Application of Experimental design for determination of insulin analogs

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Introduction

Capillary electrophoresis (CE) is a popular method permitting separation of analytes from small molecules to large biomolecules. Its advantages include simplicity, high separation efficiency, short analysis time and low sample and solvent consumption, making this a powerful alternative method to HPLC.

DoE technique was employed to increase method performance, optimization, and development of CE methods for the determination of insulin and its analogs. This methodology provided information on the impact of key factors on method development as well as the interaction of significant components in terms of reducing analysis time. of higher molecular weight transformation products of higher molecular weight transformation products.

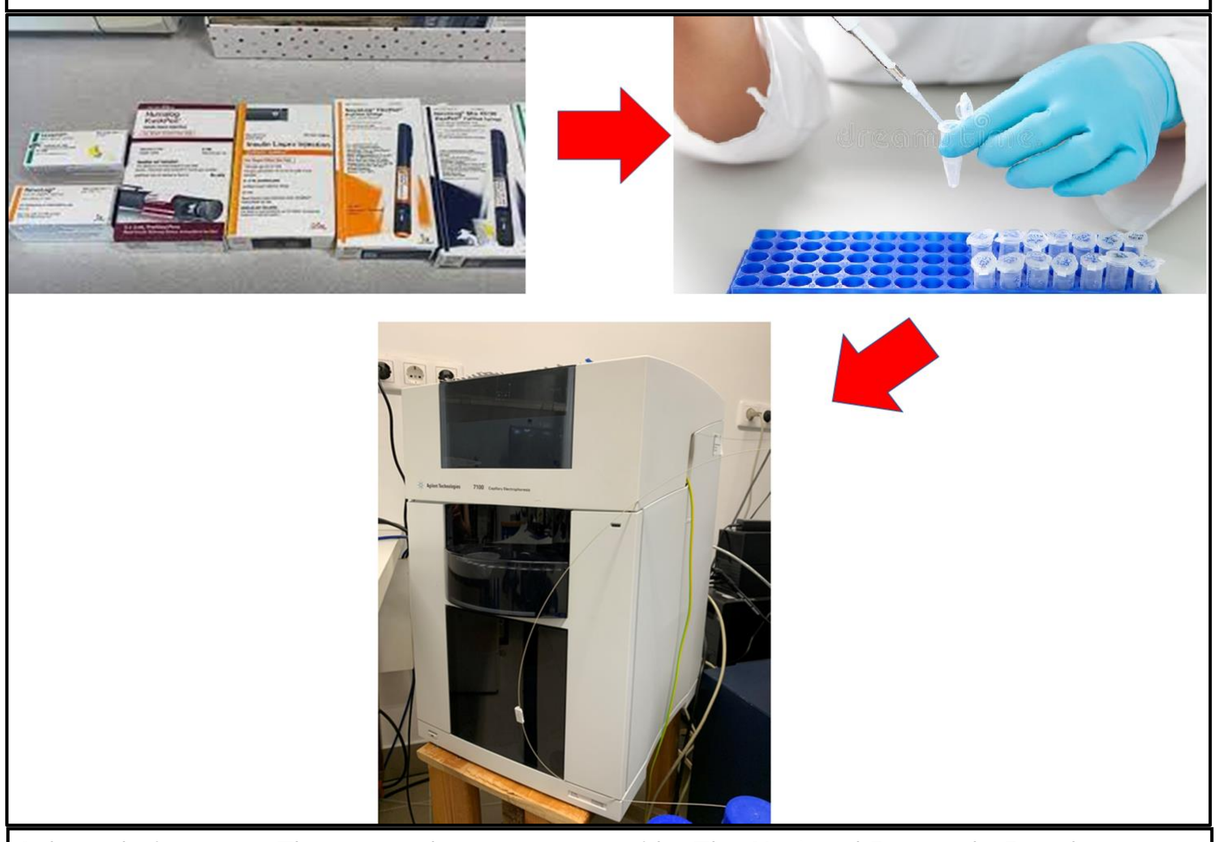
In this study, we demonstrate research of the main factors influencing migration behavior of insulin and its analogues. For this purpose, application of Response Surface Methodology enabled the optimization of a CE method with the objective to improve the separation of insulin analogs.

