Capillary electrophoresis as a second dimension to Off-Gel™ isoelectric focusing for peptide separation

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CZE analysis of OGE-IEF peptide fractions using a positively coated capillary and an acetic acid buffer. Chitosan is a hydrophilic polyelectrolyte, derived from chitin.[1] In comparison to cationic detergents or other polyelectrolytes used for capillary coating, chitosan can be rather strongly bound to the capillary.[2] Thus, it is neither necessary to add the polyelectrolyte in the running buffer nor to regenerate the coating between each run. Moreover, the reversed electroosmotic flow (eof) provided by the adsorption of chitosan on the capillary walls is rather high and allows a fast analysis of positive peptides in a counter-electroosmotic mode. For these reasons, chitosan-modified capillaries have first been considered to assess the orthogonality of CZE and OGE-IEF (Figure S-1). Also, for comparison purpose, the z scale of the below figure is the same than the one used for drawing figure 3 of the main manuscript.

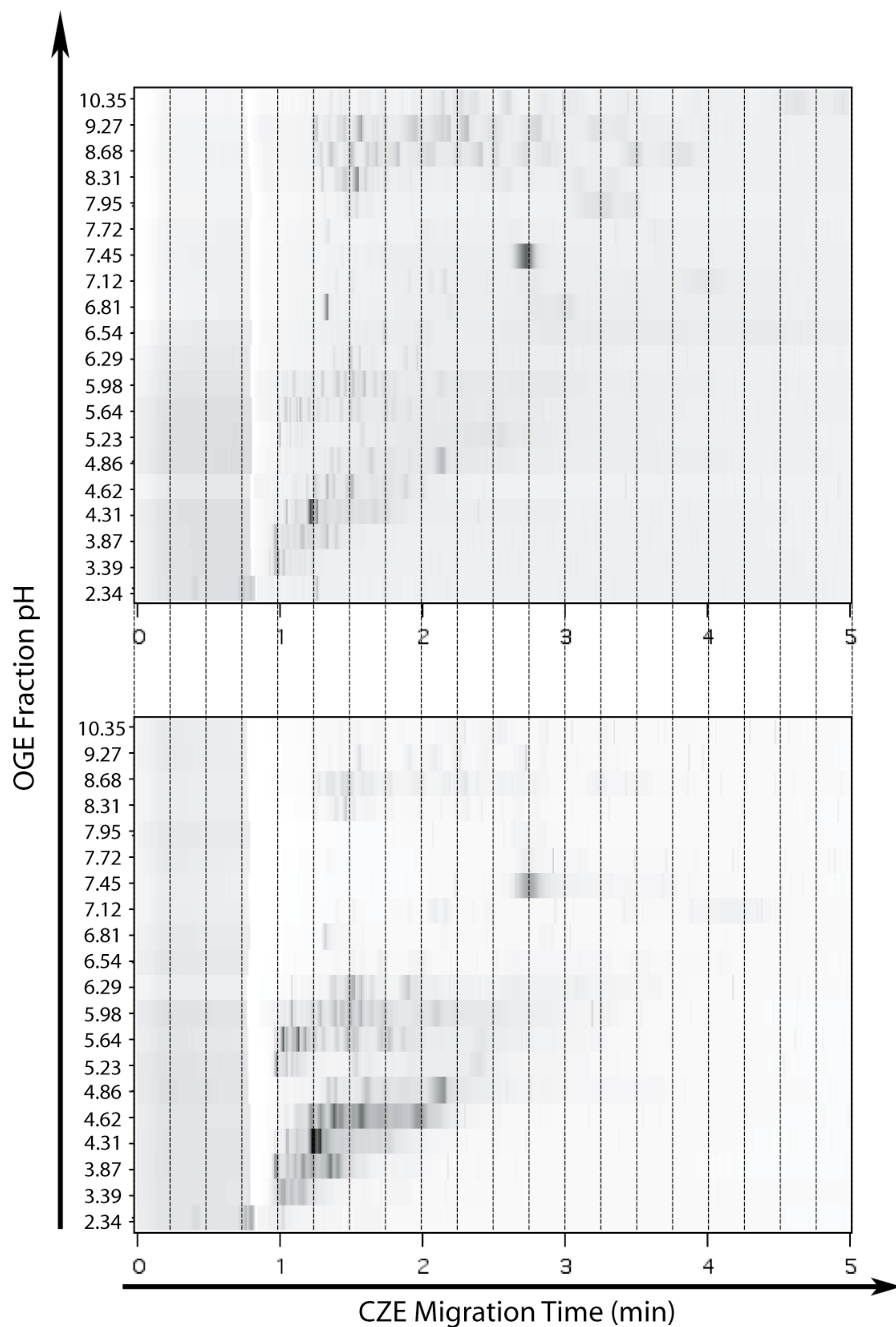

Figure S-1. Fractions from OGE-IEF analyzed by CZE (positively coated capillary, acetic acid 10%). Conditions: voltage -30 kV, observed current -23 μA, temperature 25°C, UV

absorbance at 200 nm. Concentration of each tryptic digest before the OGE fractionation: 0.02 $g.L^{-1}$

