PRECONCENTRATION AND SEPARATION OF AMINO ACIDS BY ONLINE-COUPLING OF ISOTACHOPHORESIS AND CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY

T. Melzer*, C. Huhn

Institute of Physical and Theoretical Chemistry, University of Tuebingen
Auf der Morgenstelle 18, 72076 Tübingen; tanja.melzer@uni-tuebingen.de

Capillary electrophoresis-mass spectrometry of samples in a low concentration is generally difficult due to low injection volumes. Therefore, effective preconcentration and matrix separation strategies have to be developed to improve detection limits.

In this work, isotachophoresis (ITP) is coupled to capillary electrophoresis-mass spectrometry via a microfluidic glass chip interface to enable online-preconcentration of amino acids for subsequent separation. The hyphenation of isotachophoresis and capillary electrophoresis via this interface allows the application of different buffers in each dimension, as well as the flexible exchange of the attached capillaries for the separation steps. The interface provides a low to zero dead volume and completely circular microchannels.

A method for isotachophoretic separation of all proteinogenic amino acids as cations based on non-aqueous electrolytes was investigated for detection limits and maximum capillary load to improve the preconcentration effect of the first dimension. Parameters investigated were capillary inner diameter, injection conditions and analyte concentration. ITP-MS analysis was used for this investigation revealing ITP-MS detection limits between 1 and 10 µg/L.

Furthermore, in order to optimize the transition from ITP to CE, capillary electrophoresis-mass spectrometry of amino acids was established with analyte plugs having transient ITP conditions comparable to the plug which is transferred from the first dimension of the two-dimensional setup. Therefore, leading and terminating electrolyte were added to the sample and their influence on the CE separation was examined. Results indicate that the addition of 40 % of each leading and terminating electrolyte does not affect the separation regarding migration time and peak area, so that a robust 2D system can be expected.

The most critical part of the hyphenation of both methods is the determination of voltage switching from first to second dimension. Capacitively coupled contactless conductivity detection was applied to monitor and thus control the migration of the analyte plug through the microfluidic glass chip interface and optimize the sample transfer step.