Separation of Magnetic Nanoparticles by Cyclical Electrical Field Flow Fractionation

T. O. Tasci[1], E. Manangon[2], D. P. Fernandez[2], W. P. Johnson[2], and B. K. Gale[3]

[1]Department of Bioengineering, University of Utah, Salt Lake City, UT 84112 USA
[2]Department of Geology and Geophysics, University of Utah, Salt Lake City, UT 84112 USA
[3]Department of Mechanical Engineering, University of Utah, Salt Lake City, UT 84112 USA

In this study, the potential of Cyclical Electrical Field Flow Fractionation (CyEFFF) for the separation of magnetic nanoparticles is investigated. We demonstrated for the first time that by the application of appropriate voltage waveforms, one can separate gold nanoparticles with sizes less than 50 nm. By using suitable voltage waveforms, the detrimental effect of the particle diffusion is suppressed and particles in the range of 10 nms can be fractionated. In addition, it is shown that CyEFFF is capable of separating lipid and polystyrene sulfonate coated magnetite nanoparticles with the same hydrodynamic radius of 50 nm.

***Index Terms*—Magnetic nanoparticles, magnetite, separation.**

I. Introduction

FIELD FLOW FRACTIONATION (FFF) is a powerful method for the separation and characterization of macromolecular, colloidal and micron-sized particles [1].

Cyclical Electrical Field Flow Fractionation (CyEFFF) is one of the subtechniques of FFF which separates the particles according to their sizes and electrical mobilities [2]. In CyEFFF, the separation channel is composed of bottom and top electrodes which are separated by a thin spacer.

A typical schematic of the CyEFFF system can be seen in Fig. 1. In this system, oscillating voltages are applied to the electrodes which result in a cyclical electric field inside the channel. As a result of the cyclical electric field, particles move back and forth between the electrodes. Particles with high electrophoretic mobilities will move longer distances away from the channel walls and they spend more time in the faster fluid regions. As a consequence, they elute earlier than the lower mobility particles.

Earlier studies showed that diffusion of the nanoparticles is a limiting factor in CyEFFF. It gives rise to band broadening in the UV fractogram and prevents the achievement of high resolution separations. We address and solve this problem by changing the shape of the applied voltage waveform. In the earlier works, researchers used square wave voltages with dc offset voltages. In this work, we don't apply any dc offset voltages but we use square wave voltages with higher duty cycles (i.e., the duration of the positive voltage is larger than the duration of the negative voltage).

In the literature, magnetic SPLITT and magnetic FFF systems have been used for the separation of magnetic nanoparticles [3], [4]. In a recent work, separation with alternating magnetic fields was investigated numerically [5]. Unlike those works, in this study, we use alternating electric fields for the fractionation of magnetic nanoparticles instead of magnetic fields.

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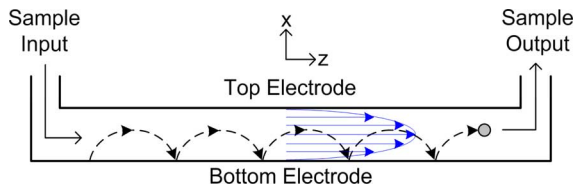

Fig. 1. Cyclical EFFF System. Dashed line show the particle trajectory resulting from the cyclical field. (Operation principle: Oscillating square wave voltages are applied to the electrodes which result in a cyclical electric field inside the channel. As a result of the cyclical electric field, particles move back and forth between the electrodes. Particles with high electrophoretic mobilities move longer distances away from the channel walls and they spend more time at the faster fluid regions. As a consequence, they elute earlier than the lower mobility particles.)

II. Experimental Procedure

To investigate the separation capabilities of the CyEFFF system with the application of high duty cycle voltage waveforms, five experiments were done by using different types of nanoparticles. Nanoparticle types, particle coatings, hydrodynamic sizes and electrophoretic mobilities are summarized in Table I.

The cyclical EFFF channel used in the experiments was same as the one used in the earlier works [6]–[8]. The EFFF channel had a length of 64 cm, height of 178 μm and a width of 2 cm.

For all of the experiments, de-ionized water (18.2 $\mathrm{M\Omega/cm}$) was used as the carrier, which was pumped by the HPLC pump (Alltech model 426, Alltech Associates, Inc., IL, USA). The flow rate used in the experiments was 1 ml/min, except for the experiment-3, in which the flow rate was 0.5 ml/min. Resulting void time in experiment-3 was 4.6 min and void time for the remaining experiments was 2.3 min.

