BLOOD AND PROTEIN SEPARATIONS USING A MICROMACHINED ELECTRICAL FIELD-FLOW FRACTIONATION (μ-EFFF) SYSTEM

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Abstract

This work demonstrates the application of a micromachined electrical field- flow fractionation (μ-EFFF) system to the separation of biological components: blood and adsorbed proteins. These experiments demonstrate the potential of this system to become a major component of μ-TAS systems. The μ-EFFF system is demonstrated by separating polystyrene beads with and without attached proteins. Analysis such as this is critical to biocompatibility studies. The system is also demonstrated by separating blood components. Cells, proteins, and platelets are clearly separated and available for further analysis.

Keywords: Electrical field- flow fractionation, blood separations, protein adsorption, sample preparation

1. Introduction

Electrical field- flow fractionation (EFFF) is a particle separation technique used to separate and measure parameters of colloids, emulsions, and other suspensions. EFFF utilizes an electric field perpendicular to the separation direction, as shown in Figure 1, to separate particles based on size and electric charge. Since EFFF is an elution method, all separated samples can be collected for later analysis. Thus, EFFF is an ideal device for sample preparation (which has generally been neglected by the MEMS community) and for use with a perpendicular analysis system.

There is a wide range of applications for μ-EFFF systems beyond characterization of polymers, which has been the extent of their use to date [1,2]. Macroscale EFFF systems have been used for the characterization of polymers [3], colloids [4,5], sugars [6] and clays [7]. Other FFF systems have been demonstrated on such diverse biological materials as cells, bacteria, viruses, proteins, DNA, starches, lipid emulsions, liposomes, micelles, and vesicles. Other potential biological and medical applications, which have not been demonstrated, include studies of DNA and protein adsorption on surfaces, analysis of drug delivery vehicles such as vesicles, micelles, emulsions, and liposomes, organelle separation and characterization, and diagnostic tests (separation of viruses and bacteria). One purpose of this paper is to demonstrate the capabilities of this system in this area. μ-EFFF systems, however, are not limited to biological applications, but are indeed available for particle analysis in

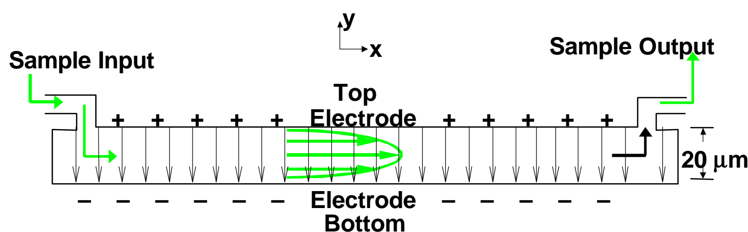

Figure 1. Diagram of operation of EFFF system showing input and output ports, application of electric field, parabolic flow profile, and relative channel dimensions.

a growing list of fields. A relatively well-developed field is environmental water monitoring where EFFF has been applied with some success [7], as well as other FFF systems.

The theory behind the operation of EFFF systems is well developed and the motivation for miniaturization of EFFF systems has been explored extensively in previous publications. Accordingly, the theory and motivation for this system will not be examined here [1,2,8]. Instead, the μ-EFFF system will be demonstrated using two biological applications of interest. The first application is differential retention of particles with and without adsorbed or attached proteins. The second application will involve separation and retention of blood and blood components.

2. Methods

The basic methods for separation and analysis were similar to those reported earlier in publications [2,8]. Only information specifically relevant to this study is given here.

For the protein attachment studies, 280 nm particles particles coated with Protein A were purchased from Bangs Laboratories of Fishers, IN (Inv# L990212A). The particles are stored in a 100 mM borate buffer at a pH of 8.5 with 0.1% BSA (Bovine Serum Albumin), 0.5% Tween and 10 mM EDTA. The particles have a binding capacity of 74.7 μg of human IgG/mg, which gives a relative measure of the concentration of attached proteins. The particles were stored and used directly as indicated on the bottle. Both mixed and separate runs were made with plain 280 nm polystyrene particles and the protein coated particles. The runs were all performed in a platinum μ-EFFF system with an applied voltage of 1.392 V and a measured current of 10-12 μA, which is a relatively low power separation for this system.

