Abstract

Heparin is an acid mixture of glycosaminoglycans with high negative charge density which naturally occurs in human body. Due to its ability to bind antithrombin III and thus accelerate inhibition of thrombin it has anticoagulant effect. This is abundantly used in clinical practice for operations, in case of embolia or heart-attacks. Protamine is a mixture of small basic peptides, which is used in clinical practice as a heparin antidote. The interaction between heparin and protamine is electrostatic and is also used for determination of heparin in human plasma or blood using affinity methods. In my study it was found that if protamine and heparin are mixed in one vial, a complex is formed. Its resulting charge depends on concentration ratio of protamine and heparin. On the other hand, in case the protamine is injected as a sample and heparin is added to background electrolyte as a protein-binding ligand, it is possible to determine heparin from decreasing protamine peak area. Because of the complexity of protamine-heparin interaction, tetraarginine was used as structurally close model of protamine to increase repeatability of measurements. The method for determination of heparin was optimalised. It uses 20 mM or 60 mM ortho-phosphoric acid as background electrolyte, 1 mg/mL solution of tetraarginine as heparin-binding protein and UV detection at 200 nm. The measurements were performed in an uncoated silica capillary of 50 μm I. D. and 50,0 cm of length (41,5 cm to detector) using a temperature of 25 °C. After protamine is injected, the voltage of 30 kV is applied for 30 s, then the system is mobilised using a pressure of 5 kPa. In 20 mM ortho-phosphoric acid the limit of detection is 1.9 μg/mL, the limit of quantification is 6.3 μg/mL and the upper limit of linear dynamic range is 29.5 μg/mL. Relative standard deviation of tetraarginine peak area with no added heparin is 0.9 % (n = 10). In 60 mM ortho-phosphoric acid the limit of detection is 0.9 μg/mL, the limit of quantification is 2.9 μg/mL and the upper limit of linear dynamic range is 16.9 μg/mL. Relative standard deviation of tetraarginine peak area with no added heparin is 7.8 % (n = 10).

Keywords: Heparin, protamine, tetraarginine, capillary zone electrophoresis, affinity electrophoresis