

FABRICATION AND CHARACTERIZATION OF PHOTOPATTERNED POLYMER MEMBRANES FOR PROTEIN CONCENTRATION AND DIALYSIS IN MICROCHIPS

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ABSTRACT

Unique capability for processing protein samples in microchips is demonstrated via fabrication of photopatterned nanoporous polymer membranes with engineered pore size. Proteins are electrophoresed into membranes and concentrated by over two orders of magnitude before being injected for analysis. Specific analytes can be extracted from complex samples by flowing sample past a long dialysis membranes and perfusing in counterflow. Protein processing performance is used to characterize membrane porosity and specific diffusivities.

INTRODUCTION

Real-world biological samples require extensive pretreatment before they can be analyzed in a miniaturized device. These pretreatment steps may include preconcentration or desalting of protein solutions before analysis.

Sample preconcentration enables identification and quantification of trace analytes. Previous work with electrophoretic concentration at a barrier[1] has been used to concentrate DNA; however, the nonlinear concentration results in previous work are indicative of dielectric breakdown, leading to perturbative effects on downstream analysis and poor concentration reproducibility.

Desalting and other size-specific extraction techniques are often necessary because small ions reduce the sensitivity and stability of protein analytical tools. Previous work has separated analytes via diffusivity variations [2]; this technique is effective only for simple systems with large diffusivity variations and cannot be used in the more efficient counterflow configuration. Dialysis using sandwiched commercial membranes[3,4], dialysis tubes[5], or dialysis probes[6] does not allow the flexibility or ease

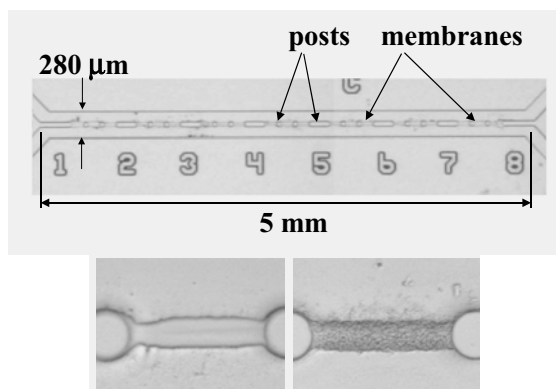


Figure 1. Top: a counterflow microdialysis channel fabricated in-situ with membranes interspersed between silica support posts. Sample is injected at upper right and exits at upper left; perfusion liquid is injected at lower left and exits at lower right. Bottom: blowup of 35 μm thick 2 kD MWCO (left) and 100 kD MWCO membranes

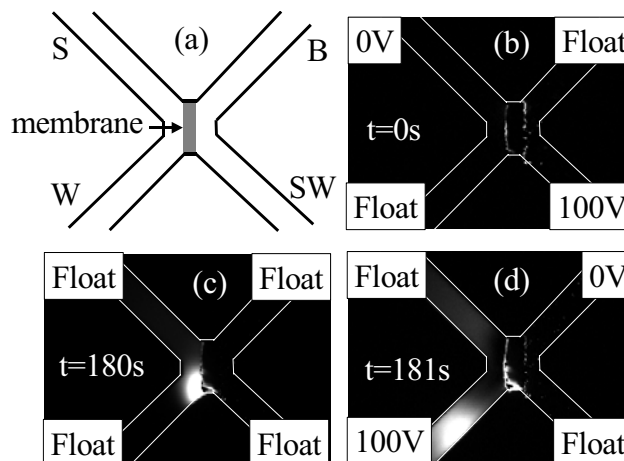


Figure 2. (a) Capillary electrophoresis cross channel with membrane at the junction. The channel width is 80 μm . (b) the beginning of sample concentration. (c) the end of concentration. Negatively-charged Alexa-Fluor 488-labeled BSA stacks at the membrane. (d) Concentrated BSA is injected into the channel.

of integration required to process subnanoliter volumes of nascent analytes on-chip.

We present a novel method to overcome these limitations and integrate protein concentration and dialysis on microchips by laser-patterning polymer membranes within silica microchannels.

FABRICATION

Thin (5–50 μm) nanoporous polymer membranes are fabricated within glass microchannels by using projection optics to define a thin 355 nm laser sheet that photoinitiates polymerization and phase-separation in a thin region within channels previously filled with a monomer/solvent/ photoinitiator solution. The nanoporous membrane remains after unreacted monomer is flushed away. The monomer is 2-(N-3-sulfopropyl-N,N-dimethylammonium) ethyl methacrylate crosslinked with methylene bisacrylamide and the resulting polymer is covalently attached to the glass surface through organosilane surface acrylate functionalization. Membrane shapes and sizes are controlled by shaping the laser sheet, while pore size and molecular weight cutoff are controlled by manipulating the polymer solubility and phase-separation process through changes in the solvent.