Experimental

The CE instrument was a 7100 model (Agilent, Waldbronn, Germany). Electrophoretic separations were carried out using fussed silica capillaries with an internal diameter of 50 µm and a total length of 65 cm in positive mode with -50 mbar pressure. The capillary was conditioned between each injection for 18 min with 0.1M NaOH, 6 min with acetonitrile and 24 min with the running buffer.

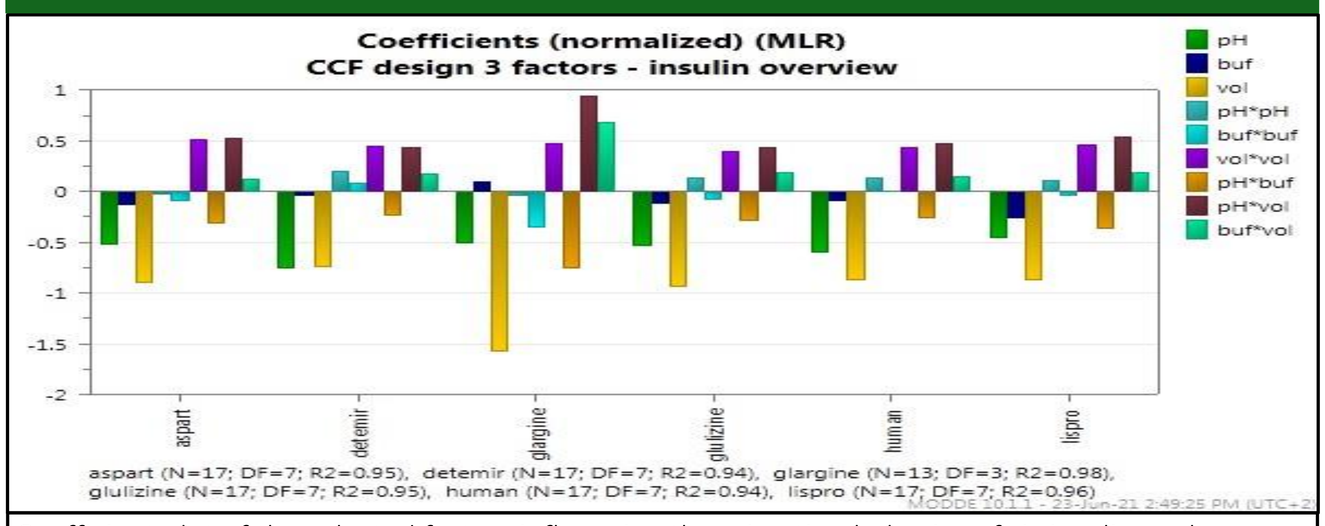
The factors with higher influence in the migration behavior of each insulin analogue were selected and analyzed using Experimental design.

The impact of the above-mentioned factors separately and their interaction (combination of the factors) were analyzed by RSM design called CCF.. The levels of factors selected for optimization were the following: BGE pH (8, 9, 10), BGE concentration (40, 50, 60), voltage (10, 20, 30). The number of total experiments calculated for these 3 factors was 15.

