Nanoparticle Separations Using Miniaturized Field-flow Fractionation Systems

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ABSTRACT

In this work, a μ-ElFFF system is demonstrated with complimentary on-column electrical conductivity and resonance light scattering (RLS) detection systems. The fabrication of a μ-ElFFF system is described and test results from performing separations of gold RLS Particles™ and Protein A coated/uncoated polystyrene nanospheres in the system are presented. The particles ranged in size from 40-280 nm in diameter, and 0.1 μL samples, with particle concentrations as low as 1.09 x 10^{-11} M, were injected into the input port of the microchannel, separated and detected. A CCD camera mounted on a microscope was used to obtain images that clearly showed the front and tail ends of the samples. The images correlated well with the response of the conductivity detector measurements. Integration of an optically based signal generation and detection method allows real-time visualization of separations and correlation of optical and electrical detector signals.

Keywords: MEMS, RLS, FFF, nanospheres, detection

1 INTRODUCTION

Field-flow fractionation (FFF) is a separation technique that can be used as a stand-alone analysis system or as an integrated component for sample preparation/purification. There are numerous subtypes of FFF including electrical, thermal [1], sedimentation [2], flow [3], and acoustic [4]. One of the first subtypes to be implemented in a micro format is electrical FFF (ElFFF) [5]. In all FFF systems, the driving force behind the separations is applied normal to the fluid flow direction. In μ-ElFFF an electric field is applied normal to the direction of flow in a microchannel, forcing similar particles in the channel to equilibrate at a specific distance/height from the channel wall. The parabolic flow velocity profile of the microchannel makes particle fractions at different average heights flow at different velocities, resulting in a temporal and spatial separation (Fig. 1).

High-resolution detection of the separations can be achieved by performing electrical and optical detection on-chip. In previous work, μ-ElFFF systems with on-chip electrical detectors were shown to be capable of efficiently separating several types of particles and molecules [6]. Additionally, resonance light scattering has been shown to be capable of detecting particles in the 10-1000 nm (in diameter) range [7].

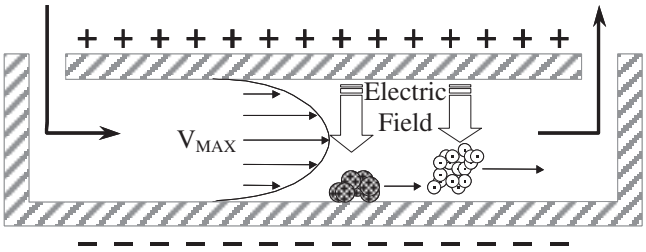


Figure 1. Diagram showing the FFF separation principle. Maximum flow velocity (V_{MAX}) is at center of μ-channel. In this case, an electric field is used to generate the driving force behind the separation.

Finally, integration of an optically-based signal generation and detection method allows real-time visualization of separations as well as correlation of the optical and electrical detector signals.

This paper presents the fabrication of μ-FFF systems, and test results from performing separations of gold RLS Particles™ and Protein A coated/uncoated polystyrene nanospheres.

2 MATERIALS AND METHODS

2.1 Fabrication of the μ-ElFFF System

To enable visualization of the separations, glass was used as the substrates in the fabrication of the μ-FFF channels. After cleaning the substrates with a mixture of H_2SO_4/H_2O_2 (7:3) for 10 minutes, followed by immersion in 1% HF for 10 seconds, DC sputtering was used to deposit 500 Å Ti/1000 Å Au onto each substrate. To create the top and bottom surfaces (electrodes) of the channel, the substrates were spin-coated with photoresist (Shipley 1805, Shipley, USA) for 32 seconds at 1000 rpm. After exposing and developing (Shipley 354 Developer, Shipley, USA) the resist, the exposed gold and titanium were removed through wet etching in their respective etchants. The substrates were then rinsed with deionized (DI) water and the resist was removed by rinsing the substrates with acetone.

