2D interference-free MS detection of proteins from cIEF - methodical and instrumental aspects

B. R. Rudisch*, C. Huhn

Institute for Physical and Theoretical Chemistry, University of Tübingen, Auf der Morgenstelle 18, 72076 Tübingen
* benjamin.rudisch@uni-tuebingen.de

Capillary isoelectric focussing (cIEF) is a promising alternative for classical gel isoelectric focussing. Some of the main benefits of cIEF over classical gel IEF are an easy automation and the possibility for a further characterization as well as quantification, e.g. using mass spectrometry. For gel cIEF, this would require to recover analytes from the gel. [1] One major drawback when coupling cIEF to MS is the impairment of the ionization efficiency and fouling of the ion source due to the ampholytes necessary to form a stable pH gradient. Solutions to this issue were described in literature, including the use of very low ampholytes concentration or dialysis interfaces to remove small ampholytes. [2] A third method that uses a second CE dimension to separate proteins and ampholytes from a heart cut of the cIEF dimension has been used by our workgroup.

Here we show the influence of three different ampholyte mixtures on the detection of three model proteins representing different pI ranges. The results show different levels of suppression in protein signal as well as electrospray stability. In the future, narrow ampholyte cuts in the range of the protein's pI will be used to better model the heart cut performed in the first dimension. Also, by using different buffers and pH conditions the separation might be further optimised. Heart cutting of the first dimension will be performed in a new interface designed as a capillary multiplexer, which is made entirely of glass. Based on a SlipChip principle[3] the interface will allow for flexible and fast cuts with minimal band broadening due to dead volumes. Using glass as material, optical detection methods can be implemented for intermediate analyte detection increasing cut precision.

[1] A. S. Zarabadi, T. Huang, J. G. Mielke, *J. Chrom. B.,* **2017**, 1053, pp 65-71.
[2] J. Hühner, M. Lämmerhofer, C. Neusüß, *Electrophoresis*, **2015**, 36 (21), pp 2687-2694.
[3] W. Du et al., *Lab Chip*, **2009**, 9 (16), pp 2286-2292.