

Separation of peptides using capillary electrophoresis: comparison between simulation and experimental determination

ABSTRACT

Theoretical and experimental electropherograms of nine peptides in a standard mixture have been matched. The capillary electrophoresis separation of the peptides mixture was carried out in 80 mM phosphate buffer, adjusted to pH 2.3 with 1.0 M lithium hydroxide, in the range of 25 – 30 kV. The capillary was performed in 50 cm × 50 μm i.d. × 365 μm o.d. uncoated fused silica capillary. All experiments were carried out 25°C. The theoretical peptides charge were calculated using the Hendersen-Hasselbach equation at the experimental pH using the individual amino acid pKa values as report by Rickard et al. (1991). The theoretical electrophoretic mobility of each peptide was then calculated using the multi-variable model described by Janini et al. (2001). The theoretical mobilities were subsequently converted to migration times using the experimental conditions used in this study. The peaks were modelled with Gaussian functions assuming peak area for each peak is proportional to the number of peptide bonds in the peptide and peak widths based on half the maximum theoretical efficiency as described in Kenndler and Schwer (1992). The simulated electropherogram showed a close similarity to the experimental electropherogram. The correlation between the theoretical and experimentally determined mobility of all the nine peptides was good, i.e. $r^2 = 0.9781$. In this study, closest-neighbour algorithm correction on the test peptides theoretical mobilities did not show any improvement of the correlation.