With the metal patterned, both the top and bottom substrates required further processing. For the top substrate, input and output ports were mechanically drilled

using a diamond tipped, 1.6 mm diameter bit at a speed of 3100 rpm and a feed rate of 2 mm/min. A photdefinable epoxy (SU-8-5, MicroChem, USA) was spin-coated onto the bottom substrate to a thickness of 30 μm and a three-step, 50 minute prebake was performed at 55°C (first 10 mins), 85°C, and 55°C (last 10 mins). An exposure dose of 360 mJ/cm^2 was used to expose the SU-8, immediately preceding a post exposure bake (PEB) at 70°C for 30 minutes in a convection oven. Following the PEB, the SU-8 was developed (SU-8 Developer, MicroChem, USA) for 3 minutes, and subsequently rinsed with isopropanol and DI water.

A pre-bond was performed by aligning the top and bottom substrates, clamping them together and baking the assembly at 125°C for 1 hour in a convection oven, with a cool down bake at 65°C for an additional hour in a second oven. The final bond was formed by allowing capillary forces to draw a two-part, solvent-free epoxy (3713, Epoxies, Etc., USA) into open areas outside of the channel walls (Fig. 2a).

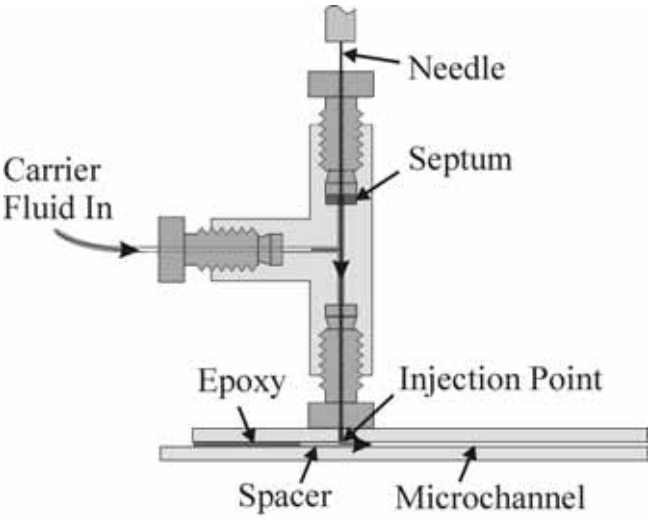


Figure 2. Schematics showing (a) a side view of the inlet interconnect with sample injection port and (b) a top view of a μ-ElFFF system.

To complete the system, interconnects for tubing were attached to the input and output ports using a standard 5-minute epoxy, with the interconnect at the input port allowing for samples to be injected at the channel entrance (Figs. 2a & 2b).

2.2 Testing Protocol

Two types of on-column detection were performed: optical and electrical. For optical detection, the μ-FFF system was placed on a microscope stage and illuminated with white light from the backside. To avoid saturation of the particle images obtained through the 50X objective, a prism was used below the system to angle the incoming illumination light. A CCD camera was mounted to the microscope and connected to a PC, enabling digital capture of the separations.

Electrical detection was also performed on-column, using integrated detector electrodes. Conductivity measurements were made using a pair of opposing electrodes on the top and bottom channel surfaces. A 2 kHz, 2.475 Vrms sinusoidal waveform was applied to the detector electrodes and the current was monitored using a multimeter (HP 3458A, Hewlett-Packard, USA) that was attached to a PC for data storage.

A voltage ranging from 0-2.5 volts was applied to the main electrodes to create the electric field, driving the separations.

The carrier solution (DI water) was pumped through the systems, using a syringe pump, at flow rates between 0.6 and 2.1 mL/hr.

For the separations, 0.1-0.5 μL samples of Protein A coated and uncoated polystyrene beads of 280 nm diameter and gold RLS Particles^{TM} of 40-80 nm diameter, respectively, were injected into the channel. For the RLS Particles^{TM}, solution concentrations as low as 1.09 x 10^{-11} M were uses and the polystyrene bead solutions were less than 10% solids.

