PRECISE MICROSCALE POLYMER GRADIENTS APPLIED TO ISOELECTRIC FOCUSING AND PORE LIMIT ELECTROPHORESIS

G.J. Sommer, A.K. Singh, and A.V. Hatch
Sandia National Laboratories, Livermore, CA USA

ABSTRACT

We demonstrate the power of our technique for establishing and immobilizing well-defined polymer gradients in microchannels by fabricating two miniaturized analytical platforms: microscale immobilized pH gradients (µIPGs) for rapid and high resolution isoelectric focusing (IEF) applications, and polyacrylamide porosity gradients to achieve microscale pore limit electrophoresis (µPLE) in which species are separated based on molecular size by driving them toward the pore size at which migration ceases. Both separation techniques represent the first microscale implementation of their respective methodologies.

KEYWORDS: Gradient, polyacrylamide gel, isoelectric focusing, pore limit electrophoresis

INTRODUCTION

We have previously demonstrated fabrication of microscale immobilized pH gradients (µIPGs) for rapid and high resolution isoelectric focusing (IEF) applications [1,2]. Here we extend the fabrication technique to realize microscale pore limit electrophoresis (µPLE) using crosslinked polyacrylamide porosity gradients. In PLE, analytes are spatially separated based on molecular size through by electrophoretically driving them toward their effective pore limit – the pore size at which migration ceases [3,4]. Both microscale implementations provide inherent advantages over conventional approaches: speed, reduced sample volume, and simple on-chip incorporation for multidimensional analysis.

EXPERIMENTAL

Microscale polymer gradients were fabricated within glass channels using the method shown in Figure 1a. First, a thin membrane is photopolymerized within the central channel to prohibit fluid flow through this segment, while allowing formation of a monomer gradient across the segment via diffusion. Different aqueous monomer solutions are then fed continuously through flanking channels serving as both source and sink of monomer. For µIPG fabrication, the solutions contain acrylamide/bisacrylamide, photoinitiator, and calculated concentrations of acrylamido monomers of varying pK to generate a pH gradient (pH 3.8 – 7.0 for the results shown). For µPLE applications (constant pH), the solutions instead contain different concentrations of acrylamide and bisacrylamide to establish a porosity gradient (10%T, 2.6%C and 40%T, 12%C). Following equilibration, the established gradient is immobilized by photopolymerization. Spiking one boundary solution with fluorescent dye verifies precise gradient formation by tracking its temporal distribution and comparing the results with diffusion-based computational estimates (Figure 1b).
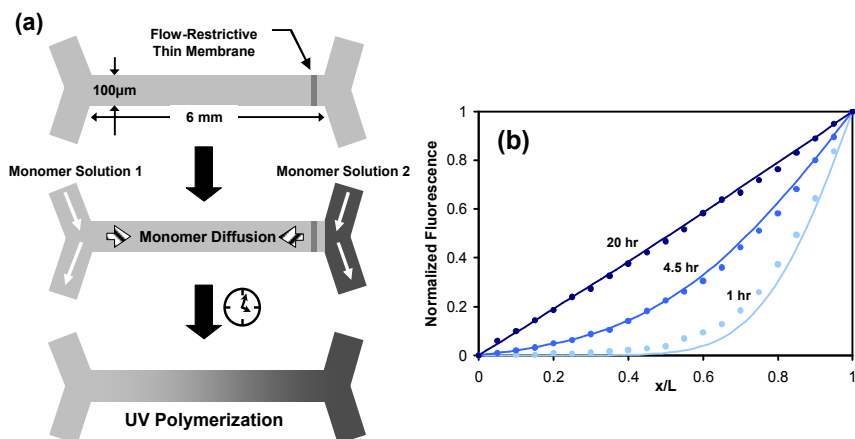
Figure 1. (a) Polymer gradient fabrication scheme. (b) Time-dependent concentra-tion profiles measured with fluorescent dye and compared with analytical estimates.

