

RAPID FREE FLOW ISOELECTRIC FOCUSING VIA NOVEL ELECTRODE STRUCTURES

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ABSTRACT

This work presents the first implementation of nanoporous and hydrogel materials as an electrical interface for micro free flow electrophoresis devices. Packed polymer beads or agar were used to isolate the sample from the electrochemical reactions at the metal electrode surface. These materials allow for applied voltages two orders of magnitude larger than what is possible with patterned metal electrodes. This versatility in voltage allows a wide variety of samples to be rapidly focused.

Keywords: isoelectric focusing, free flow electrophoresis, agarose, packed beads

1. INTRODUCTION

Complex biological samples must typically be purified to increase assay sensitivity and reduce non-specific interactions. Free flow isoelectric focusing (IEF) is ideal as an initial separation step: it rapidly concentrates while separating samples, and it can continuously sort a sample into sub-fractions based on pI. Free flow isoelectric focusing has been previously shown to perform separations of biological material [1-3]. However, it has been limited by the ability to apply field strengths high enough to facilitate rapid separations similar to capillary IEF techniques. The major barrier to high electric field strengths is due to the electrolysis of water at the surface of a metal electrode. At low potentials, hydrolysis products remain soluble, but at high potentials, the formation of bubbles disrupts the electric field and fluid flow. The transverse IEF device reported by Xu et al [1] addresses the need to isolate the electrodes, but has been improved upon in several ways: the open sample channel reduces sample adsorption; the electrode material nearly eliminates fluid flow between the electrode reservoirs and sample channel, and the high conductivity within the electrode leads to a more homogeneous field.

2. THEORY

For any species undergoing electrophoresis, the general conservation equation is given by Equation 1, below:

$$\frac{DC_i}{Dt} = \nabla \cdot (D_i \nabla C_i - \mathbf{E} \mu_i C_i) \quad (1)$$

The species concentration, C_i , is both a function of position and time. Moreover, C_i depends on the diffusion constant, D_i , electrophoretic mobility, μ_i , and the local electric field, \mathbf{E} . In the case of isoelectric focusing, the sign of μ changes as the species enters regions of different pH, reversing the electrophoretic flux at the isoelectric point. The quality of free flow IEF depends on both the diffusive and electrophoretic fluxes exerted on the sample. Using this general formulation, a free flow IEF model was developed based on a well-defined ampholyte composition in order to describe and predict the experimental results.

3. EXPERIMENTAL

The layout of the free flow IEF device is shown in Figure 1. The bulk of the device is made of PDMS and is fabricated using standard soft lithography techniques. The post structures (Figure 1b) along the sample channel are used to pattern the surrounding electrode material, eliminating the need for additional photolithography techniques. With these devices, a variety of samples were successfully focused.

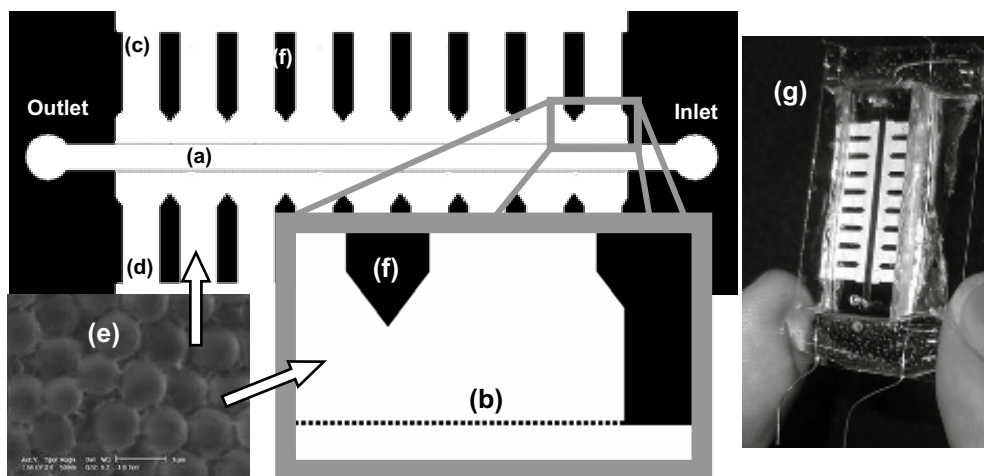


Figure 1: Layout of transverse IEF device. Sample channel (a) is defined by 40 square μm posts (b) and is 1mm by 20 mm. Upper and lower electrodes are the anode (c) and cathode (d), respectively and are filled with agar or packed PMMA beads (e). Larger structures (f) aid in supporting the fragile post array. Photo also shown (g). Features are 50 μm high.

