Abstract

In this doctoral thesis, a novel method for the determination of primary bile acids cholic acid and chenodeoxycholic acid is presented. Bile acids play various vital roles in the mammalian body. Moreover, their determination is extremely helpful in liver and biliary disease diagnosis and management. These saturated organic compounds lack strong chromophores and fluorophores in their structure, and thus are usually hard to detect in spectroscopy. For this reason, either instrumentally advanced but expensive methods, such as mass spectrometry, or less reliable enzymatic methods are commonly employed in bile acids quantitation. Hence, the demand for simple and reliable methods for their determination is strong. Bile acids are also known to be virtually inert for direct electrochemical oxidation. Herein, a simple method for their chemical activation for electrochemical oxidation on bare electrode materials was developed, optimized and applied to cholic acid and chenodeoxycholic acid determination. The activation is based on a dehydration reaction of a primary bile acid with 0.1 mol L^{-1} $HClO_4$ in acetonitrile (water content 0.55%) that introduces double bond(s) into the originally fully saturated steroid core. This naturally increases the electron density in the structure, and thus allows electrochemical oxidation of the bile acids under unchanged conditions directly in the dehydrating medium at +1.2 V (*vs.* $Ag/AgNO_3$ in acetonitrile). Increased electrochemical activity of cholesterol under equivalent conditions was also observed. The mechanism for the activation reaction was proposed based on results of CE-MS experiments. This activation reaction was optimized and implemented into an electroanalytical procedure employing differential pulse voltammetry on boron-doped diamond electrode, and the bile acids were determined in artificial and real-life serum samples with good selectivity. The results of the differential pulse voltammetric method were compared to the ones obtained with a previously proposed method (HPLC with fluorescence detection) confirming good accuracy of the present method. The limits of detection were estimated to be 0.5 μmol L^{-1} for cholic acid and 1.0 μmol L^{-1} for chenodeoxycholic acid. Moreover, application in liquid-flow techniques was envisaged. Pilot experiments with flow-injection analysis with amperometric detection in wall-jet arrangement using boron-doped diamond working electrode were performed with limits of detection of *ca* 1 μmol L^{-1} for both compounds, thus confirming favourable potential of the method in the amperometric mode of operation. An innovative, simple and fast electroanalytical approach to the detection of primary bile acids with a potential of clinical application was developed in this doctoral thesis.