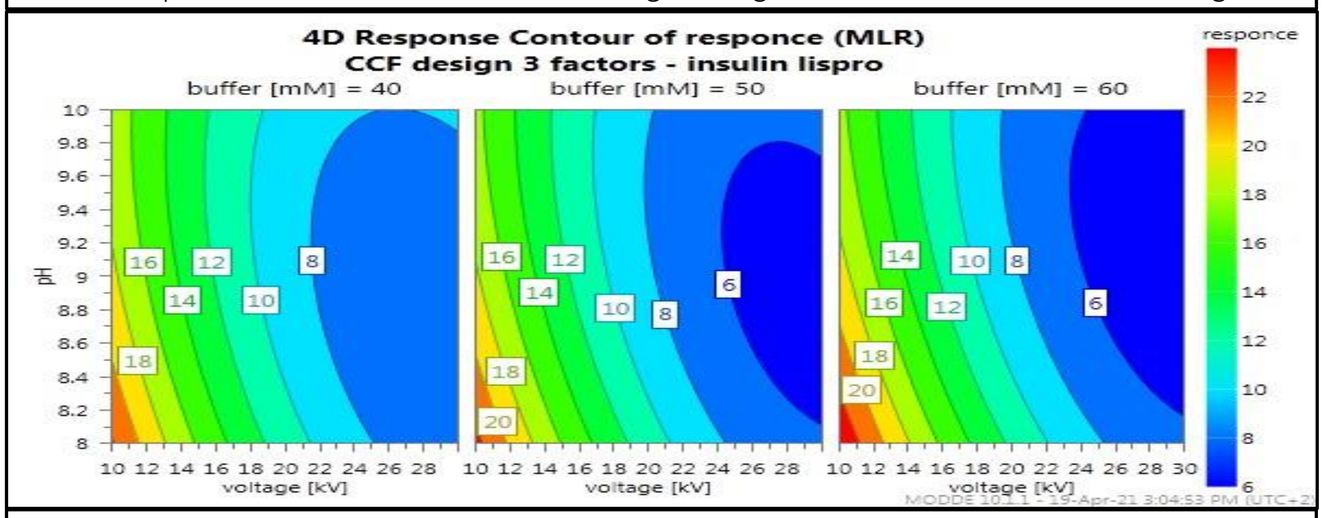


Acknowledgement: The research was supported by The National Research, Development and Innovation Office, Hungary (K127931)