Application of ac and dc voltages was done by using Agilent signal generator (Model 33120A) and Agilent dc power supply (Model E3640A). For the detection of nanoparticles UV/Vis detector (ESA-Model 520) was used. The UV detector data, the electrical current flowing through the separation system and potential difference between the channel walls were measured by LabView (National Instruments) data acquisition card.

Each experiment began with the injection of the sample in to the EFFF channel at $\mathrm{t} = 0$. Immediately following the in-

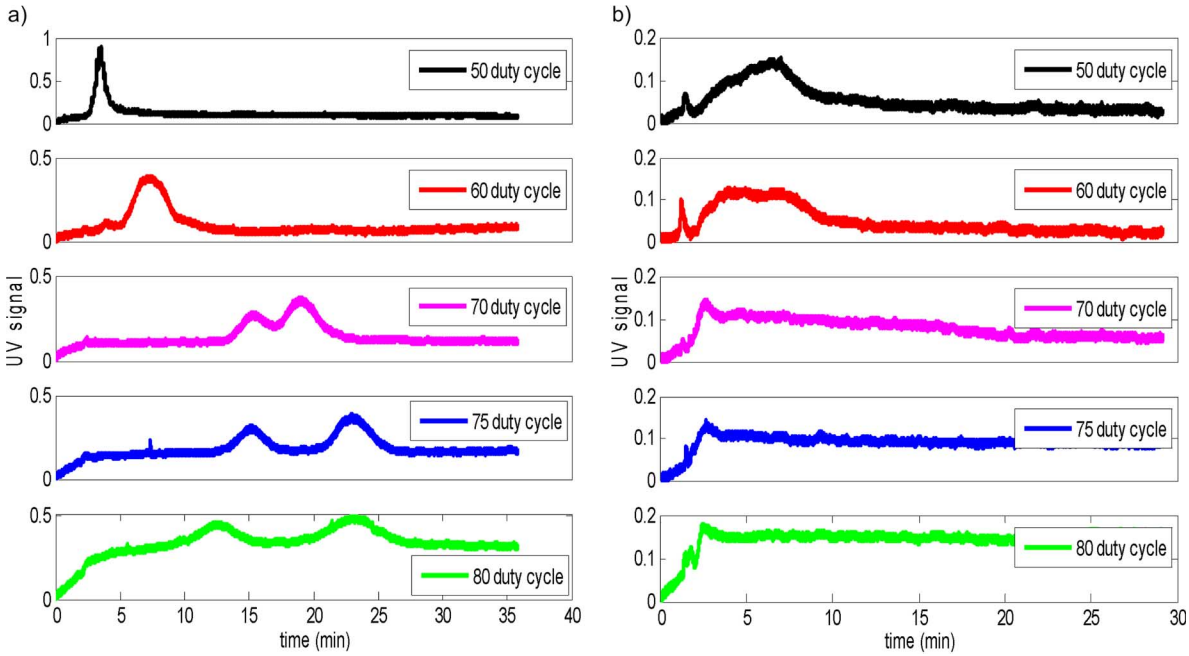

Fig. 2. a) UV fractograms for 15 and 40 nm gold nanoparticle mixture (exp1) b) UV fractograms for MACS magnetic particles (exp2).

jection, at $\mathrm{t} = 0^{+}$, we turned on the power supply to apply 1 V dc voltage for 1 minute. By the application of this constant voltage, we made sure that all the nanoparticles were attracted to the channel wall (accumulation wall). At $\mathrm{t} = 1\ \mathrm{min}$, we turned on the HPLC pump to start the carrier flow. At the same time we also turned on the signal generator to apply the square wave voltage to the system. After observing the peaks in the UV detector, electrical power and pump were turned off. This recipe was followed in all of the experiments conducted. As a quick note, in this recipe, different than the earlier CyEFFF works [7]–[10], we only use the dc voltage in the first 1 min period, and after that, square wave voltages with high duty cycles are used alone. In previous studies, researchers used dc offset voltages until the end of the separation experiments together with the 50% duty cycle square wave voltages.

Details of the separation experiments are given below.

Experiment-1: Mixture of 15 and 40 nm mean diameter gold nanoparticles (NanoComposix, CA, USA) were used. Square wave voltages (10 Hz, 10 Vpp) with duty cycles ranging from 50% to 80% were applied.

