Abstract

Heparin is a mixture of sulfonated polysaccharides which is negatively charged. Heparin is a substance which is important in organism and fundamentally affects its physiology. Main attribute of heparin is anticoagulation – it prevents the complete blood coagulation. This anticoagulant effect balances the hemocoagulation by influencing the coagulation pathway. In some cases a pharmacological application of heparin is needed so the heparin is administrated as a injection of physiological solution of sodium or calcium heparine salt. Monitoring of level of the heparin in blood is problematic – methods used today are based on the measurement of a time required for blood clot formation. The result evaluation is done by comparing a sample with reference solution. These methods are relatively imprecise, can not be used in „on-line" setting and are highly influenced by general health condition of patient.

In this work some principles of affinity capillary electrophoresis were adapted from another work – heparin was determined indirectly by monitoring of decrease of the peak area of protamine. Protamine is medically used antidote of heparin because they create a stable complex together. In this work protamine was replaced by well defined tetraarginine because the most frequent amino acid in protamine is arginine.

The method was optimized – 10 mM phosphoric acid is used as background electrolyte. Two zones are hydrodynamically (5 kPa) injected into the capillary. First is heparine (120 s) and second tetraarginine (3 s). After injection 30 kV voltage is applied for 30 seconds – the zones migrate through each other so the complex is formed. Zones are then mobilized through detector out of capillary. The electropherogram of unattached tetraarginine is recorded spectrophotometrically (200 nm). The concentration of heparin in solution is calculated according to the decrease of the peak area of tetraarginine.

To improve sensitivity and run-to-run repeatibility a known amount of heparin was added to calibration solutions and hydroxyethylcelulose was added to background electrolyte. Using these conditions LOD was calculated as 1,1 μg/ml and LOQ as 3,8 μg/ml.