3 RESULTS AND DISCUSSION

The completed microchannel had a channel height of approximately 25-30 μm. The width and length of the microchannel were 0.5 cm and 6.2 cm, respectively. The particles used for this work ranged in size from 40-280 nm in diameter and were either composed of gold RLS Particles^{TM} or polystyrene beads. Sample sizes ranged from 0.1 to 0.5 μL with particle concentrations as low as 1.09 x 10^{-11} M. For each separation, deionized water was used as the carrier fluid.

Electrical detection was used for separations of the uncoated and Protein A coated polystyrene beads and the results showed a resolution slightly less than one but clearly distinguishable (Fig. 3). For each run, the void peak (representing the time unretained components pass the detector) is seen about 350 seconds before the peak due to

the uncoated beads, which eluted about 200 seconds before the coated beads. The higher retention of the coated beads may be explained, in part, by the fact that the protein coating increased the diameter of the particles. It is common in many FFF systems (when operated in normal mode) for larger particles to be more strongly retained than smaller ones (Fig. 4).

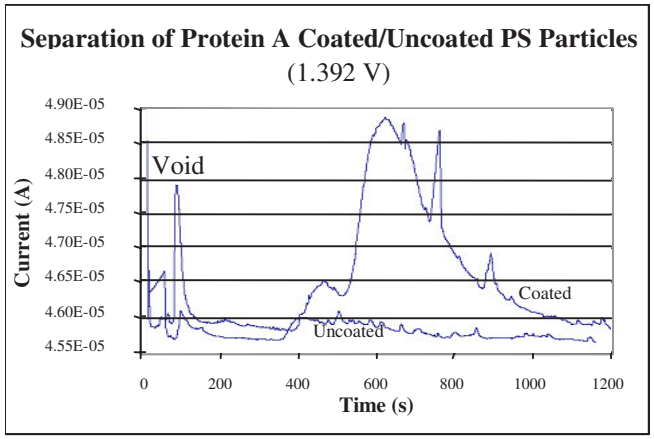


Figure 3. Conductivity measurements for separations of a mixture of polystyrene beads coated with Protein A and uncoated beads (top trace) and a sample containing only uncoated beads (bottom trace).

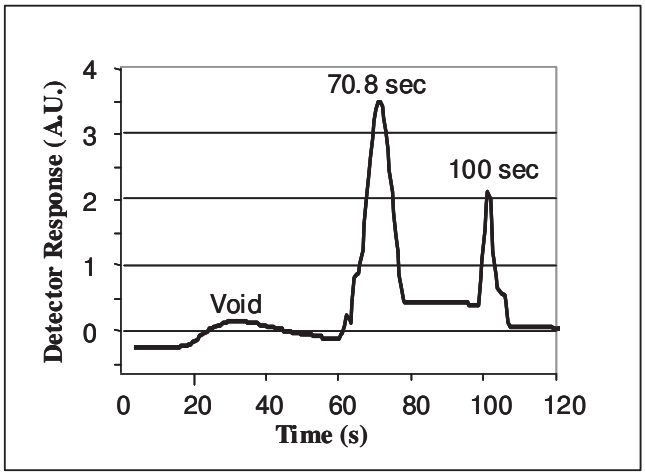
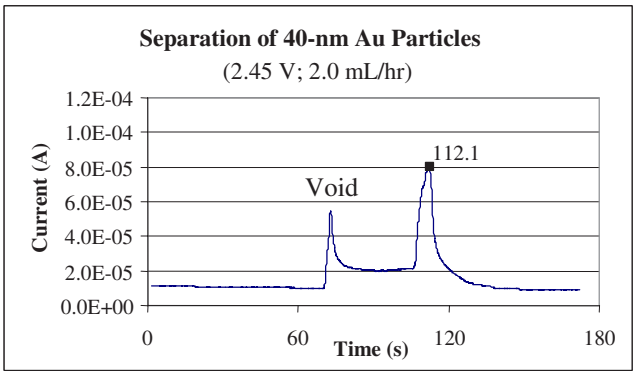