The blood analysis runs were performed using heparinized dog blood as provided by the Animal Resource Center at the University of Utah. The samples were kept refrigerated until ready for use. Two different samples were tested in the μ-EFFF system. The first run was performed using whole blood without any processing. The second run was performed using whole blood that had been ultrasonicated for 40 minutes to lyse and destroy all cells. Several runs were performed using each sample type to verify the results. During the test runs, fractions were collected using a glass slide. About every 30 seconds, the glass slide was touched to the outlet port of the system in a new location and the effluent from the system was allowed to wick onto the glass. Thus, samples were available for later observation under the microscope to determine what particles were eluting at a particular time. The runs were all performed in the platinum electrode system with an applied voltage of 1.392 V and a measured current of 10-12 μA.

3. Results and Discussion

The results from all of the biological analyses performed in the μ-EFFF system were very promising. The results of the tests comparing particles with and without attached proteins are shown in Figure 2. Both fractograms show a peak at about 400 seconds indicating the elution of the bare particles. For the run using particles with attached protein A, there is a small peak at 400 seconds indicating elution of some particles without any attached protein, and then a much larger peak at about 600 seconds indicating the elution of the particles with protein A attached. Thus, in this case, the protein increased the retention time of the particles either by increasing the diameter, increasing the charge, or some of both. For the run with protein A, the signal throughout the run was much higher than for other runs indicating that the protein increased the detectability of the sample in the AC detection mode. Clearly, the system was able to differentially retain identically sized particles based only on the quantity of protein attached at the surface. In fact, bare particles included in the run with the protein A modified particles produced a peak that was easily distinguished, though the

resolution was not quite one. It is likely, that if the signal on the second peak had not been so strong, the two peaks might have been resolved to an even higher degree.

The results of the blood analysis were equally promising. The results of two blood analyses are shown in Figure 3. The fractogram of the whole blood shows a series of peaks early on in the separation, followed by a short lull, and then a very strong peak out near the area where the steric transition point is anticipated to be. Analysis of the fractions on the glass slides seemed to indicate that the cells were eluted in the void peak as might be expected due to their relatively large size compared to the channel. This assumption was corroborated by the slightly reddish tint found in the fractions expected to coincide with the void peak. There was significant difficulty in finding cell bodies in these fractions, potentially due to their extreme dilution in the separation system and the later drying.

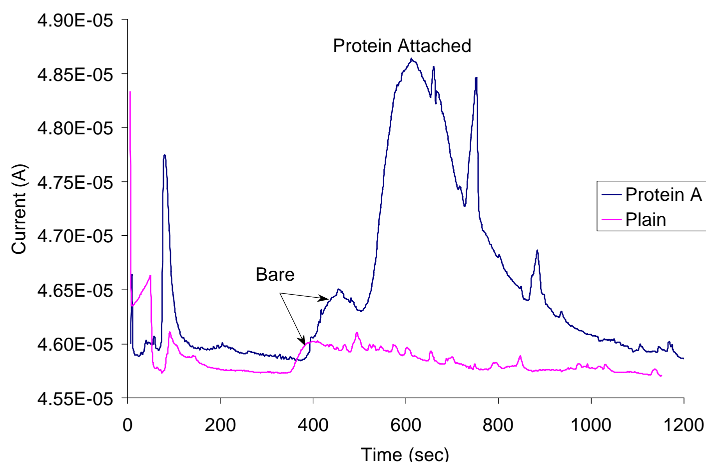

Figure 2. Fractograms showing differential retention of bare particles and particles with attached proteins.

