B100 Evaluation of Poly(ethylene oxide) as a High-Speed, High-Resolution Sieving Matrix in Shortened Capillaries or Microchips for Forensic STR Analysis

Joan M. Bienvenue, MS, Kate Wilson, BS, Jerome P. Ferrance, PhD, and James P. Landers, PhD, University Of Virginia, Department Of Chemistry, Mccormick Road, Charlottesville, Va 22904*

The goal of this research project is to demonstrate the use of poly(ethylene oxide) (peo) as a high-resolution sieving matrix for forensic analysis of DNA.

This presentation will impact the forensic community and/or humanity by discussing the evaluation of an alternative polymeric sieving matrix for high-resolution separation of DNA, as well as its potential inclusion in next-generation microchip technology for DNA analysis.

Capillary electrophoresis is now the sanctioned method for separation and analysis of short tandem repeat (STR) fragments of DNA for forensic application and genetic mapping. Increased speed, separation efficiency, and automation have made the technique indispensable in the forensic laboratory. In order to accomplish the high-resolution separations required for STR analysis, a limited number of polymeric solutions have been evaluated systematically for suitability in this application. Linear polyacrylamides have become the separation matrix of choice because of their stability, reliability and ability to yield high-resolution separation DNA fragments differing in length by a single base. However, the high cost of commercial products, poor resolution over shorter capillary distances resulting in longer separation times, and the high viscosity of some polymers, has created the need to search for alternative sieving matrices. Though current commercial products accurately and reproducibly separate fragments with high resolution, in lengthy capillary systems, their cost and separation efficiency over short distances become prohibitive when making the shift from capillary-based separations to microchip-based systems. These considerations become increasingly significant when attempting to translate conditions from capillary-based methods to the microchip platform, where channel length and total device area of are utmost importance. As the forensic community moves towards fully integrated micro devices capable of sample preparation and full genetic analysis, choice of separation matrix will play a major role in device design, analysis time, and ease of use. Thus, as polymers are evaluated using capillary electrophoresis, attention must be paid to the feasibility of the sieving matrix for microchip-based analysis. Critical evaluation of polymer viscosity, resolution over short distances, device preparation, reproducibility of separations, ease of polymer preparation, and cost of analysis must occur when developing a polymer for separation of STR fragments for forensic genetic analysis with the goal of translation to the micro device.

The work presented here describes the evaluation of poly(ethylene oxide) (PEO), an inexpensive, commercially-available polymer, for separation of STR fragments in capillaries and potentially for inclusion in the microchip platform. Accurate, repeatable separations of STR fragments with single base pair differences are reported using a denaturing, lowviscosity, low-molecular weight solution of PEO. The effects of buffer composition, capillary temperature, polymer weight and concentration and capillary length are described. A variety of polymer weights are explored at varying concentrations to determine the polymeric conditions that result in the highest resolution separation. Shortened capillaries were used to simulate separation lengths similar to feasible microchannel lengths in analytical microdevices for evaluation of polymer properties. Reproducibility of the separation is evaluated, as well as the use of epoxypoly(dimethylacrylamide)(EPDMA)-coated capillaries for minimization of electroosmotic flow (EOF). Urea concentration and capillary temperature are also evaluated and optimized conditions for high-resolution separation of samples in shortened channels are presented. Comparisons of PEO to conventional commercial products are highlighted and the appropriateness of this sieving matrix for translation to the microdevice is appraised.

DNA, Capillary Electrophoresis, Microchip

 ** Presenting Author*