4. RESULTS AND DISCUSSION

Focusing of small amphoteric dyes, proteins, and mitochondria is shown in Figure 2. Each sample in Figure 2 required a different voltage range in order to focus. With applied voltages above the range of the sample, agglomeration and surface adhesion became significant. The three types of samples have similar electrophoretic mobilities (1 to 3×10^{-4} $\text{cm}^2/\text{V}\cdot\text{s}$), but they have diffusion constants spanning two orders of magnitude (8×10^{-6} to 1×10^{-8} cm^2/s). According to the model, it is this difference in diffusive flux that results in the large voltage range necessary for focusing. Depending on the conductivity of the sample introduced to the device, the voltage drop across the channel was calculated to be 66 to 21% of the total applied voltage, a 5 to 15 fold improvement than reported in [1]. Figure 3 shows the simulated and actual focusing of FTIC labeled BSA.

5. CONCLUSIONS

This work presents new electrode materials as a simple, reliable way of applying high DC fields to micro free flow electrophoresis devices, and creates the foundation for using chemically modified hydrogel electrodes for more sophisticated charge-based separations. These materials may also be used as electric interfaces for other high voltage microfluidic devices.

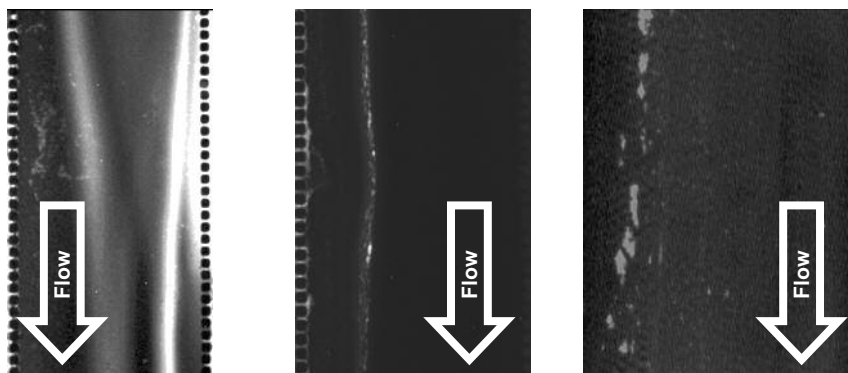


Figure 2: Free flow IEF of three different samples. A) Low molecular weight amphoteric dye, 12s at 300V. B) FITC-BSA 20s at 40 V. C) HeLa mitochondria 50s at 5V. The anode is on the left side of the channel; the cathode is at the right.

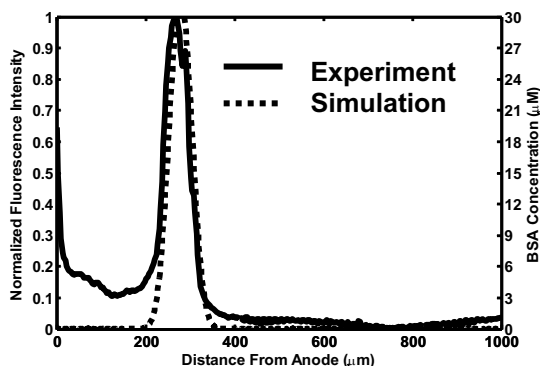


Figure 3: Focusing of FITC tagged BSA. Solid line indicates normalized fluorescent intensity across channel after 20 seconds at 40V. The dashed line is the calculated steady state focusing profile.

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