On figure S-1, the white band that can be seen in both images at a time slightly below 1 min corresponds to the EOF water peak. It proves that the use of chitosan as a capillary modifier provides a rather strong and stable electroosmotic flow (n = 40: average eof = $43.6.10^{-9}$ $m^2.V^{-1}.s^{-1}$; RSD = 1.4%) Concerning the peptide bands, the overall pattern is highly comparable. In particular, the number of bands in low populated fraction visually demonstrates the similarity of the electropherograms. For example, in the fractions 12 and 14 presenting respectively a pH of 6.81 and 7.45, bands presenting the same migration times can clearly be observed. Similarly, in the OGE fraction presenting a pH of 3.87, the same bands are visible on both images at the same migration times. From a resolution point of view, if we consider the top image on figure 2, approximately ninety bands are visualized whereas more than 120 peptides are expected in the peptide test mixture. Following this observation, we then evaluated other conditions to enhance the resolution of the CZE separation.

Background noise induced by the CAs presence. During the OGE fractionation, CAs are contained in the sample mixture at 1% (v/v). At this low concentration, the CAs do not hinder significantly the detection of the separated peptides. However, CAs can still be detected in CE with UV detection. Figure S-2 shows the background noise induced by the CAs in CE when used at 1% (v/v) during the OGE fractionation. In this example, the CE separation has been carried out in a neutrally coated capillary with 10% acetic acid as BGE.

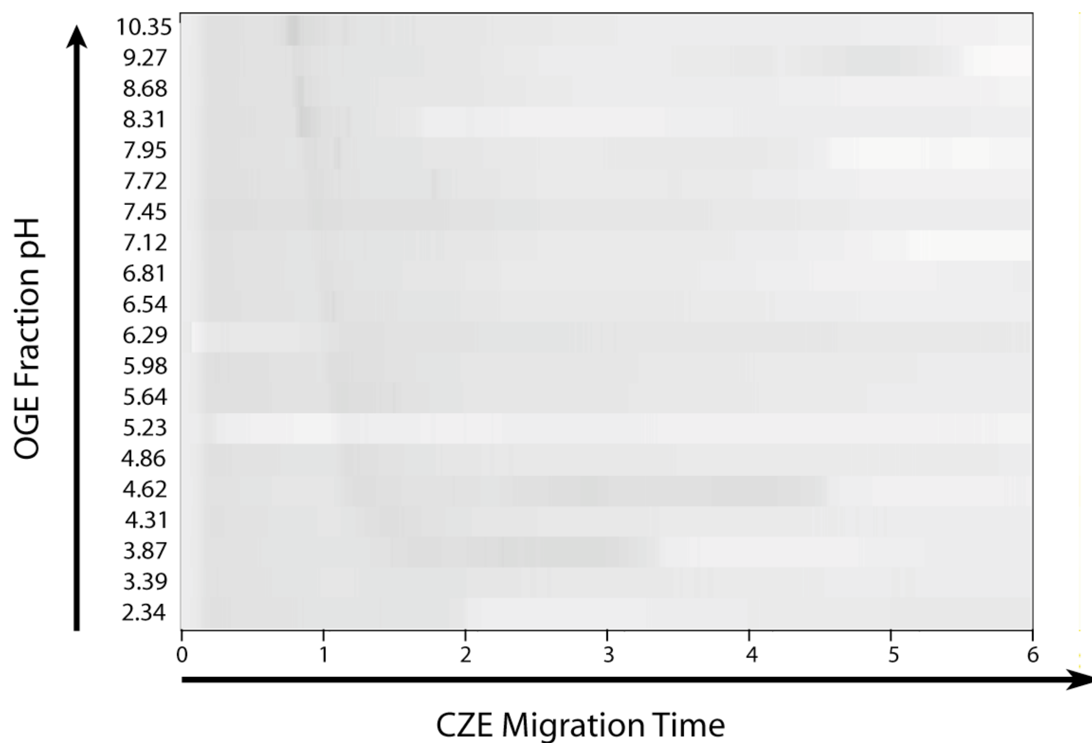

Figure S-2. Fractions from OGE-IEF analysed by CZE (HPC coated capillary, acetic acid 10%). Conditions: voltage 30 kV, observed current 23 μA, temperature 25°C, UV absorbance at 200 nm. Concentration of the wide pH range CAs mixture (3-10) before OGE fractionation.

(1) Muzzarelli, R. A. A. *Natural chelating polymers*; Pergamon Press: New York, 1973.
(2) Yao, Y. J.; Li, S. F. Y. *Journal of Chromatography A* **1994**, *663*, 97-104.