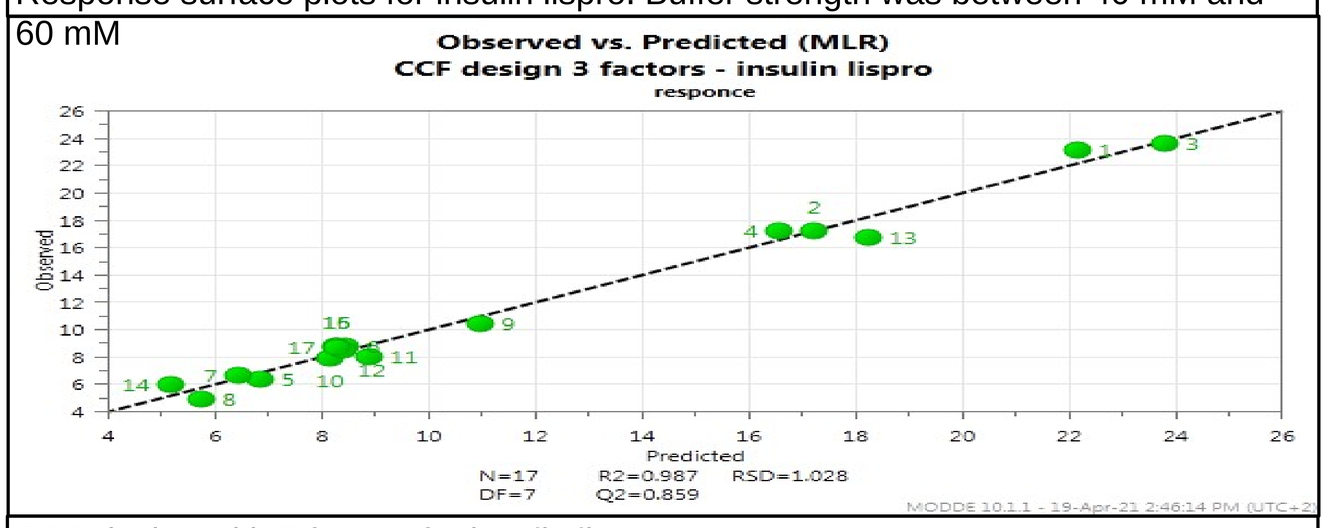
Results



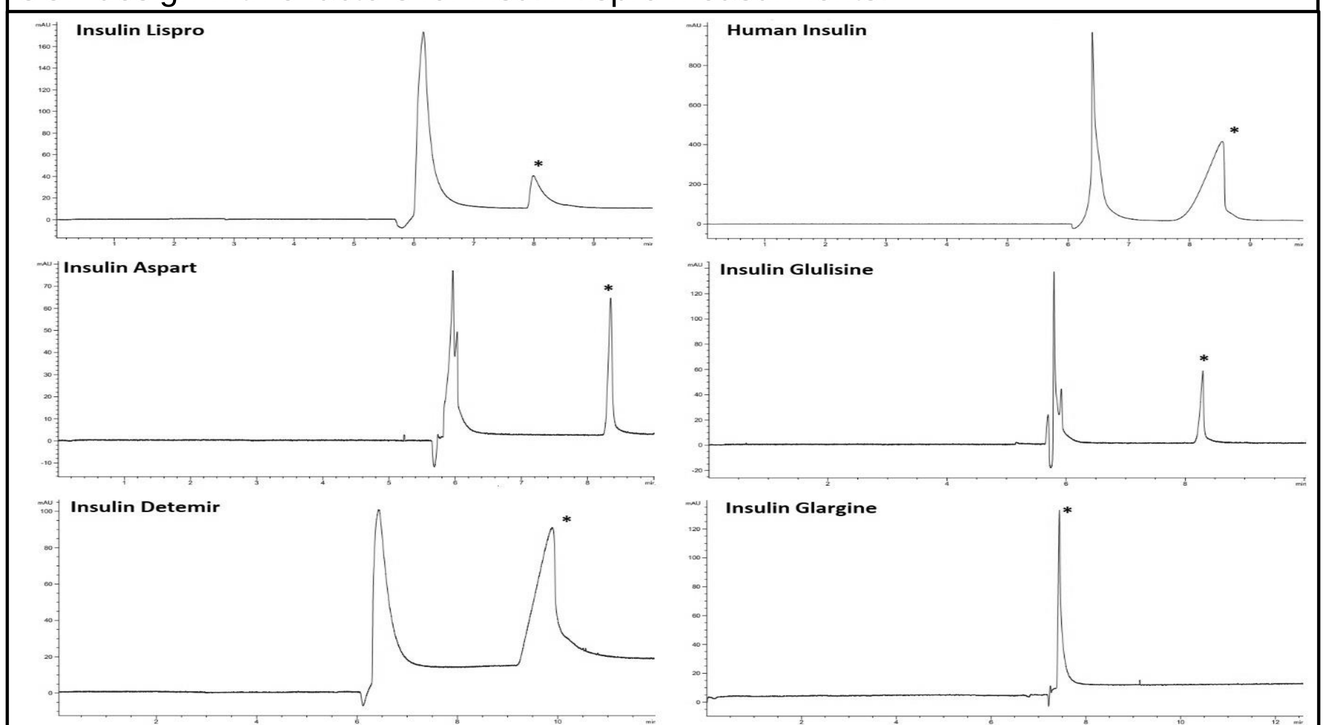
Coefficient plot of the selected factors influencing the migration behavior of six insulin analogs.



Response surface plots for insulin lispro. Buffer strength was between 40 mM and 60 mM



CCF design with 3 factors for insulin lispro measurments.



CE electropherograms of separation of insulin analogues. *= the main compound (insulin). Conditions: BGE: 40mM ammonium acetate, pH 9, voltage 20 kV, temperature 25°C.

Conclusions

Applying the RSM design, was able to find the optimal range of the most crucial factors including buffer pH and concentration, in relation of analysis time reduction. In consideration of the fact that when determining proteins and peptides with CE, a long preconditioning time is necessary to remove the adsorbed molecules in capillary wall, the appropriate analysis time for this determination would be 8-10 min. After several analysis for each insulin analogue, the optimal scope of specified factors to obtain acceptable analysis time were: applied voltage 20-25 kV, the buffer pH: 8.5-9.5 for the buffer concentration 40 mM ammonium acetate.