Experiment-2: MACS anti-mouse IgG1 microbeads were used as the injected sample. Those are superparamagnetic particles which conjugated to epitope tag specific antibodies. Experimental conditions were the same as the experiment 1.

Experiment-3: Similar to experiment 2, MACS particles were injected. Applied voltage had amplitude of 10 Vpp, frequency of 10 Hz and a duty cycle of 60%. In this experiment, besides the UV detector, DAWN HELEOS II light scattering detector was used to measure the rms radius of the particles.

Experiment-4: Lipid (fluidMAG-Lipid) and polystyrene sulfonate (fluidMAG-PS) coated 100 nm (hydrodynamic size) magnetic nanoparticles were injected. Electrical parameters were 10 Vpp, 5 Hz and 70% duty cycle.

Experiment-5: Lipid (fluidMAG-Lipid) and polystyrene sulfonate (fluidMAG-PS) coated 50 nm (hydrodynamic size) magnetic nanoparticles were injected. Electrical parameters were 6 Vpp, 10 Hz and 75% duty cycle.

For each separation experiment, resolutions of the separations were calculated according to

$$Rs = \frac{t_2 - t_1}{2(\sigma_1 + \sigma_2)} \tag{1}$$

where t_1 and t_2 are the positions of the peaks and σ_1 and σ_2 are the standard deviations of the peaks as they are approximated to a Gaussian curve.

Finally, to determine the operation modes of all separation experiments, mean excursion distances of the particles were calculated. Mean excursion distance is the length travelled by the particle across the channel thickness during the negative cycle of the voltage. We denoted this length by l_{e-} which was calculated according to the (2). Where f (Hz) is the applied frequency, dc is the duty cycle of the voltage waveform, μ_p $(\mathrm{m}^2/\mathrm{Vs})$ is the electrophoretic mobility of the particle and E_{eff} (V/m) is the effective electric field inside the channel

$$l_{e-} = -\mu_p \int_{\frac{1}{f*dc}}^{\frac{1}{f}} E_{eff}(t)dt. \tag{2}$$

In (2), integral of the electric field inside the channel was calculated for the negative cycle of the applied voltage and this result was multiplied by the electrophoretic mobility of the particle to obtain the length travelled by the particle.

The effective field represented in (2) was calculated by using the (3) below

$$E_{eff}(t) = \frac{I(t) \times R_{bulk}}{h}. \tag{3}$$

TABLE I
PROPERTIES OF THE PARTICLES USED IN THE EXPERIMENTS

	Exp1	Exp2	Exp3	Exp4	Exp5
Particle types	Gold (NanoComposix)	Magnetite (MACS)	Magnetite (MACS)	Magnetite (Chemicell)	Magnetite (Chemicell)
Average Size(s) (nm)	15 and 40	280	280	100	50
Coating	Tannic acid surfactant	antibody	antibody	Lipid / polystyrene sulfonate	Lipid / polystyrene sulfonate
Electrophoretic mobility (μmcm/Vs)	-3.7 -3.6	NA	NA	-2.6 -5.8	-3.2 -4.9

For MACS particles, electrophoretic mobilities couldn't be obtained since zeta potential measurement didn't meet the quality criteria for those particles.

TABLE II
RESOLUTIONS AND MEAN EXCURSION LENGTHS CALCULATED FOR THE EXPERIMENTS

	Exp1	Exp2	Exp3	Exp4	Exp5
Resolutions	dutycycle=50% 0.00 dutycycle=60% 0.00 dutycycle=70% 0.85 dutycycle=75% **1.71** dutycycle=80% 1.19	dutycycle=50% 0.00 dutycycle=60% **0.53** dutycycle=70% 0.00 dutycycle=75% 0.00 dutycycle=80% 0.00	0.00	0.00	**0.67**
Mean excursion lengths l_e- (μm)	dutycycle=50% 45.5 (for 15nm) 44.3 (for 40nm) dutycycle=60% 38.5 (for 15nm) 37.5 (for 40nm) dutycycle=70% 22.8 (for 15nm) 22.2 (for 40nm) dutycycle=75% 16.3 (for 15nm) 15.9 (for 40nm) dutycycle=80% 10.9 (for 15nm) 10.6 (for 40nm)	NA	NA	32.2 (for lipid) 71.9 (for PS)	13.3 (for lipid) 20.4 (for PS)

Mean excursion lengths for experiments 3 & 4 couldn't be calculated, since electrophoretic mobilities of MACS nanoparticles was not available (see Table I).

where, $I(t)$ is the measured current in Amperes, $h(m)$ is the channel height and R_{bulk} (Ω) is the resistor representing the electrical resistance of the carrier liquid between the channel walls. R_{bulk} value was calculated according to the methods explained by Srinivas *et al.* [7].

