4cf Novel Polymeric Networks for on-Chip Electrophoretic DNA Purification from Cell Lysate and High-Performance DNA Separations

Thomas N. Chiesl and Annelise E. Barron

We have created a family of water-soluble block copolymers of acrylamide and N-alkyl acrylamides (Hydrophobically Modified Polyacrylamides, or HMPAMs) that can selectively remove proteins from DNA via microchannel electrophoresis. It was hypothesized that the inclusion of hydrophobic subunits into a polymer chain could provide materials that would show substantial protein adsorption, due to interactions with the protein's hydrophobic amino acids. A series of acrylamide co-monomers with varying N-alkyl chain lengths (C4, C6, C6-6, C8) were synthesized via the reaction between acryloyl chloride and the respective alkyl amines and characterized by reverse-phase HPLC and 1H NMR. Copolymers were synthesized via an aqueous "micellar" polymerization technique with up to 4 mol% of hydrophobic subunit, as verified by 1H NMR. Copolymer molar masses were controlled with chain-transfer agents and ranged from 300,000 g/mol to 7 million g/mol as determined by tandem GPC-MALLS. Capillary and chip electrophoresis of bovine serum albumin proteins in these media reveals that N,N-dihexyl and N-octyl acrylamide-containing copolymers show the most significant protein adsorption. N-hexyl acrylamide copolymers showed moderate adsorption, while butyl acrylamides exhibited none. All HMPAM copolymer matrices allowed the rapid electrophoretic passage of double-stranded DNA molecules. These copolymer matrices are now being tuned for selective on-chip removal of proteins and lipids from cellular lysate, while allowing the rapid passage of DNA for subsequent genetic analysis.

HMPAMs also form a novel class of DNA separation media: "physically crosslinked" polymer networks, which could meet the demands of increased separation performance within the bioanalytical community. Poly(acrylamide-co-dihexylacrylamide) comprising as little as 0.13% mol dihexylacrylamide (DHA) yields remarkably improved electrophoretic DNA separations when compared to a linear polyacrylamide (LPA) of matched molar mass. Single-molecule DNA imaging reveals a novel separation modality during gel electrophoresis, resembling "inch-worm" movement, which we have termed "stationary entanglement coupling." Physically crosslinked HMPAM gels exhibit three distinct concentrations regimes that have dramatic consequences on DNA a ,ηseparation mechanism. At polymer concentrations below C concentration similar to C* (the overlap threshold) in unmodified polymers, DNA separations are faster than LPA and have equal resolution. At concentrations above CT, the concentration where polymer chains become "elastically effective" (hydrophobically associated, intermolecularly), the separation of DNA is comparable over most sizes of DNA; however, improved separation performance is seen for DNA < but below CT, theηthan 30 base pairs. At concentrations above C separation performance of DNA in LPA-co-DHAs is remarkably better than what is seen in a matched LPA network. Physically crosslinked HMPAMs have advantages over both linear polymers and covalently linked crosslinked gels in terms of improved separation performance (or speed), while unlike chemically crosslinked gels, physically crosslinked networks can be "broken" (reversibly) by applied shear and loaded into microchannels. Using these media, several hundred base-pairs of DNA have been sequenced in microfluidic devices in under 10 minutes, with high peak efficiencies and excellent results compared to matched-molar mass LPA. This represents at least a ten-fold increase in throughput with far less sample consumption then conventional capillary electrophoresis.