

MICROFABRICATED CYCLICAL ELECTRICAL FIELD FLOW FRACTIONATION

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ABSTRACT

A cyclical electrical field flow fractionation (CEFFF) microsystem is demonstrated for the first time in this paper. Retention of polystyrene nanoparticles is accomplished and the effect of varying parameters such frequency is explored. This new sub-micron size particle separation technique can be used to separate cellular components, viruses and bacteria, proteins and DNA, colloids and emulsions; and thus, can be used as a lab-on-a-chip sample preparation method. Due to handling of smaller sample volumes in the system, the separation is faster than in comparable macrosystem.

KEYWORDS

Field flow fractionation, Sample preparation, Separation, Microfluidics

INTRODUCTION

Field flow fractionation (FFF) is a powerful separation method which has developed over the past 30 years. Instead of applying an electrical field parallel to the flow of carrier as in electrophoresis, FFF applies the electrical field perpendicular to the flow and employs the principles of differential electrophoretic mobilities and diffusion rates to separate particles. However, separation in FFF is achieved at the voltages three orders of magnitude lower than those used in electrophoresis; which is a major advantage of FFF. [1]

Although simple electrical FFF is a powerful method, it suffers a serious limitation due to the capacitance associated with polarization layer formed at the electrodes when a DC field is applied. This capacitance effectively reduces the electric field to less than 3% of the applied field value and thus the separation efficiency suffers. However, we have shown this problem can be countered by applying an alternating or cyclical electrical field which does not allow the build up of a double layer.

THEORY

Another advantage of FFF is the well-developed theory that allows for direct measurement of physical properties, often without calibration. The theory for cyclical FFF is no exception. An initial issue is what the effective field will be in the FFF channel. Using basic electrical theory, we can predict the field based on some of the electrical parameters of the system. If the applied voltage is denoted as $V(f,t)$, the effective field $E(f,t)$ in a microchannel of depth w , is given by the equation

$$E(f,t) = \frac{V(f,t)R_B}{w} \sqrt{\frac{1 + (2\pi f R_{DL} C_{DL})^2}{(R_B + R_{DL})^2 + (2\pi f R_B R_{DL} C_{DL})^2}} \quad \dots(1)$$

where with the bulk resistance of the carrier is R_B , the capacitance of the double layer is C_{DL} , and R_{DL} is the resistance of the double layer. For our microsystem with depth $25\mu\text{m}$, R_B is $15\text{K}\Omega$, C_{DL} is 0.0001F and R_{DL} is $12\text{K}\Omega$; the effective field is more than 97% of the applied field value for the frequencies above 0.3Hz .

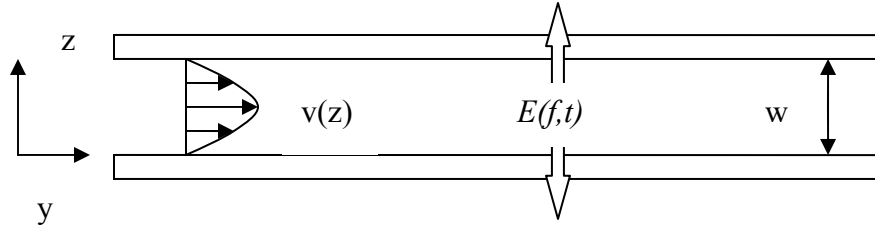


Figure 1: Microchannel geometry with electric field and flow field distributions

The particle motion under the influence of the electric field is given by multiplying the field strength by the electrophoretic mobility and integrating over time. The z -position of the particles can then be multiplied by the flow velocity at that position and integrated to determine the total elution time. Thus, particles in the microchannel have two orthogonal velocity components and under the influence of the electric field the particles elute later than when no field is applied. Depending upon the type of electric field applied (square, triangular or sinusoidal waveform), retention of the particles by the microsystem changes. The effect of such waveforms is discussed elsewhere [2]. Overall, these equations can be used to precisely predict the elution of particles in cyclical FFF.

Depending upon the distance a particle traverses in the microchannel during one cycle of the electric field, two modes of operation are defined in CEFFF; namely low frequency mode and high frequency mode. In low frequency mode, the frequency of the field is such that the particle travels to the opposite wall in less than half a cycle and remains there until the field is reversed. Whereas, in high frequency mode, the field is reversed continually so that the particle is never allowed to settle to the opposite wall and it bounces back and forth on the same wall as the polarity of the field changes.

EXPERIMENTAL

Fabrication of the Microsystem:

The input and output ports of nominal outside diameter $600\mu\text{m}$ were drilled in the microscope glass slides with a diamond drill bit and cleaned with piranha etch. The electrodes made of titanium (300\AA) and gold (1200\AA) were sputtered and annealed at 300°C for 5 minutes. The gold was etched using iodine etch ($4\text{g KI} + 1\text{g I}_2 + 40\text{ml DI water}$) and titanium was etched using 10% HF. The negative photoresist SU-8 25 (Microchem, Inc) was patterned on the electrodes to form the channel geometry.

Electrical wires were bonded with silver epoxy and the two substrates were bonded using UV-curable adhesive (Loctite, Inc). Upchurch Scientific fittings were used to make fluidic connections for sample injection and buffer flow. A schematic of the completed microchannel is shown in figure 2 and the complete device is shown in figure 3.

