Conclusions

This work demonstrates that capillary electrophoresis is a suitable method for analysis and physico-chemical characterization of biomolecules. It was exemplified by pK_a determination of a series of novel phosphinic pseudopeptides and by separation of stereoisomers of the phosphinic pseudopeptides and serine.

A self-consistent procedure for pK_a determination by CZE was carried out. In particular, various background electrolytes were tested in the acidic region at pH < 2. A set of background electrolytes was evaluated for reliable determination of electrophoretic mobilities in the 1.5 – 12 pH interval. The temperature of the solution inside the separation capillary was measured by a simplified approach, thus enabling precise determination of the electrophoretic mobilities. Additionally, a throughput of the CZE method for the pK_a determination was substantially improved, while high separation efficiency and precision of the electrophoretic mobilities was retained. The method benefits from adjustable velocity of electroosmotic flow, achieved by modification of the capillary inner surface, and efficient determination of electroosmotic mobility.

Further, a study was performed which explored the validity of Onsager model for theoretical description of concentration dependence of the electrophoretic mobilities. It turned out that the composition of BGE has a major effect on experimental electrophoretic mobilities. Subsequently, it was found that individual parameters of the analyzed ions have to be considered in order to achieve reliable limiting mobilities of the ions.

CZE separation of diastereomers of the phosphinic pseudopeptides was studied in achiral BGEs. It turned out that the diastereomers can be resolved exclusively at a pH region which covers the pK_a values of the analyte. In general, dissociation of the central phosphinate moiety of the pseudopeptides plays a leading role in separation of the diastereomers. All of the pseudopeptides were separated into two diastereomeric pairs and complete separation of many of them was achieved. Comparison of CZE and HPLC separations of diastereomers revealed that HPLC provided, in general, higher resolution, whereas CZE was able to resolve some analytes which were not resolved by HPLC.

Finally, a CZE method for determination of D-serine was developed, employing in-capillary derivatization of the analytes. This method is suitable for analysis of large series of samples in commercial CE analyzers and it was successfully applied to testing of a series of potential inhibitors of serine racemase.