Hadamard Transform Capillary Zone Electrophoresis (HT-CZE)

Benefits and Limitations of a Multiplexing Technique on an Unmodified Standard CE System

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We present the realization of a Hadamard encoded multiplexing method on a standard and unaltered Agilent 7100 CE System and the evaluation of characteristics of such a system that undergoes up to 505 injections with intermittent separation phases comparing experimental and simulated data.

HT based multiplexing methods have already been implemented in various kinds of chromatographic and spectroscopic techniques ranging from GC, HPLC and ion mobility spectrometry to NMR, IR and MS. They allow for an enhanced signal-to-noise ratio while saving analysis time through overlapping the desired signal accordingly to a specifically programmed order. Our group has successfully developed such methodologies for HPLC and GC using pseudo-random binary sequences (PRBS) of up to 2000 injections based on Hadamard matrices.[1]

In capillary electrophoresis, that approach commonly requires additional hardware to realize multiple, fast and most importantly reproducible injections, e.g. pneumatic auto-samplers, micromachined inlet ports of various shapes, optical gating and microchip voltage switches. They all have in common that injection circles can be performed without disconnecting the system from a continuous voltage application. To our knowledge there has only been one research report thus far examining and evaluating multiple injection cross-correlation capillary electrophoresis without additional instrumentation.[2] This report encouraged us to perform our own experiments in order to (1) improve the preliminary published data and (2) characterize as well as (3) optimize such a system that from a theoretical point view raises stirring questions: How do zones and their order in the capillary influence the injection of a successive zone? How do zones of multiple analytes behave and to what degree do they influence each other? Is there a way to realize high through-put in CE when due to EOF fluctuations migration velocity change in between runs?

In this talk, we will present our data on a system consisting of four nucleotides (AMP, GMP, CMP and UMP) that have been successfully measured in a HT-CZE mode with pseudo-random binary sequences of up to 9-bit (505 injections). Besides evaluations of effects of voltage application, peak capacity changes, injection methods, length of injection, time of voltage application in between injections as well as number and order of zones injected, we provide simulations using Simul X in order to discuss the behavior of analytes during voltage application of multiple injections and intermittent separation cycles. With these information in hand, the benefits and limitations of the Hadamard transform multiplexing technique in CZE will be highlighted.

References:

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[2] A. Seiman, J. C. Reijenga, *Procedia Chemistry* **2010**, *2*, 59-66.