Guanosine Gels for Chiral and Bio-separations in Capillary Electrophoresis

by

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ABSTRACT

Guanosine gels (G-gels) are self-assembled networks of hydrogen-bonded tetrads formed by guanosine nucleotides and their derivatives. The tetrads stack upon themselves to form columnar, helical aggregates that are stabilized by π-π interactions and centrally-located cations. Many G-rich oligonucleotides similarly self-assemble into G-quartet structures. In this dissertation, novel media incorporating G-gels or G-rich oligonucleotides were developed for the separation of enantiomers, DNA and proteins in capillary electrophoresis.

In the study of enantiomers, G-gels join numerous other chiral separation agents such as cyclodextrins, crown ethers, chiral surfactants, antibiotics, proteins, and polysaccharides for chiral separations. Previous work showed the effectiveness of G-gels formed by guanosine-5'-monophosphate (GMP) for separation of the enantiomers of the cationic drug propranolol using capillary electrophoresis. Subsequently, it was found that not all chiral compounds could be resolved into their enantiomers, leading us to investigate in the present work the structural features that appear to be correlated to enantiomerically selective interactions of chiral compounds with G-gels. For those compounds (anionic 1,1'-binaphthyl-2,2'-diyl hydrogen phosphate (BNPA) and net-neutral tryptophan) for which enantiomeric resolution was achieved, the effects of experimental conditions and G-gel composition were examined. For the other compounds (hydrobenzoin and neutral amino acids and derivatives), the migration times were used as an indicator of the extent of interaction with the G-gel run buffer. It was found that the extent of interaction alone does not determine the chiral selectivity of the

G-gel, indicating that the mechanism of chiral separation involves particular structural characteristics of the chiral compounds.

In the study of DNA, sieving gels are usually used in capillary gel electrophoresis to resolve DNA strands of different lengths. For complex samples, however, such as those encountered in metagenomic analysis of microbial communities or biofilms, length-based separation may mask the true genetic diversity of the community since different organisms may contribute same length DNA with different sequences. There is a need, therefore, for DNA separations based on both the length and sequence. Previous work has demonstrated the ability of G-gels to separate four single-stranded DNA 76-mers that differ by only a few A/G base substitutions. The goal of the present work was to determine whether G-gels could be combined with commercial sieving gels or G-rich oligonucleotides could be attached to polymers of sieving gels in order to simultaneously separate DNA based on both length and sequence. The results are given for the four 76-mers and for a standard dsDNA ladder. Commercial sieving gels were used alone and in combination with G-gels. For the 76-mers, the combined medium was less efficient than the G-gel alone but nevertheless was able to achieve partial resolution. The combined medium was at least as effective as the sieving gel alone at resolving the denatured DNA ladder and showed indications of sequence-based resolution as well, as supported by MALDI-MS. The results show that the combined sieving gel/G-gel medium retains the selectivity of the individual media, providing a promising approach to simultaneous length- and sequence-based DNA separation for metagenomic analysis of complex systems. The oligonucleotide-modified polymeric medium was more efficient than the combined medium for separation of the 76-mers, which makes it an alternative to the

combined G-gel/Sieving gel. The successful attachment of oligonucleotide to the polymer chains was indicated by successful separation of 76-mers in capillary electrophoresis and by size exclusion chromatography (SEC).

In the study of proteins, the G-gel showed better separation of a protein standard ladder than capillary zone electrophoresis and micellar electrokinetic chromatography. G-gel separated the proteins in the ladder with a different electropheretic profile obtained from the sieving gels, suggesting that G-gels could be a good candidate to couple with sieving gels in a 2-dimensional (2-D) capillary electrophoresis system for separation of complex protein samples.