Electrokinetic Based Bioseparation in Polymeric Microfluidic Chips

Prashanta Dutta[1],‡ Keisuke Horiuchi[1], Huanchun Cui[2], and Cornelius Ivory[2]
[1]Mechanical and Materials Engineering, Washington State University, Pullman, WA
[2]Chemical Engineering and BioEngineering, Washington State University, Pullman, WA

This study presents high resolution protein separation in poly-di-methyl-siloxane (PDMS) microchip using isoelectric focusing (IEF) techniques. Microfluidic chips are developed using soft lithography and multilayer bonding techniques. In this study, the separation channel is 300 microns wide and 5 microns deep, while the length between anodic and cathodic reservoirs is varied from case to case. Microfluidic chips are prepared for IEF by dynamically coating the channel surface with 0.4% methyl cellulose (MC) in Nanopure® water to discourage electroosmotic flow. For one-dimensional protein separation, the microchannel is filled with a mixture of 4% ampholyte, 0.4% MC and 0.125 mg/mL of phycoerythrin, 0.125 mg/ml allo-phycocyanin and/or 0.03 mg/ml green fluorescent protein. Next, acid and base reservoirs are filled with anolyte and catholyte, respectively, and then current is applied along the axis of the channel until one or more bands of protein focused, usually in just a few minutes even at relatively low voltages. The focused bands are generally well-formed with sharp edges and are less than 100 microns across yielding a putative peak capacity in excess of 100 peaks in a 2-cm long channel.

The resolving power of one-dimensional protein separation is then significantly improved by first focusing protein species in a straight channel using broad-range ampholytes and then refocusing segments of that first channel into secondary channels that branch out from the first one. Experiments demonstrate that three fluorescent protein species within a segment of pH gradient in the first stage are refocused in the second stage with much higher resolution in a shallower pH gradient. A small IEF fraction containing variants of green fluorescent protein from the second stage is refocused in the third stage for further separation. A serially performed three-stage IEF is completed in less than 25 minutes under particularly small electric field strength up to 150 V/cm.

‡ Corresponding author and speaker: Tel: 509-335-7989; Fax: 509-335-4662; Email:dutta@mail.wsu.edu