

## **Abstract**

The aim of this thesis was the development of a voltammetric method for the determination of 7-dehydrocholesterol as a biomarker of congenital disease Smith-Lemli-Opitz syndrome. The concentration of 7-dehydrocholesterol in plasma will increase hundred-fold to hundreds to thousands  $\mu\text{mol L}^{-1}$  in patients suffering from Smith-Lemli-Opitz syndrome. The determination of 7-dehydrocholesterol was carried out in artificial serum in this work, which was prepared to mimic the conditions of postnatal diagnosis of Smith-Lemli-Opitz syndrome. 7-dehydrocholesterol provides a well developed voltammetric signal at approximately +0.8 V vs. Ag/AgNO<sub>3</sub> in acetonitrile. Differential pulse voltammetry with optimized parameters was used for the determination of 7-dehydrocholesterol. The presence of protein in artificial serum (human serum albumin) resulted in an unacceptably high detection limit of the method (178  $\mu\text{mol L}^{-1}$  in artificial serum). Therefore, human serum albumin was eliminated from the artificial serum samples by precipitating it with acetonitrile and subsequently centrifuging the resulting suspension. At the same time, the ratio of aqueous and organic components in the studied medium was also adjusted with acetonitrile to achieve an optimal voltammetric response of 7-dehydrocholesterol. With regard to the dilution of the artificial serum sample and the size of the voltammetric response, the optimal medium for the determination of 7-dehydrocholesterol acetonitrile – artificial serum 9:1 was selected. In this medium, the detection limit of 1.5  $\mu\text{mol L}^{-1}$  in artificial serum was achieved. This detection limit is low enough for the screening of Smith-Lemli-Opitz syndrome. Using the standard addition method, 7-dehydrocholesterol was determined in three samples with respective concentrations of 30, 100 and 250  $\mu\text{mol L}^{-1}$ . The recovery of the method was 43 – 70 %, therefore, it is clear that further optimization of the method is needed. Very good regression parameters of the concentration dependencies obtained by the standard addition method ( $> 0.99$ ) indicate that the electrochemical detection is accurate. Therefore, in the future, it is necessary to focus on the losses of 7-dehydrocholesterol caused by the sample pretreatment, especially the elimination of proteins from the studied matrix. This diploma thesis describes a simple and inexpensive method with high potential for use in point-of-care testing in facilities without a direct access to complicated instrumental laboratory equipment.

## **Key words**

7-dehydrocholesterol, boron doped diamond, oxidation, Smith-Lemli-Opitz syndrome, voltammetry