SWEEPING FLOW ELECTROPHORESIS (SFE): A NEW CONTINUOUS SEPARATION TECHNIQUE

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ABSTRACT

In this paper, a new principle for continuous electrophoretic separation is introduced. An electrokinetically pinched sample flow is swept through a microfluidic chamber. The wavelengths of the resulting sine-waves are in direct relation with the electrophoretic mobility of the compound. The principle is applied to separate Rhodamine B and Fluorescein.

KEYWORDS: Sweeping flow electrophoresis, SFE, EOF

INTRODUCTION

Free-flow electrophoresis has several advantages over capillary electrophoresis: the mobility of a separated compound can be determined time-continuously, plug-formation is not required and the analytes can be recovered after separation. A disadvantage of free-flow electrophoresis is the fact that two different actuation forces are required. These are typically pressure driven flow in forward direction and electrokinetic separation in lateral direction [1]. Here we introduce a continuous flow electrokinetic separation principle that is fully electrically controlled. The principle uses a pinched sample flow that is swept through a chamber by two guiding streams. The electrophoretic mobilities of the sample compounds are in direct relation with the wavelength of the resulting sinusoidal separation profiles. We call this technique sweeping flow electrophoresis (SFE).

THEORY

Recently, we introduced an EOF driven technique to position a sample flow in a laminar flow chamber [2]. The principle is depicted in figure 1. Two guiding flows position a central sample stream in a laminar flow chamber. The three flow streams are electroosmotically manipulated, so that control of the applied potentials results in the accurate positioning of the sample stream [2]. The guiding stream potentials are chosen such, that a sinusoidal sample flow is generated. The *y*-position of the sample stream depends on the time (*t*), the *x*-position and the sweeping frequency (*f*):

$$y(x,t) = Y \sin\left(2\pi f t - \frac{2\pi x}{\lambda}\right) \tag{1}$$

and

$$\lambda = \frac{(\mu_{eof} + \mu_{elec})E}{f} \qquad (2)$$

with Y the amplitude of the sine wave, λ the wavelength of the sine wave, μ_{eof} the electroosmotic mobility of the liquid, μ_{elec} the electrophoretic mobility of the sample compound and E the electric field. In [2] we demonstrated that it is feasible to sweep the sample stream, while keeping the electric field in the chamber constant. For this case λ becomes a constant that depends directly on the electrophoretic mobility of the separated compounds. A periodic image capture in phase with the sweeping frequency allows measurement of the wavelength.

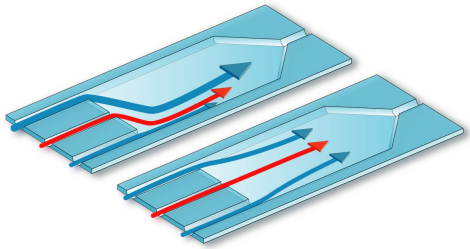

Figure 1: Principle of sample stream guiding in a laminar flow chamber. Two guiding streams sweep a central sample stream through the chamber

EXPERIMENTAL

A glass microfluidic chip is used as described in [2] (see figure 2). The chip is wet-etched with HF and contains powderblasted interface holes. Glass-glass direct wafer bonding was performed by applying pressure and annealing at 600°C. The chip is placed in a holder that contains reservoirs and electrodes for electrokinetic actuation. The principle is demonstrated for the separation of Rhodamine B and Fluorescein. The sample stream is swept with a frequency of 0.5 Hz.

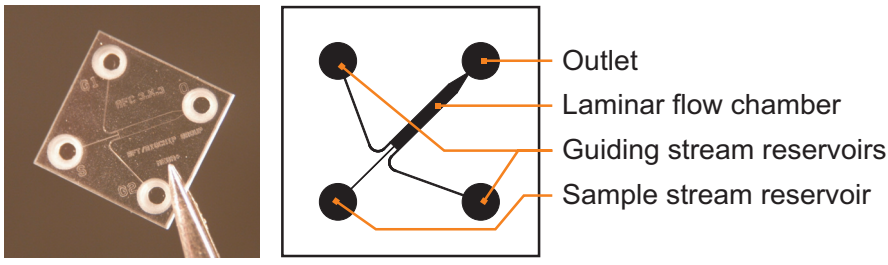

Figure 2 (a): Glass microfluidic chip with powderblasted holes. (b) The chip contains three inlet holes (one for the sample and two for the guiding streams) and one outlet hole. A positive potential is applied via external electrodes to the inlet holes. The outlet reservoir is grounded.

RESULTS AND DISCUSSION

Figure 3 shows images of the separated compounds for various parts of the sweeping period. As can be seen, the two compounds are separated from each other by a clear variation in the wavelength of the sine. Fluorescein shows a much shorter wavelength than Rhodamine B. This is in good agreement with the above formulas, since Fluorescein is typically negatively charged, while Rhodamine B is relatively neutral for the buffer used.

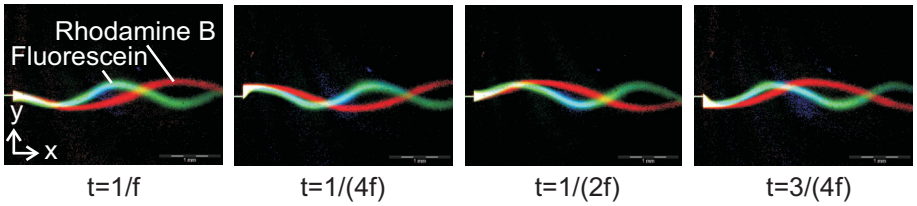

Figure 3: Sequence of separation images taken at one, one fourth, one halve and three quarter of the sweeping period.

CONCLUSIONS

A new, electroosmotically steered, continuous flow separation technique has been demonstrated. The technique uses two guiding streams to sweep a sample through a microchamber. Wavelength analysis of resulting sine waves yields information on the various electrophoretic mobilities. Rhodamine B and Fluorescein have been successfully separated. The technique can be considered an alternative to conventional capillary chip electrophoresis and free-flow electrophoresis.

ACKNOWLEDGEMENTS

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