CONCLUSIONS

The work has demonstrated that capillary zone electrophoresis and micellar electrokinetic chromatography are high-performance and high-sensitive methods for qualitative and quantitative analysis and for physico-chemical characterization of peptide hormones.

Using the strategy for the rational selection of experimental conditions for CE analysis and separations of peptides, ionogenic peptides were separated as cations in the classical and isoelectric buffers in the acidic region, pH 2-2.5, and as anions in amphoteric buffer in weakly alkaline conditions, pH = 8.1. Non-ionogenic peptides were analyzed by micellar electrokinetic chromatography with micellar phase consisting of anionic detergent sodium dodecylsulphate in alkaline conditions, pH 8.8. The experimental conditions were selected with the aim to avoid the adsorption of peptides on the capillary wall, which was particularly reached with suppression of dissociation of silanol groups on the inner capillary wall in the acidic pH region and with electrostatic repulsion negatively charged peptides from negatively charged dissociation silanol groups on the inner capillary wall in

the alkaline pH region. The pH values of background electrolytes were chosen in the region, where the biggest differences in calculated specific charges of analyzed peptides were obtained. The efficiency of separation was comparable in classical and isoelectric buffers in acidic conditions, even though better results were obtained in isoelectric buffers for gonadotropin-releasing hormones.

It was demonstrated on CZE analyses of human insulin and its octapeptide fragment labeled by fluorescence marker NBD, that the sensitivity of fluorescence detection with the detector on the base of argon laser induced fluorescence (excitation/emission 488/520 nm) is 10-times to 300-times higher than the sensitivity of UV-absorption detectors with wavelength around 200 nm.

Capillary zone electrophoresis was also used for physico-chemical characterization of analyzed peptides. A new method was developed for easy estimation of temperature inside the capillary, which managed to precisely determine effective electrophoretic mobility of peptides at standard temperature, 25˚C.

The relationships between effective electrophoretic mobility of peptides, their effective charge, size and conformation were checked by using the semiempirical models correlating these parameters of peptides in solution and their similar electrophoretic behavior was confirmed for homologous sets of peptides hormones. Using these semiempirical models the probable molecular shapes were predicted for peptides in particular background electrolytes.