IEF was demonstrated by electrophoretically loading fluorescently-labeled p*I* markers and proteins into the μIPG under either non-denaturing (buffer) or denatur-ing (buffer, urea, thiourea, and CHAPS) conditions. Carrier ampholytes were not required. Continuous voltage was applied until species focused at their respective p*I*'s.

μPLE was achieved by electrophoretically driving plugs of fluorescently-labeled proteins through the porosity gradient toward their pore limit and tracking their elu-tion distance in time. Protein mobility, μ, is governed by a quasi-Ferguson relation-ship that implies a logarithmic relationship between protein size and pore limit.

RESULTS AND DISCUSSION

Figure 2 shows fluorescent micrographs of samples separated via IEF. Resolu-tion ($\Delta(pI)_{min} \approx 0.040$) is comparable with macroscale IPG strips, while the separa-tion time shows ~50-fold improvement (< 20 min). Figure 3 shows migration pro-files for proteins separated using μPLE, and the logarithmic dependence of pore limit on molecular weight. Porosity gradients can also be used to improve CE reso-lution and both techniques can be used to preconcentrate dilute samples (results not shown).
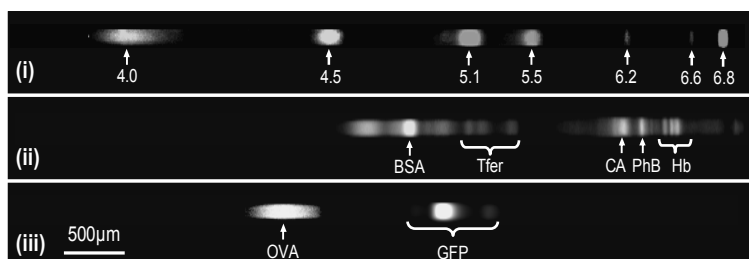
Figure 2. **IEF Results.** *Fluorescent migrographs of several fluorescent dyes (i) and proteins (ii-iii) separated along 6mm-long μIPG.*
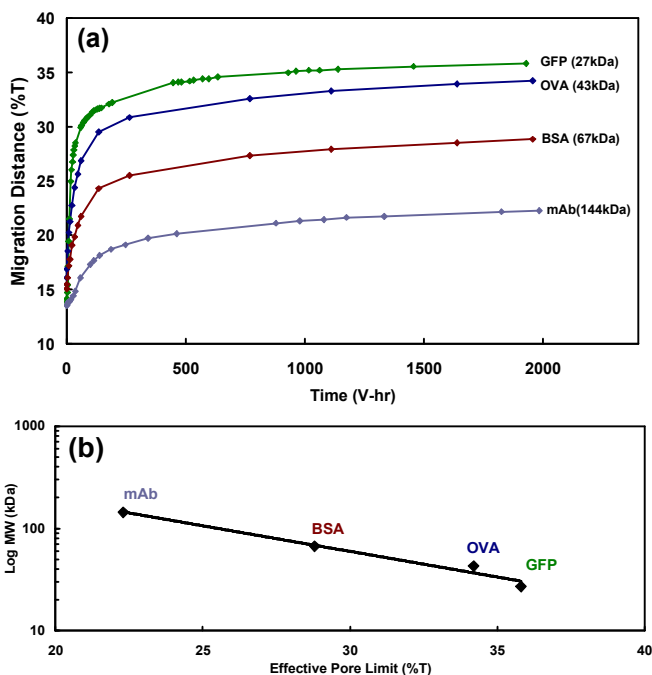

Figure 3. **μPLE Results.** *(a) Migration profiles of fluorescently-labeled proteins through 10%T, 2.6%C → 40%T, 12%C polyacrylamide gradient. (b) Quasi-Ferguson logarithmic correspondence between protein MW and effective pore limit following 2000 V-hr elution.*

CONCLUSIONS

We have developed a novel method for generating and immobilizing microscale polymer gradients. Its utility is demonstrated for μIPG and μPLE applications – two powerful separation techniques not previously realized on-chip. The gradient gels are also easily integrated into μTAS devices with promise for fully automated, multi-dimensional separations and analysis.

ACKNOWLEDGEMENTS

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