MDH activity assay based on a new nitrogen-doped graphene modified SPE for NADH detection.

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Malate Dehydrogenase (MDH) (EC 1.1.1.37) is increased in some neurodegenerative diseases such as Alzheimer's disease, and abnormal MDH activity in serum can serve as a diagnostic tool for severe liver damage (e.g. Hepatocellular carcinoma). Electrochemical detection of malate dehydrogenase activity was provided by nicotinamide adenine dinucleotide (NADH) electro-oxidation as a product of MDH-dependent L-malate conversion. This study includes the development of high performance sensor based on carbon screen-printed electrode (SPE) modified with N-doped graphene for the sensitive detection of NADH as well as optimization of conditions and procedures for determining the activity of MDH.

The surface of the working carbon electrode was modified with a layer of N-doped graphene with chitosan by drop-casting method. Electrochemical characterization of the modified electrodes was carried out by cyclic voltammetry (CV) and electrochemical impedance spectroscopy. Composition of carbon SPE modification layer was optimized as 1% of NG in 1% chitosan prepared in 1% acetic acid. The optimized sensor provides NADH detection by CV from 0.02 to 5 mM in 0.1 M phosphate-buffered saline (PBS) pH 7.5 at scan rate 0.75 mV/s and picks potential close to 0.3 V.

The modified electrode was used to determine the activity of MDH by CV in a drop of 50 µl. Optimal conditions for the analysis has been identified as MDH sample pre-incubation in the presence of 10 mM NAD^+ and 60 mM L-malate for 5 minutes. Determinations were performed in 0.1 M PBS at pH 7.5. The possibility of determining MDH activity using the developed sensor in the range of 1-5 U/ml with a detection limit of 0.72 U/ml was demonstrated.

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