formats.[4,5] Interestingly, however, whilst there have been extensive studies aimed at controlling separation conditions and capillary/channel surface chemistries, sample injection techniques and post-separation fractionation vary little from the original formats reported over 20 years ago.

As with conventional CE, controlled injection of a small sample volume into a chip-based separation channel is critical when performing high-efficiency electrophoresis. Extra-column band broadening is primarily defined by the quality of the on-column injection and detection procedures. In simple terms, the total peak variance, σ^2_{tot}, is given by

$$\sigma^2_{tot} = \sigma^2_{inj} + \sigma^2_{col} + \sigma^2_{det}$$

where σ^2_{col} is the variance generated in the separation channel, σ^2_{inj} is the variance due to injection and σ^2_{det} is the variance originating from the (finite volume) detection system. Since most on-line optical detection schemes provide for sufficiently small detector pathlengths, σ^2_{inj} dominates the extra-column contribution. Accordingly, larger injected sample plugs will significantly impact σ^2_{col} and will result in a decrease in the theoretical plate number. In short, higher theoretical plate numbers are contingent on small injection volumes and associated plug lengths.

The primary methods for introducing a sample plug into a separation channel are hydrodynamic or electrokinetic in nature. During hydrodynamic injection, a defined sample plug is either driven directly into the capillary or into a cross-channel intersection on a planar chip by means of a pressure difference. Hydrodynamic injections are reproducible and also permit accurate quantification of the contained components (due to a lack of injection bias).[6] However, pressure injections require external pumps and valves for on-chip separations, thus increasing system complexity and instrumental footprint. During electrokinetic injections, sample is driven into the separation channel under an applied electric field. A variety of schemes for injection are possible in chip-based systems, however the majority involve an orthogonal crossed channel geometry where sample is electrokinetically conveyed across the separation channel after which the applied field is switched so that only the sample within the intersection is injected for subsequent separation.[7] Importantly, this general approach allows the reproducible injection of extremely small volumes of sample.

Unfortunately, electrokinetic injection schemes are normally biased towards the introduction of small, high-mobility molecules, even when using cross-channel injectors in chip-based systems.[8] Accordingly, quantitative analysis of resulting electropherograms can often misrepresent the sample content. To illustrate the hidden complexities of electrokinetic injection schemes Jin and Luo presented a numerical analysis of electroosmotic flow in such systems.[9] Figure 1 illustrates calculated electric potential distributions during a pinched injection (Figure 1A) and subsequent separation (Figure 1B) using a standard cross-channel injection scheme. It can be seen that during the loading step the potential gradient acts to drive sample towards the sample waste vial while preventing it from leaking into the separation channel. During the dispensing and separation stages the potential distribution shifts to permit the plug at the intersection to enter the separation channel with excess sample being "pushed back" towards the sample and sample waste reservoirs. Accordingly, the ratio of electric field strengths in the separation and sample channels control both the confinement of the sample at the intersection and the volume of each species injected. Additionally, it should also be noted that variable surface conditions (a common scenario) on each arm of the cross-piece will cause significant variations in local electroosmotic flow and complicate optimisation of injection protocols. Experimental support of these ideas was provided by Alarie and co-workers who found that pinched injection using a cross-channel injector exhibited a bias towards neutral molecules.[8] They found that bias occurs during both loading and dispensing steps. Indeed, a variation in injected volume of up to 27% was observed, with the degree of bias
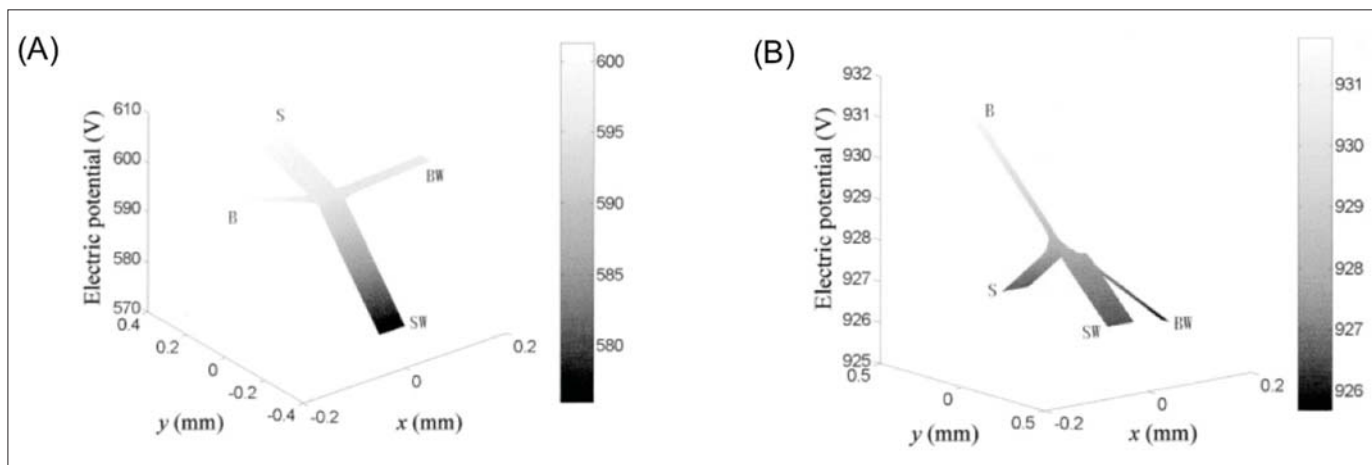
Figure 1: The calculated distribution of electric potential at a microfluidic cross-region during a pinched injection on a chip-based electrophoretic device. (A) Potential distribution during loading. (B) Potential distribution during dispensing and separation steps. Images reproduced from reference [9]

