Capillary zone electrophoresis and capillary isotachophoresis for physicochemical characterization of peptides

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Capillary zone electrophoresis (CZE) and capillary isotachophoresis (CITP) will be shown as powerful methods for physicochemical characterization of peptides [1].
CZE was employed for investigation of the acid-base and electromigration properties of polyprotic oligopeptides, particularly for determination of thermodynamic acidity constants (pK_a) and electrophoretic mobilities of human and salmon gonadotropin-releasing hormones (GnRHs) and insect antimicrobial peptides (AMPs) [2, 3]. Apparent mixed acidity constants, p$K_{a,mix}$, and actual ionic mobilities of GnRHs and AMPs were determined by a nonlinear regression analysis of the pH dependence of their effective mobilities measured in a series of the background electrolytes within a wide pH range (1.80-12.10), at constant ionic strength (25 mM) and reference temperature (25°C). Then, the p$K_{a,mix}$ values were recalculated to the thermodynamic pK_as using the Debye-Hückel theory.
CITP and CZE were applied to estimate the effective charge of linear poly-α-L-glutamic acid, linear poly-α-L-lysine, and the first five generations of dendrigraft poly-L-lysines [4, 5]. The procedure is based on the linear dependence of CITP zone length of the analyte on its concentration and effective charge and on the determination of ionic mobility of the analyte by CZE. The applied cationic and anionic CITP systems provided sharply separated zones with a good linearity and repeatability of the zone lengths. The effective charge number per one lysine residue for linear poly-*L*-lysine was in a good agreement with Manning theoretical value (0.5). Slightly reduced value 0.36 was found for poly-α-L-glutamic acid, whereas dendrigraft poly-*L*-lysines showed a dramatic decrease of the effective charge number per lysine residue with the increasing generation number, from 0.84 for short oligolysines of the first generation down to 0.08 for the fifth generation.

References

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