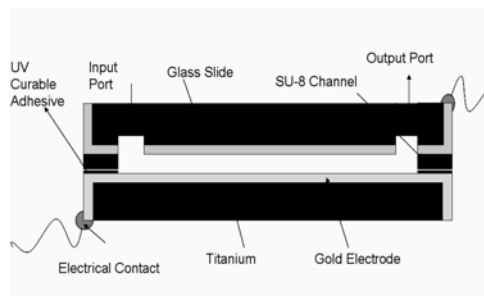


Figure 2: Cross section of Microsystem

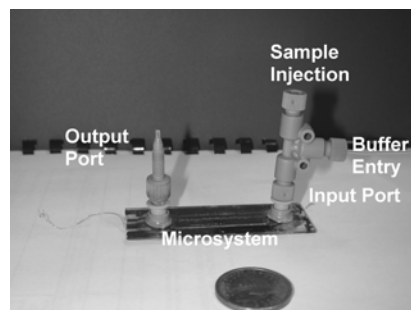


Figure 3: CEFFF Microsystem

Retention:

Experiments were performed with 280nm size polystyrene (PS) particles (Bangs Laboratories of Fishers, IN) at a concentration 0.1% by weight. The particle sample volume of 0.2 μ l was injected using a Hamilton microsyringe and the buffer (in this case DI water) was pumped through the microsystem continuously using a syringe pump (KD Scientific) at a flow rate of 1.0ml/hr. An HP 33120A function generator was used to generate a square waveform, an HP 34401A digital multimeter was used to measure the current, and an Agilent E3630A power supply was used for DC tests. The presence of particles was determined at 225 nm on a UV/VIS absorbance detector Model 520 (ESA, Inc, MA) with a flowcell volume of 1.2 μ l. The detector data acquisition was done in real time using a GPIB interface and LabVIEW software (National Instruments). Experiments were performed in both low and high frequency modes at several square wave peak-to-peak voltages (VPP). For high frequency mode experiments, a DC offset voltage of 0.1 V was applied to ensure that the particles were attracted towards one of the walls. Each experiment was run several times for repeatability.

RESULTS AND DISCUSSION

The particles showed retention in both low and high frequency modes and over a wide range of frequencies and voltages (Figure 4). At voltages higher than 4VPP, the microsystem showed very little particle retention, possibly due to electroplating and/or electrolysis bubbles disrupting the field and flow.

For low frequency mode, as predicted by CEFFF models, elution times decrease as the frequency of the field is increased in this mode. The magnitude of the particle

peaks also decreases as the frequency is decreased. This results in band broadening, which in most cases should be minimized. The effect is primarily due to diffusion [2].

In high frequency mode, as the frequency of the field is increased, the elution time increased. This fact was in agreement with the theory. However for the experiments in high frequency mode, an initial offset voltage was required to force the particle to settle near one of the electrode walls. The initial runs with no offset voltage showed no retention of the particles due to the fact that the particles diffused to the center of the channel very rapidly and eluted out with the maximum velocity flowlines. This shows the importance of diffusion and initial position of the particles in microscale systems. Similar effects have also been observed earlier in macroscale systems. [2]

When theory and results are compared, the values predicted by the theory are very close for low frequency mode, but the elution times in high frequency mode have been somewhat higher than the theory predicts, for what are currently unknown reasons. In this case, extra retention might be considered valuable, but until the reasons for the increased retention are clear, the theory for retention in high frequency mode is suspect.

CONCLUSION

μ CCEFFF was shown to be a viable technique for the analysis of nanometer size particles. The results agree with the predicted trends in elution time in different modes of operation. However, a full characterization is needed to use this system for charge based separations. Diffusion effects were much more significant in the microscale system and may lead to inefficiencies that need to be further studied and possibly controlled.

REFERENCES

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- [2] S Merugu and B K Gale, "Cyclical electrical field flow fractionation" *Proc. FFF*, Enschede, Netherlands, **2002**

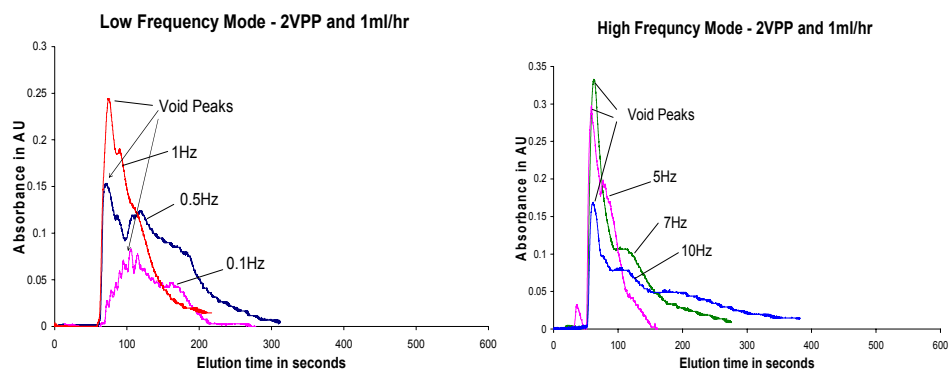


Figure 4: Elution times for 280nm size PS particles in low and high frequency modes