2D INTERFACE FOR INTERFERENCE-FREE MS DETECTION OF PROTEINS FROM CIEF - OPTIMISING THE SECOND DIMENSION

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Multidimensional separation techniques are powerful tools to tackle difficult analytical problems. Therefore, employed techniques should be as orthogonal as possible to achieve the best results and improved performances.For protein analysis, capillary-based isoelectric focussing (cIEF) has proven its great potential, mainly because of the short run times and lower sample consumption compared to conventional gel-electrophoresis. The need for ampholytes, however, is still a large drawback as it perturbs the detection of the analytes, especially in mass spectrometry. Therefore, a 2D setup was developed by our workgroup, coupling cIEF with subsequent capillary zone electrophoresis (CE) to cut, transfer and separate analytes from ampholyte background. Here we show the optimisation of the CE-dimension to prove the potential of this approach for interference free MS detection of analytes from cIEF.

For cIEF, commercially available ampholyte solutions and model proteins are used. To hyphenate cIEF and CZE-MS, a fused-silica chip manufactured using selective laserinduced etching (SLE) is employed to create an interface to cut-out analytes from the first and transfer them to the second dimension. Different ampholytes were used to test their behaviour in CE-ESI-MS.

Simulations suggest that the best separation of analytes and ampholytes is achieved using a BGE system with a pH value contrary to the analyte's pI value. Three model proteins with acidic to basic pI values were used in the measurements. Different commercially available ampholyte solutions exhibited very different behaviour in CE separation and signal-discrimination in MS detection. Combining the found relations between separation conditions and efficiency, our results are a first step towards a system optimised for both dimensions. There is, however, a need for a careful optimisation of the CE-MS dimension to obtain an optimal separation. In future, different BGE systems and narrow pH span ampholytes will be used to further understand ampholyte behaviour and optimise the subsequent CE separation.