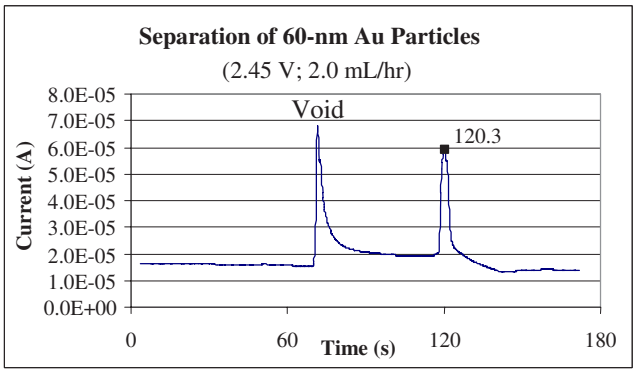
Figure 4. Separation results from a μ-thermal FFF system from a mixture of 204- and 272 nm diameter polystyrene beads, showing elution times of 70.8 and 100 seconds, respectively.

Both single and multiple particle separations were performed in the μ-FFF systems using gold RLS Particles™ and both showed excellent resolution, much greater than one.

Results of single particle separations of 40- and 60 nm gold RLS Particles™ at a flow rate of 2.0 mL/hr were consistent with the separations of the polystyrene beads in that the larger particles were more strongly retained (Fig. 5). Similar results were seen in the high-resolution, low flow rate (0.2 mL/hr), multiparticle separations of 40-, 60-, and 80 nm diameter gold RLS Particles™ (Fig. 6).



(a)



(b)

Figure 5. Conductivity measurements for single particle separations of (a) 40- and (b) 60 nm diameter gold RLS Particles™. For each run, the sample was injected at 60 seconds.

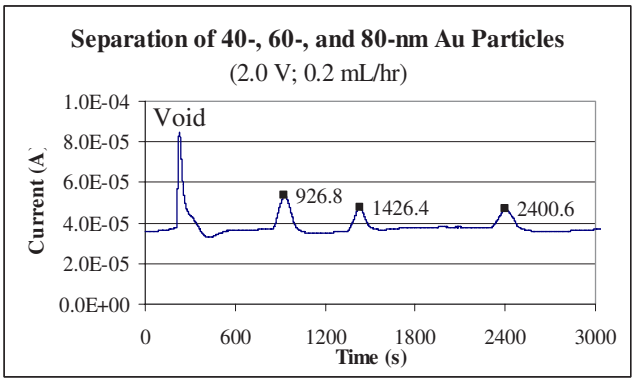


Figure 6. High-resolution, low flow rate, multiparticle separation of 40-, 60-, and 80 nm diameter gold RLS Particles™ in a μ-ElFFF system with a channel width of 1.0 cm. Sample was injected at 60 seconds.

Simultaneous electrical and optical detection was performed on the RLS Particle™ separations and the conductivity measurements with the corresponding captured images were recorded (Fig. 7).

