Characterization of Two Novel Thermoresponsive Capillary Gel Electrophoresis Mediums

by

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ABSTRACT

In this thesis, we analyze the physical characteristics of novel sieving mediums for use with Capillary Gel Electrophoresis (CGE) for DNA separations. Six different molecular weights and types of a triblock copolymer known by the trade name Pluronic, and a tunable thermoassociative hydrogel made from guanosine and guanosine derivatives were characterized. These materials are well known for applications other than chemical and biological separations, and have already shown great promise as novel mediums to increase separation resolution in the case of Pluronic, and to allow separations of DNA based on sequence in the case of the guanosine gels. The broader merit of this work is to advance both the field of Metagenomics, and a separation method used for single strand conformation polymorphisms known as CE-SSCP. The scope of the characterization of these gels was to find the working limits and physical properties of the gels that will affect their separation characteristics. Using Gel Permeation Chromatography (GPC), we were able to understand the effect of molecular weight and diblock copolymer on gelation temperature of Pluronic, and find a consistent abundance of diblock contamination in the polymer sample. Using various rheological studies, we were able to find the gelation onset, offset and midpoints of Pluronic hydrodrogel.

We also applied rheology to find the dissociation temperature for the six different ratios/concentrations of guanosine hydrogels. Because these two mediums have differing properties that allow Pluronic to have high resolution, and guanosine based gels to separate DNA by primary nucleotide sequence, we have also assessed the potential for these two gels to be incorporated as a single medium for high resolution of DNA based simultaneously on fragment length and fragment sequence.