

ULTRA-FAST PRESSURE INJECTION FOR ELECTROPHORESIS SEPARATIONS ON MICROCHIP

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Abstract: In this paper, we present a new type of sample introduction using pressure in an original designed electrophoresis microchip. This highly flexible injection technique is also characterized by a tunable sample plug volume and by very short injection times (< 1 sec.). The simplicity of the injection system makes it very attractive for a wide range of microchip based analytical applications. Finally, the high repeatability of the pressure pulse injection is demonstrated through electrophoresis experiments of a mixture composed of 3 calcein derivatives and fluorescein separated in about 5 seconds.

Key words : sample loading, plug, hydrodynamic, CE

Introduction. Capillary electrophoresis (CE) in the microchip format has demonstrated superior capabilities over conventional electrophoresis for high-throughput separations [1,2]. Mostly, electrokinetic sample injection is used, but sample introduction by electro-osmotic pumping has a strong limitation: the composition of the injected plug may differ from the original sample composition [3,4]. To prevent this so-called biased injection, alternative procedures have been presented to load the sample by pressure using valves and pumps or hydrostatic pressure [5-7]. To preserve the main advantages offered by miniaturization, we have designed an electrophoresis microchip dedicated to pressure injection, which enables to inject, in a simple way, a sample plug of controlled and variable size within a few hundreds milliseconds.

Chip layout. A schematic diagram of the pressure injection microchip is shown in figure 1. Microchannels are structured in the upper (figure 1(a)) and lower glass wafer (figure 1(b)), after which the two chips are fusion bonded. The chip layout (figure 1(c)) is a classical cross-shaped channel (separation channel between reservoir I and II; injection channel between reservoir III and IV) with an additional reservoir (V) linked to the injection channel. The sample is placed in reservoir III and the injection is done using a pressure pulse on the sample reservoir. Electro-osmotic pumping is used to dispense the sample plug in the separation channel and to repel the sample waste in reservoirs IV and V.

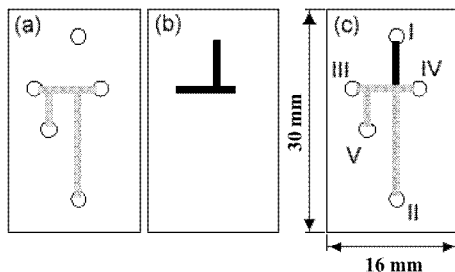


Figure 1: Schematic view of the CE microchip dedicated to pressure injection and composed of microchannels etched on two slides (a) and (b). The resulting chip layout after alignment and bonding of (a) and (b) is represented in (c).

Pressure pulse injection system for CE microchip. The pressure generation system is a very simple system consisting of two parts: a PDMS membrane and a mechanical actuator, which induces pressure by deflecting the membrane during a certain time, as schematically represented in figure 2. The PDMS membrane is dimensioned to provide an hermetic seal of the sample reservoir up to an applied pressure of about 4 kPa. When the membrane is deflected, the pressurized air pushes the liquid in the injection channel. The injection is very fast and very small quantities of liquid are involved. The air volume in the sample reservoir (about 30 μL) is much larger than the volume of the sample flushed in the injection channel (below 0.2 μL), which gives a small pressure drop during injection (see figure 3). One notes that there is no negative pressure after the 0.8-second pressure pulse in figure 3, which would have its origin in a back force originating from a too rigid PDMS membrane. We have on purpose used a flexible membrane, to avoid or strongly reduce ‘back sucking’ effects.

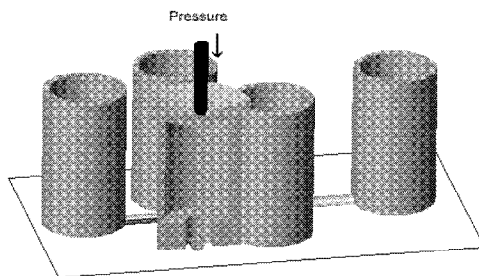


Figure 2: Schematic view of the pressure injection principle, showing the deflection of a thin membrane placed on top of the sample reservoir, thereby creating a momentary pressure rise.

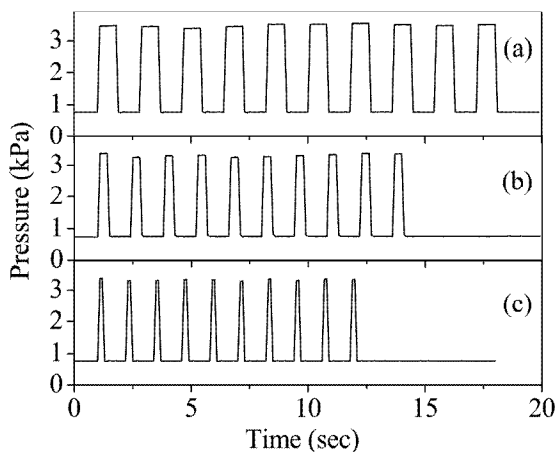


Figure 3: Pressure measured in the sample reservoir by a pressure sensor (Fujikura) on a dedicated chip during pressure injection sequences with 10 pulses of (a) 0.8 sec., (b) 0.4 sec. and (c) 0.2 sec. The background pressure corresponds to the hydrostatic pressure of the reservoir.

High speed injection-separation sequence. Our technique allows freely choosing the sample plug volume by simply changing the pressure pulse length (figure 4). By increasing the plug injection volume, higher fluorescent intensities are obtained and lower concentration samples can be detected.

Moreover, the pulsed pressure injection is characterized by a very high repeatability, allowing multiple injections and separations in a single channel in a matter of seconds (figure 5).

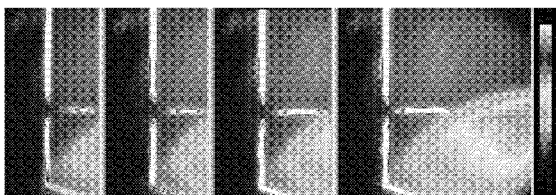


Figure 4: CCD camera capture of fluorescein injections with an increasing pressure pulse length (starting from the left image: injection time = 0.3 sec, 0.35 sec, 0.4 sec, 0.45 sec).

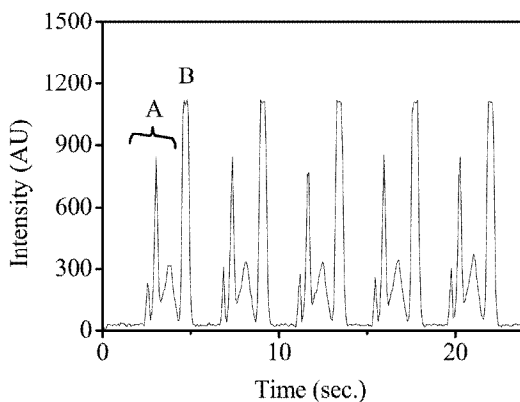


Figure 5: Electropherogram showing the separation of 3 calcein derivatives (A) and the fluorescein (B) after 5 sequential pressure injections of 0.4 sec.

Conclusion

Pulsed pressure injection represents a serious alternative to electrokinetic sample loading for microchip-based separations. Pressure enables analyte transport within the injection channel, independent of charge or electrophoretic mobility. This technique is characterized by a tunable sample plug volume, by very short injection times (0.1 - 1.0 sec.) and a high repeatability. By its simplicity, the pulse pressure injection system opens the way to parallel hydrodynamic injections without the need of integrated valves, multi-port injection systems or pumps.

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