ABSTRACT

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Title of Diploma Thesis: Use of capillary electrophoresis with contactless conductivity detection for the analysis of proteins

Nowadays there exist many methods for the determination of proteins. The aim of this work was to develop, optimize and characterize method for the analysis of a model mixture of proteins (α-lactalbumin, β-lactoglobulin, carboanhydrase, lysozyme and ribonuclease) by capillary electrophoresis with conductivity detection and to compare it with conventional capillary electrophoresis with UV detection.

The influence of the capillary inner wall coating on the adsorption of proteins, the composition and concentration of convenient background electrolyte, and the voltage and temperature were examined as experimental conditions possibly affecting the separation. A 50-cm fused-silica capillary (effective length 35 cm for conductivity detector and 41.5 cm for UV detector) with internal diameter 50 μm was used for the analysis. The UV detection wavelength was 210 nm. The static coating of the inner capillary wall realized by successive multiple soaking with ionic-polymer (polybrene-dextran sulphate-polybrene). The separation of five proteins was achieved in background electrolyte of 1.5 M acetic acid (pH 2). The separation voltage of -25 kV was applied and the temperature was maintained at 25 °C.

The calibration curves measured in the concentration range ≈ 0.03 - 1 mg/ml were linear for all analytes (correlation coefficients 0.9899 - 0.9976). The detection limits for conductivity detection ranged from 4.8 μg/ml (carboanhydrase) to 10.0 μg/ml (β-lactoglobulin) and for UV detection from 3.4 μg/ml (carboanhydrase) to 8.8 μg/ml (β-lactoglobulin). Repeatability expressed as % RSD of effective mobilities (n=6) ranged between 0.3 - 0.7 % (conductivity detection) and 0.3 - 0.9 (UV detection). For corrected peak areas the RSD values were 0.8 - 4.6 % (conductivity detection) and 0.4 - 5.7 % (UV detection). The limits of detection as well as method repeatability were found comparable for both detection approaches.