Abstract

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Title of Thesis: Separation of molecular probes by capillary electrophoresis

Oligonucleotides are organic molecules which find their application in fields such as biotechnology, molecular biology, diagnosis and therapy. For the use of oligonucleotides (ON´s) as molecular probes, it is necessary to covalently modify the ON´s for example with a fluorescent dye and a quenching moiety. The products of this labelling reactions are the required double labelled ON probe, but also side products like the unlabeled ON and the ON´s labeled either with the fluorescent dye or the quencher. Consequently, the products of this reaction must be separated, which represents the focus of this diploma thesis.

The separation of these double labeled ON probes has been so far only performed by chromatographic approaches. The aim of this thesis is to explore the use of capillary electrophoresis for that separation. Specifically, the use of capillary electrophoresis was investigated for the separation of a) single-labeled ON probe with BHQ quencher from a double-labeled ON probe with a BHQ quencher and a fluorescent dye 6-FAM (BHQ-FAM) and b) single-labeled ON probe with an azaphthalocyanine quencher (PCQ) from a double-labeled probe with azaphthalocyanine quencher and fluorescent dye 6-FAM (PCQ-FAM) or Cy5 (PCQ-Cy5). Noteworthy, the planar and hydrophobic structure of the azaphthalocyanine quencher is different from the types of quenchers used so far and thus the separation of azaphthalocyanine labelled molecules can be considered as a new challenge.

The experimental conditions for the electrophoretic separation of the single-labeled BHQ from double-labeled BHQ-FAM probes as well as of the single-labeled PCQ from double-labeled PCQ-Cy5 probes were completely optimized. However, the separation of the PCQ from PCQ-FAM probes required 40 minutes. The used electrophoretic conditions for the separation of BHQ from BHQ-FAM and PCQ from PCQ-Cy5 are more suitable than when HPLC is used. However, separation by HPLC under isocratic conditions and with acetonitrile as an organic solvent appears to be more suitable for the separation of PCQ and PCQ-FAM.