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CAPILLARY ELECTROPHORESIS FOR THE STUDY OF LIGAND- PROTEIN INTERACTIONS

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Capillary electrophoresis in format of frontal analysis is a relatively new opportunity for binding studies. Applied technique affords the accurate determination of binding parameters using commercial CE instrumentation and guided by the separation principles of zone electrophoresis. The characterization of interactions between drugs and serum proteins is essential for understanding of their distribution and action mechanism. The application of capillary electrophoresis at current work were focused on the characterization of interactions between some structurally different flavonoids and human serum albumin (HSA). Flavonoids have recently attracted much attention due to potential beneficial effects on human health, including antioxidant and free radical scavenging activities.

The experiments were performed at closed to physiological conditions (67 mM phosphate buffer, pH 7.4, temperature 36.5°C) and using optimized injection time (50mbar during 40s). According to results, studied flavonoids displayed moderate affinities towards the human serum albumin (binding constants in the range $10^3 - 10^4 \text{ M}^{-1}$) and was as followed: flavone > quercitrin > rutin. The difference of the structure characteristics between the flavonoids has significant effect on their binding properties to HSA. Results of the displacement experiments performed with warfarin and ibuprofen revealed the flavonoid binding position at site I on the HSA molecule. The acting forces playing the considerable role in the complex formation were identified as hydrophobic interaction and electrostatic forces.

The capillary electrophoresis was performed as a powerful method for characterization of binding parameters. Due to the rapidity of analysis, possibility of automatization, small sample consumption and accuracy of results CE-FA could be one of the alternatives to traditional techniques widely used.