366a Plenary: Scanning Temperature Gradient Focusing for Simultaneous Concentration and Separation of Complex Samples

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Temperature gradient focusing (TGF) has been demonstrated to provide very high resolution separations in very short microfluidic channels. However, one potential drawback to TGF and other counter-flow gradient focusing methods is the limited peak capacity; only a small number of analyte peaks (~ 4) can be simultaneously focused and separated. For many applications, it is necessary to separate and quantitate a large number of components from a complex sample mixture. In this paper we report on a new method that can be used with TGF to provide separations with both high resolution and high peak capacity in short microfluidic channels. Briefly, TGF works by balancing the electrophoretic motion of an analyte against the bulk flow of buffer through a microchannel. The new method reported here is implemented by ‘scanning' the bulk flow rate over time, sequentially focusing different ranges of analytes. The scanning is accomplished by controlling the pressure applied to the microchannel. The focused analyte peaks are detected as they pass a fixed detection point near the end of the gradient zone, giving a signal vs. applied pressure plot that is similar to a conventional chromatogram. There are a number of advantages to this method. First, it gives an increased peak capacity as a large number of analytes can be sequentially focused and detected. For example the full range of amino acids can be analyzed. Second, because a constant scan rate effectively injects and focuses each analyte for a controlled amount of time, separations are more repeatable and quantitative. Peak areas (which are proportional to the sample concentration) have a typical reproducibility of 10% or better. Third, it simplifies the detection scheme required for the technique, making it compatible with single point LIF and UV absorbance detection typically used with CE. Arguably, the most important advance offered by scanning TGF is the ability to adjust detection limits as needed by changing the scan rate. For relatively high concentration samples, a scan can be run fairly quickly (~5-10 min.), so that each analyte is in the focusing window for only a short time. To decrease the detection limits for the analysis of very dilute samples, the scan can be run at a slow scan rate, giving each analyte a long time in the focusing window. A combination approach can also be used, with a rapid scan rate over relatively abundant peaks and a slower scan rate over selected portions of the separation to analyze the trace components.