being dependent on the ratio of electric field strengths applied during each step. Other authors have since demonstrated similar biasing phenomena using other modes of electrokinetic injection, and although some geometries and the use of large sample reservoirs alleviate the problem they do not completely eliminate it. The only injection formats not displaying sample bias are those based on pressure differences. Consequently, there is a need for alternative injection mechanisms that are simple to implement and enable reproducible delivery of specific sample volumes without bias.

Droplet-based or segmented-flow microfluidics has been the subject of much attention over the past five years.[10,11] In such systems femtoliter – nanoliter droplets can be generated at high speed by combining two immiscible phases (typically aqueous and oil-based). The formation of droplets is a spontaneous process and is normally a result of shear force and interfacial tension at the liquid-liquid interface. Importantly, the formation of droplets within microfluidic channels can be achieved at rates of up to several kHz with exquisite control over both droplet size and composition. Based on these features such platforms are ideally suited to processing millions of individual reactions in ultra-short times and with superb reproducibility.[12] It is therefore unsurprising that a number of recent studies have assessed the utility of integrating electrophoretic and chromatographic separations with droplet microfluidics, for both sample injection and post separation fraction collection. For example, a sample can be fractionated into droplets post reaction or separation and transferred to a subsequent analytical process, or defined sample volumes can be injected into separation channels using the droplet as an injector. This approach, in theory, allows injection of the representative samples required in quantitative analysis.

Whole droplet injection also ensures that sample wastage is negligible and electrokinetic bias (encountered in continuous-flow injection) is eliminated. Recently, several novel approaches have been described employing droplet microfluidics to bridge continuous-flow devices.

