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Combination capillary isotachophoresis and capillary zone electrophoresis in small nucleic acid fragment analysis of separation and hybridization

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Analysis of ions and substrates in biological material was followed by a great development of protein and enzyme diagnostics more than two decades before. Now, the nucleic acids (DNA and RNA) are commonly applied in diagnostics and therapeutics in human and veterinary medicine. The basic instrument in analysis of nucleic acid is a hybridization of two complementary single-stranded sequences of nucleic acid. Today, it is possible to measure several copies of the nucleic acid by amplification techniques, for example by PCR in picomoles and attomoles level. But the nucleic acid diagnostics requires a new way of thinking and more sensitive and faster methods, especially in medical area. The ITP is modern amplification-free analytical technique, offers sub-nanomolar sensitivity, cheap, robust and simple-mode operation, short time analysis and huge pre-concentration of analytes. We are proposing a combination of ITP with Capillary Zone Electrophoresis (CZE) to the analysis of small fragments of nucleic acid by principle hybridization target with the oligonucleotides detection probe by UV-VIS detection. For hybridization study we used DNA oligonucleotides synthesized in our laboratory (targets-probes) with no secondary or secondary (hairpin) structure. All DNA oligonucleotides used in hybridization study were full complementary. We used the concentration range of oligonucleotides from 1 pM to 100 nM. For the ITP-CZE experiments, we used Tris-HCl based electrolytes with MgCl2, and 0.1% hydroxyethyl cellulose (HEC) and 0.2-2% linear acrylamide (AA). We used HEC for suppression of Electro-Osmotic flow (EOS) of AA such as separation sieving matrix, and equimolar concentrations of Mg2+ to promote of hybridization. We experimentally demonstrate the hybridization and separation models for on-line combinations ITP-CZE with used the model DNA oligonucleotides. We are able to separate model DNA non-complementary oligonucleotides with an identical length, but the different base composition. Our experimental method delivers results in less than 20 minutes with the limit of detection (LOD) of 15 pM. We analyzed hybridization and separation of short DNA oligonucleotides that had similar sequences to mature miRNAs. The control of temperature was a critical step for the preservation of the double-stranded structure of DNA hybrids in our experiment. Our future research activities include performing ITP-CZE clinical applications for miRNA cancer diagnostic assay.

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