III. RESULTS AND DISCUSSION

UV fractograms obtained from experiments 1 & 2 can be seen in Fig. 2.

As shown in Fig. 2(a), as we increase the duty cycle of the applied voltage, we obtain 2 separate peaks, corresponding to 15 and 40 nm gold nanoparticles. The highest resolution was achieved at a duty cycle of 75%. As presented in Table II, the resolution corresponding to 75% duty cycle condition is 1.71, which is much higher than the resolutions of other duty cycle experiments.

As we look at the electrophoretic mobilities of the 15 and 40 nm particles, we observe that they are close to each other. This shows us that considerable amount of the separation is due to the diffusion coefficients of the particles.

Fig. 2(b) is the experimental result obtained for MACS nanoparticles. It is clear that as the duty cycle of the applied voltage is increased, magnetic particles retained more in the channel. Maximum retention was obtained for 80% duty cycle case, but a small separation was obtained for only the 60% duty cycle condition, with a separation resolution of 0.53 (Table II, exp2)

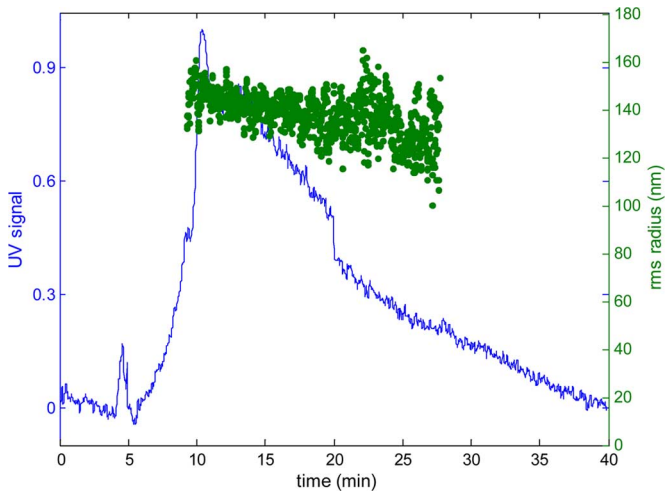

Fig. 3. UV absorption fractogram and rms radius data of the MACS nanoparticles (exp3). Voltage: 10 Vpp, 10 Hz, 60% duty cycle. Flow: 0.5 ml/min.

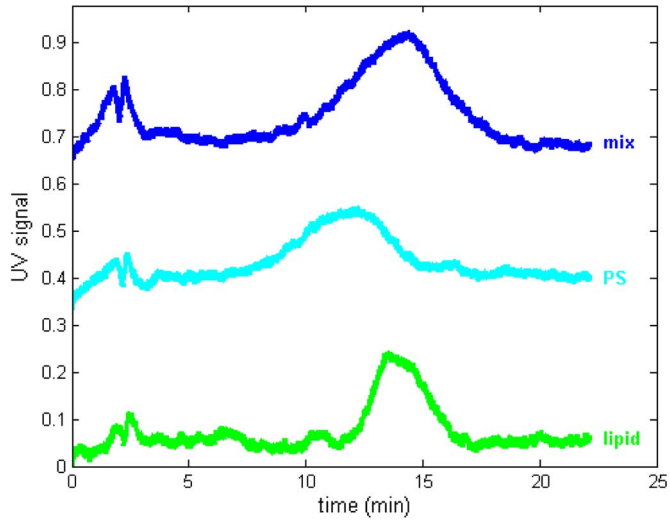

Fig. 4. UV fractograms of 100 nm lipid and polystyrene sulfonate (PS) coated magnetite nanoparticles (exp4). Voltage: 10 Vpp, 5 Hz, 70% duty cycle. Flow: 1 ml/min.