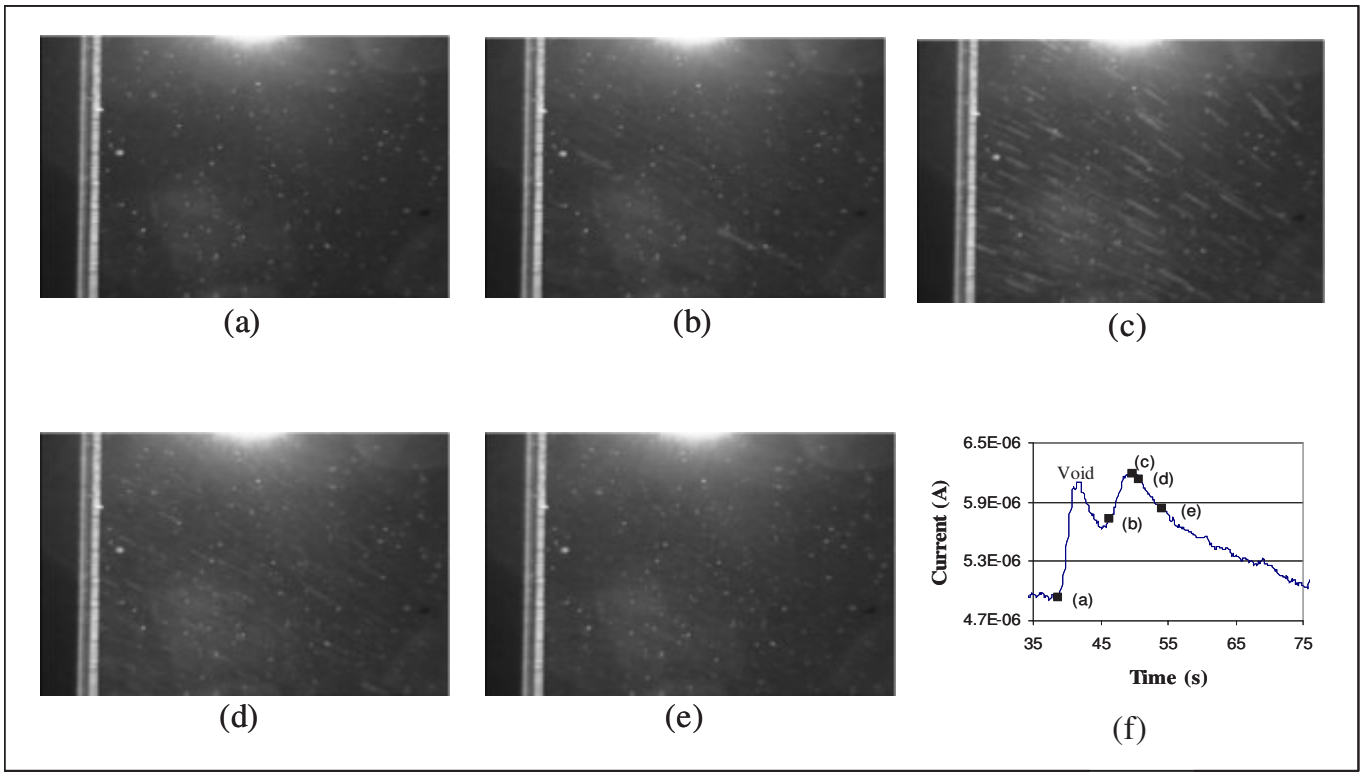


Figure 7. Results from a single particle separation of 80 nm diameter gold RLS Particles™ showing (a-e) images obtained near the detector electrodes at the times indicated on the (f) conductivity measurement graph. Sample was injected at 0 seconds. The width of the microchannel was 1.0 cm. The applied voltage for this separation was 2.2 V, with a flow rate of 1.5 mL/hr.

For this separation, the resolution was less than one. A possible explanation might be that the voltage was disproportionately low (2.2 V) for the flow rate (1.5 mL/hr) at which the run was performed.

4 CONCLUSIONS

Several μ-ElFFF systems were fabricated using micromachining technologies. These systems were used for performing single and multiple particle separations on gold RLS Particles™ and uncoated and Protein A coated polystyrene beads.

All particle separations showed that the μ-ElFFF systems were operating in normal mode, with larger particles being more strongly retained. High speed separations have been demonstrated, signifying the possibility of using these μ-ElFFF systems for rapid, high-resolution separations.

On-chip electrical detection was combined with on-column optical detection and each was shown to be an effective detection method for separated particles in real time. The on-chip electrical detector's response correlated well with the optical signals, showing distinct peaks for each analyte.

5 REFERENCES

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