One of the first attempts to controllably introduce the contents of an isolated droplet into an electrophoretic channel was described by Edgar and co-workers.[13] The reported microfluidic device consisted of a droplet generation region and a separation channel separated by an immiscible partition at the point of injection. Fusion of an incoming

aqueous droplet with the immiscible boundary effectively injects the droplet content into the separation channel to allow separation of a precisely defined volume (Figure 2A-F). Although separation of fluorescently labelled amino acids contained within 10 fL droplets was successfully achieved, the authors highlighted several disadvantages of their design including the need for precise pressure control and selective channel pre-treatment (to generate hydrophobic and hydrophilic surfaces) and oil contamination that results in a reduction in electroosmotic flow. Subsequently, Roman et al. described a microfluidic device employing a K-shaped element to transfer sample from segmented
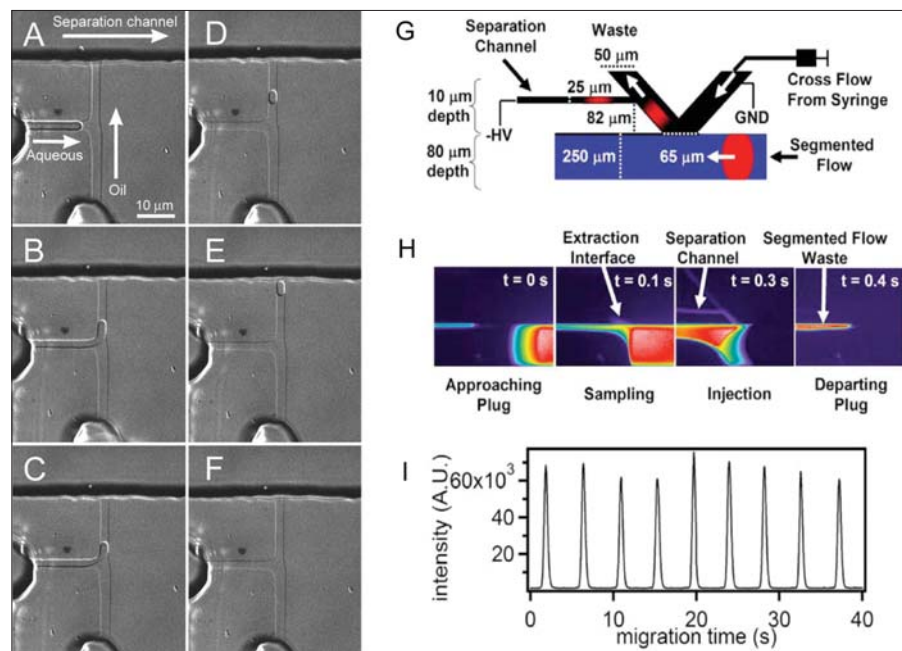

Figure 2: (A-F) Sequence of images depicting the generation, transport and injection of an aqueous droplet into an electrophoretic separation channel. Images in (A-F) reproduced from reference [13]. (G) K-shaped microfluidic interface for sample injection. The device includes a segmented flow channel, a V-shaped cross-flow channel and a separation channel. (H) Micrographs illustrating the droplet-injection approach and the coalescence of plugs using a cross flow of 100 nL/min. (I) Series of electropherograms resulting from sampling and injection of droplets (containing 1µM serine derivatised with FITC) using the discrete injector and separation on a 5 cm long electrophoresis channel at 500 V/cm. Images in (G-I) reproduced from reference [14].
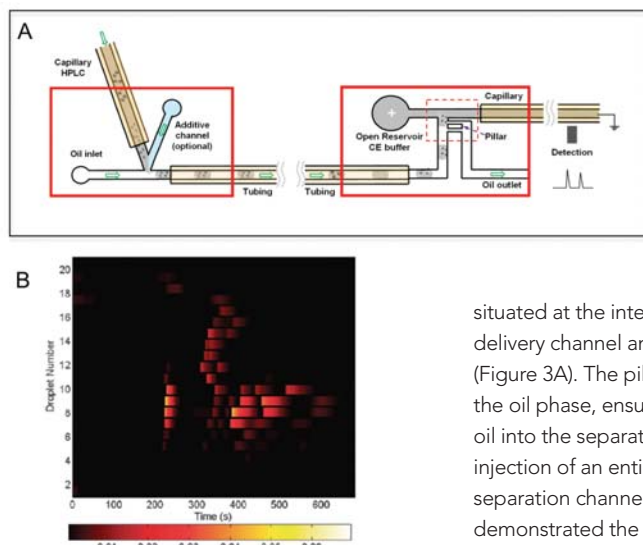
Figure 3: (A) Schematic of the droplet interface used to connect a first dimension HPLC instrument to a second dimension CE instrument. Oil extraction was achieved using a pillar array positioned at the point of droplet injection into the CE separation channel. (B) Two-dimensional separation profile obtained for the analysis of a 5-component peptide mixture using the 2D interface device. Images reproduced from reference [15].