Fig. 3 shows the UV fractogram and light scattering data for exp3. Mean rms radius of the magnetic nanoparticles is measured as 140 nm, and the particles eluted later have slightly less rms radiuses compared to the ones eluted earlier. According to Fig. 3, MACS nanoparticles have an average rms radius of 140 nm with a narrow range of ± 10 nm. In addition, these particles have broad range of electrophoretic mobilities (as determined from the wide range of retention times). We predict that high variation in the electrophoretic mobilities can be resulted from the difference in the number of attached antibodies to the nanoparticles.

Result of experiment 4 can be seen in Fig. 4. In this experiment, injection of lipid and polystyrene sulfonate (PS) coated 100 nm magnetite particles were used. As shown, both PS and lipid coated particles have high retention times (more than 10 minutes). Since lipid coated magnetite particles have smaller average electrophoretic mobility (shown in Table I), lipid coated particles have a higher retention time compared to the PS coated ones. The UV fractogram obtained for the mix shows that we couldn't get a separation at these operating conditions. We have a single and broader peak for the injection of the particle mixture. Electrical parameters used in this experiment were 10 Vpp, 5 Hz and 70% duty cycle. These parameters should be further optimized with more experiments to obtain separate peaks in the UV fractogram. In summary, in experiment 4, by high duty cycle (70%) application in CyEFFF, high retention times were obtained for both lipid and PS coated magnetite particles. Further experiments are needed to obtain a reasonable separation between those 100 nm magnetite particles.

UV fractograms corresponding to experiment5 can be seen in Fig. 5. In this experiment, lipid and PS coated 50 nm magnetite particles were used. As shown, similar to experiment 4, both PS and lipid coated particles had high retention times (again more than 10 minutes). As can be seen in the UV fractogram corresponding to the particle mixture, we can see two peaks instead of a single peak. The resolution of the separation was 0.67 as tabulated in Table II. Thus, this result shows that Cyclical EFFF is capable of separating same sized magnetic nanoparticles (50

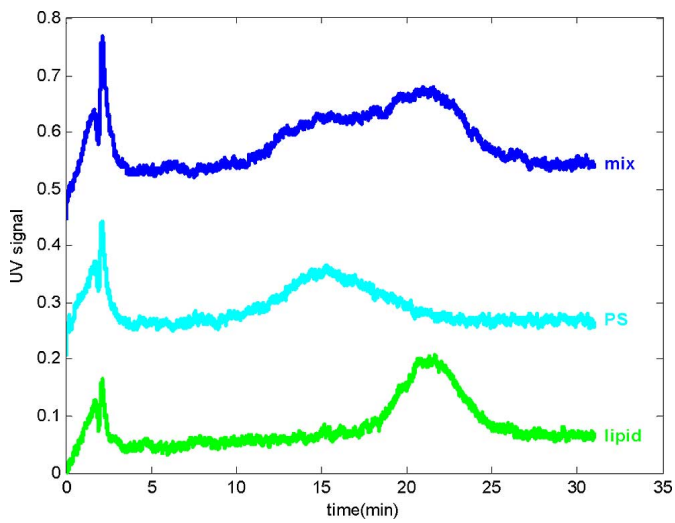

Fig. 5. UV fractograms of 50 nm lipid and polystyrene sulfonate (PS) coated magnetite nanoparticles (exp5). Voltage: 6 Vpp, 10 Hz, 75% duty cycle. Flow: 1 ml/min.

nm in this particular experiment) with different coatings such as lipid and polystyrene sulfonate.

Finally, as we looked at the mean excursion lengths (l_{e-}) of the particles in Table II, we see that all lengths are less than the channel thickness. As a result, in each experiment the operation mode of the FFF system was 'mode I'. Meaning that particles don't reach the opposite channel wall during each cycle of the voltage waveform. Different than mode I, in modes II and III of the FFF systems, particles reach the opposite channel wall in each cycle of the square wave voltage.

IV. Conclusion

It has been shown for the first time that Cyclical Electrical Field Flow Fractionation can be used for the size and electrophoretic mobility analysis of the magnetic nanoparticles. As

we increase the duty cycle of the applied voltage, magnetic nanoparticles gain higher retention times in the separation channel. By applying higher duty cycles, the detrimental effect of particle diffusion is suppressed and separations of particles less than 100 nm could be possible. Mainly, separation of same sized (50 nm) magnetite nanoparticles with different coatings is achieved. As a future work, Cyclical EFFF experiments will be conducted with other various coating types and particle sizes.

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