Abstract

This diploma thesis deals with the determination of creatinine using a combination of flow injection analysis (FIA) or high-performance liquid chromatography (HPLC) with pulse amperometry, an electrochemical technique based on the application of potential pulses on a gold working electrode.

The determination was performed in a basic environment of borate buffer with creatinine concentration of $1 \cdot 10^{-4}$ mol·l^{-1}. The lenght of the cleaning and activation pulse was optimized as well as the pH of the running buffer. A cleaning pulse of +1.8 V was first applied to the electrode for 100 ms, then an activation potential of -0.5 V was applied for 150 ms and then a measuring potential of +0.2 V for 300 ms. The optimal pH was selected as pH=9,4.

Methanol and acetonitrile were added to the borate buffer to test whether creatinine could be determined in presence of these organic solvents and whether flow injection analysis could be transformed into HPLC. Methanol in the system caused peak deformation, acetonitrile did not cause the peak deformation in the system, at higher contents the baseline was destabilized.

Furthermore, the calibration dependence in the range of concentrations from $2.5 \cdot 10^{-4}$ mol·l^{-1} to $5 \cdot 10^{-6}$ mol · l^{-1} was measured using PAD in combination with FIA. At higher concentrations, peaks splitted.

Creatinine solution of the concentration of $1 \cdot 10^{-4}$ mol·l^{-1}, alone and in the mixture with uric acid and ascorbic acid with a concentration of $1 \cdot 10^{-4}$ mol·l^{-1} were measured by PAD with HPLC. The measurement conditions were the same as the selected optimal conditions for the PAD with FIA. The calibration dependence was also measured by this method, in the concentration range from $2.5 \cdot 10^{-4}$ mol·l^{-1} to $5 \cdot 10^{-6}$ mol·l^{-1}. Creatinine was also determined by PAD with HPLC in urine sample. The found values coincided with the result of the reference method - the Jaffé method.