154a A Microfluidic System for Capillary Electrophoresis/Esi-MS of Intact Proteins

Aaron Timperman and Trust Razunguzwa

Proteomics is a vast new field that is defined by global or high-throughput analysis of many proteins to gain a better understanding of cell function and dysfunction. Currently, we are developing a microfluidic system for two-dimensional separations of intact proteins with on-line ESI-MS analysis. A critical component of this system are microfluidic channels that are effectively terminated by nanocapillary membranes (NCM). The NCMs are used as analyte concentrators, and have been used to achieve concentration factors in excess of 300-fold. Small ~1 nm diameter ions can be concentrated in front of membranes with 10 nm diameter capillaries. The use of the larger pore (10 nm) nanocapillaries has many practical advantages, but the mechanisms of this electrokinetic concentration process are not fully understood and are being investigated. Additionally, we are developing polyethylene glycol terminated self-assembled monolayers (SAMs) for minimizing non-specific adsorption on the surfaces of the glass microfluidic channels. The PEG SAMS are effective in preventing protein adsorption, and concomitantly reduce the electroosmotic flow (EOF). The electroosmotic flow on PEG coated channels is reduced by 90% compared to the native glass surface and the separation efficiencies achieved with these coatings are comparable to other commercially available permanent coatings. Because the coatings reduce the EOF, the microfluidic chip ESI-MS interface that is tailored for low EOF separations has been developed. The interface utilizes rapid voltage switching and a laser micromachined hydrodynamic flow restrictor (HDR). The rapid voltage switching between two electrodes is used to provide the ESI voltage and prevent migration of the analytes to these electrodes. A hydrodynamic flow restrictor (HDR) allows for hydrodynamic introduction of a make-up solution while preventing the electrokinetic flow in the separation channel from being overpowered. A make-up solution is used to change the solution composition prior to introducing the sample into the MS and to stabilize the flow. The interface therefore provides stable spray, compatibility with low EOF and is capable of changing solution composition.