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ACCEPTED MANUSCRIPT

A novel electrochemical biosensor for ultrasensitive detection of

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ABSTRACT

Serum total bile acids (TBA) level is used as a sensitive and reliable index for hepatobiliary diseases in clinics. Herein, a novel electrochemical biosensor was fabricated using enzymatic reaction coupling with the double oxidation circular amplification strategy for the detection of human serum TBA. With the catalysis of 3α -hydroxysteroid dehydrogenase (3α -HSD), 3α -bile acids reacted specifically with nicotinamide adenine dinucleotide (NAD⁺). And then, the reduced nicotinamide adenine dinucleotide (NADH) was produced. After that, the NADH reacted with the electron mediator of tris(2,2'-bipyridine) ruthenium(III) (Ru $(bpy)_3^{3+}$), which was then transformed to $Ru(bpy)_3^{2+}$. Ultimately, $Ru(bpy)_3^{2+}$ was further oxidized to $Ru(bpy)_3^{3+}$ under a certain voltage, which was detected by the chronoamperometry assay. The detection was performed using a disposable unmodified screen-printed carbon electrode (SPCE) without sample preparation. The proposed biosensor showed high sensitivity and accuracy with the linear range from 5.0 to 150.0 pmol/L in 10^6 -fold dilution serum. The established method had a good correlation with the enzymatic cycling method (r = 0.9372, P < 0.001, n = 72) commonly used in clinic. The electrochemical biosensor is simple, ultrasensitive and without sample pretreatment, showing great potential for point-of-care testing (POCT) of serum TBA in clinical

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