Since it appears that the cells were not significantly retained, the question arises as to what was detected during this separation. Although the answer is not yet definitive, several reasonable conclusions can be made. The blood contains dozens of different proteins that could have potentially been retained in the system, especially if they are of high charge. This conclusion appears more likely when the homogenized sample is examined. Additionally, platelets, while still being of significant size compared to the system, may have been retained and are the likely source of the strong signal near the steric transition point for this separation.

Figure 3 also shows the results obtained using the homogenized blood. To enable comparison, this figure was greatly magnified. Of immediate note is the extremely strong peak that occurs at 350 seconds. The peak at 350 s reached an amplitude of 950 µA, which would drown out any other signal and is the highest signal recorded using this detector. This peak corresponds to hemoglobin subunits released into the sample upon cell lysis. Hemoglobin complexes are known to dissociate rapidly upon release from red blood cells, so this peak would correspond to individual hemoglobin molecules. The later and much smaller peaks would then correlate

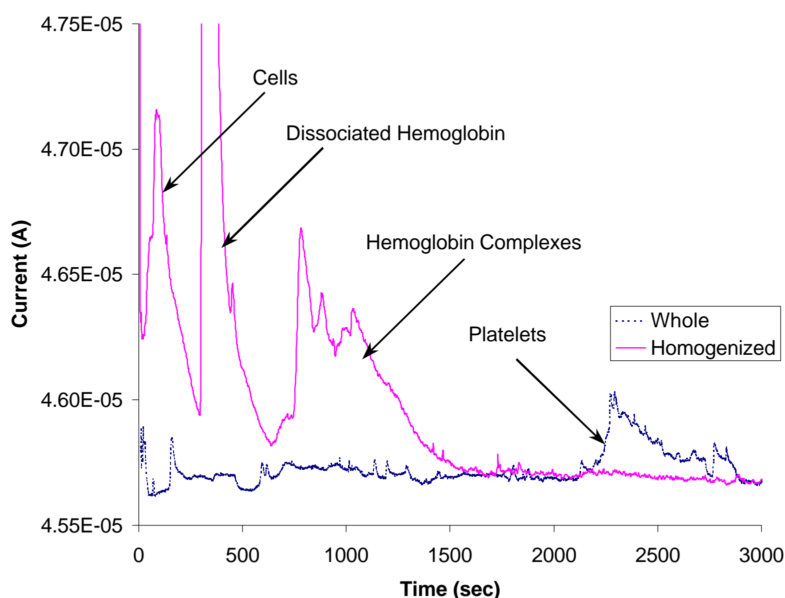

Figure 3. Fractogram from µ-EFFF system showing separation of blood components.

to hemoglobin complexes (two or more complexed hemoglobin molecules). The large magnitude of these peaks makes sense since hemoglobin is highly-charged and is found in significant quantities in red blood cells. Thus, a very strong signal would be generated in the electrical detector. These results represent the first reported separation of proteins using an EFFF system with nonmembrane channel walls.

One other feature to notice in Figure 3 is that there are no significant particles eluted after about 1600 seconds, as opposed to that for whole blood. Thus, it would appear that these larger particles that eluted in the whole blood where destroyed during the homogenizing process. If as suggested earlier, this is the when platelets would elute, then it can be assumed that the platelets were broken down as well as any other "large" particles that would elute at this time.

4. Conclusion

Biologically relevant separations were demonstrated in the μ-EFFF system showing the applicability of the system to a wide range of biological problems. Protein coated particles were easily distinguished from bare particles of identical size demonstrating the ability of the system to separate particles based on surface coating. Protein adsorption studies are critical to a variety of fields and the μ-EFFF system may be especially suited to analyses of these types. The microscale system also demonstrated the capability to retain particles from both a normal and a homogenized whole blood sample. Thus, the system is clearly valuable for biological analysis, but there remains much work to be done in the characterization of the system for these applications. Additionally, dozens of other potential biological applications for this system remain to be explored.

Acknowledgements

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