For sample preconcentration, 50 μm thick membranes were cast at the junction of a capillary electrophoresis cross microchannel that was pretreated with organosilane-linked linear polyacrylamide to suppress electroosmotic flow. For microdialysis, sets of 35 μm thick membranes were fabricated inside 5 mm long fused silica microchannels (Fig 1). Water:2-methoxyethanol solutions were used as the solvent and different ratios of these two

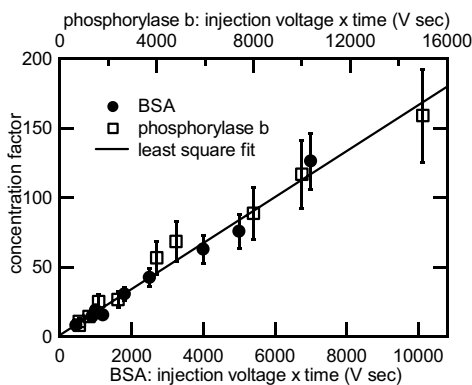


Figure 3. Linear concentration of two proteins by electrophoresis into a nanoporous membrane. Proteins may be concentrated by over two orders of magnitude; linear response facilitates metered concentration and evinces efficient small-ion transport.

components were used to generate membranes with different pore sizes.

RESULTS AND CHARACTERIZATION

Protein concentration. Semipermeable membranes patterned at the junction of an electrophoresis cross chip (Fig 2a) enable linear, metered concentration of proteins before injection. The membranes allow the small charge-carrying salt and buffer ions to pass through (membrane electrical resistance is immeasurably small) but prevent passage of proteins above the MWCO. Upon application of voltage, electrophoresis drives proteins toward the membrane where they concentrate (Figs 2b,c). Voltage switching injects the concentrated sample for downstream analysis (Fig 2d). Here the membrane obviates complicated buffer systems required for other concentration techniques (e.g., isotachopheresis, isoelectric focusing). Protein concentrations in injected samples increase linearly ($r^2=0.92$) with voltage and time over two orders of magnitude (Fig 3) and the extent of concentration is limited only by protein precipitation. The low electrical resistance of the membrane and the linear concentration both result from efficient small ion transport and support the assertion that the concentration process does not affect the buffer electrolyte distribution. This performance is a significant improvement over other electrophoretic concentration techniques [1], which show strongly nonlinear response indicative of dielectric breakdown rather than size-selective membrane transport.

Counterflow mass exchange. Long, 150:1 aspect-ratio membranes (Fig 1) enable counterflow microdialysis, an efficient means for size-selective extraction of analytes from a complex sample. Spatial variation of FITC-labeled analytes (as measured by fluorescence microscopy) during counterflow microdialysis was used to quantify the molecular weight cutoff (MWCO) and analyte-dependent specific diffusivity of the membranes. Samples were injected with pressure at upper right at 10 nl/min and perfused with pressure from lower left with water at 35 nl/min. Concentration profiles observed for two different membranes with several analytes (Fig 4a,b) were combined with a 2D control volume analysis to infer molecular-weight-dependent specific membrane diffusivities (diffusivity in the membrane normalized by that in solution; Fig 4c). Defining the MWCO as the geometrically-interpolated MW at which the specific diffusivity drops to 50% of the value for Rhodamine 560, the two membranes show MWCOs of 2 kD and 100 kD with uncertainties defined by the interpolation. The specific diffusivities of Rhodamine in the 2 kD- and 100 kD-cutoff membranes are 0.3 and 0.6, respectively,

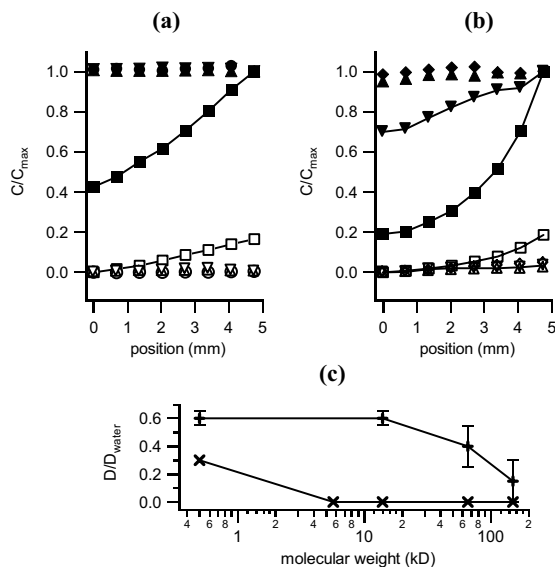


Figure 4. Counterflow mass transport. Top: concentration profiles with membranes precipitated from (a) 3.7:1 water:2-methoxyethanol and (b) 0.3:1 water:2-methoxyethanol. Closed symbols: sample channel, flow enters at $L=5\text{mm}$ and exits at $L=0\text{mm}$. Open symbols: perfusion channel, flow enters at $L=0\text{mm}$ and exits at $L=5\text{mm}$. ■ Rhodamine 560 (0.5 kD); ● monomeric insulin (5.7 kD); ▼ lactalbumin (14 kD); ◆ BSA (66 kD); ▲ anti-biotin (150 kD). Some lines omitted for clarity. Bottom: (c) specific diffusivity through membrane as a function of analyte molecular weight. × 2 kD membrane. + 100 kD membrane.

which are on the same order as specific diffusivities of dyes in agar and polyacrylamide gels.

CONCLUSIONS

The successful fabrication of thin semipermeable membranes patterned within silica microchannels allows for rapid protein sample processing steps, including concentration and dialysis. This architecture allows concentration using the same voltage injection scheme typically used on capillary electrophoresis microchips, and improves on other concentration work by generating linear concentration without perturbing the buffer electrolyte. This architecture shows dialysis performance comparable to commercial membranes and the ability to photopattern allows increased design flexibility, as multiple membranes with varying properties can be constructed in close proximity in a single microanalytical device for processing of nascent analytes.

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