flows into an electrophoresis channel.[14] Figure 2G shows a schematic of this device which consists of a 250 µm wide segmented flow channel, a V-shaped cross-flow channel and a separation channel. The cross flow channel serves two functions; first it counterbalances the pressure exerted by the segmented flow channel at the interface with the separation channel, and second it allows washing of excess sample from the mouth of the separation channel (effectively terminating the injection). The presence of oil at the interface leads to the formation of a "virtual wall" which disappears when an aqueous droplet occupies that region of space, causing an injection into the cross-flow channel (Figure 2H). The sample is then moved to the mouth of the separation channel and a plug is injected using an applied electric field. Using this device the authors demonstrated the serial injection and free solution separation of a mixture of amino-acids (Figure 2I). However, this format of droplet injection incurs appreciable sample wastage as only a small portion of the droplet is injected. Moreover, the transferred sample is further diluted in the cross-flow channel with the injection volume being difficult to control.

To address some of the aforementioned shortcomings Niu and colleagues subsequently demonstrated a fully functional droplet connector for two-dimensional separations.[15] Specifically, this microfluidic connector utilised droplet generation after a first dimension separation (nano-liquid chromatography) with oil depletion and droplet merging prior to a second dimension separation (capillary electrophoresis). Importantly, the interface is passive in its operation and employs a pillar array situated at the intersection of a droplet delivery channel and a separation channel (Figure 3A). The pillar array actively extracts the oil phase, ensuring negligible transfer of oil into the separation capillary, and enables injection of an entire droplet into the separation channel. The authors demonstrated the efficacy of the interface through two-dimensional separations of peptide mixtures (Figure 3B) and 'heart cutting' separations of yeast cell proteins. The ability to finely partition peaks originating from a first separation dimension is significant since it ensures that no chemical or biological information is lost during dimensional transfer. Furthermore, reagents can be added and mixed to the formed droplets, therefore allowing the facile integration of sample preparation.

Droplet injection into separation channels and capillaries has also been demonstrated using digital microfluidic systems. In 2009, Gorabatsova et al. [16] and Abedelgawad et al. [17] described the interfacing of digital microfluidic devices for chip-based electrophoretic systems. Both groups successfully demonstrated injection of sample from µL-volume droplets and free solution separation of their contents. Interestingly, in the following the year the device reported by Abedelgawad was refined to allow the creation of a multilayer interface where droplets could be dispensed and diluted prior to zone electrophoresis on-chip.[18]

Conclusions
It is evident that droplet-based microfluidic tools are beginning to provide new opportunities for the controlled injection, transport and isolation of ultra-small sample volumes. Although their integration with both upstream and downstream analytical separations has been proved much work remains. As has been seen, a key requirement for the delivery of droplets to a continuous flow is effective oil extraction. Current approaches require either complex local treatment of channel surfaces or delicate pressure control. More robust oil extraction techniques are therefore required. Furthermore literature reports have only demonstrated droplet interfacing to separation channels that perform free solution electrophoresis. The use of capillary gel electrophoresis, although more relevant to fields of proteomics and genomics, introduces more challenges to the design of such an interface. These include the practical problems associated with loading and replacing high viscosity sieving matrices in interface devices with complicated geometries and surface chemistries. Despite these challenges, the use of droplet interfacing tools has opened new paradigms for the analysis of ultra-small volumes. The ability to control and process such volumes in high-throughput is likely to find significant application in the fields of proteomics, genomics, metabolomics and disease diagnosis.

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