UNIVERSITY OF BUCHAREST
FACULTY OF CHEMISTRY
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ABSTRACT

Ph.D. THESIS

MODIFIED ELECTROCHEMICAL SENSORS FOR DETECTION OF NICOTINAMID ADENINE DINUCLEOTIDE

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Numbering of figure is identical to that of the Ph.D. thesis.

Nicotinamide adenine dinucleotide is part of a redox system, which plays an important role in enzymatic reactions catalyzed by dehydrogenases. The structure of this coenzyme is based on two nucleotides derived from adenine and nicotinamide (Figure I.1-1).

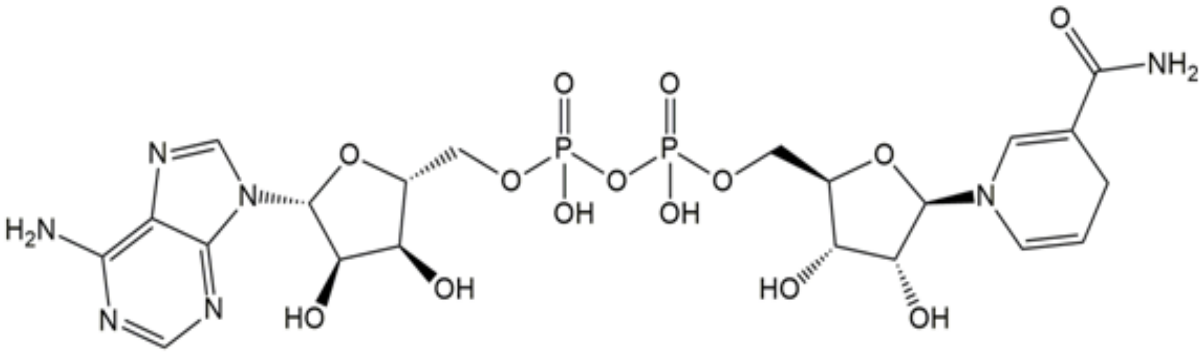

Figure I.1-1. Nicotinamide adenine dinucleotide

The nicotinamide is responsible for the redox properties of this compound, and acts as a mediator in many biological systems. It make possible the electrons transfer between two enzymatic system using the oxidized form (NAD^+) and reduced form (NADH). The NAD^+/NADH couple is involved in more than 300 enzymatic reactions catalyzed by dehydrogenases. Quantification of this coenzyme is important for studies on biochemical process as well as for developing of analytical tools used in food quality control, environmental control or clinical laboratory. Direct oxidation of NADH at the classical electrode surface requires a high oxidation potential due to its high activation energy and it is followed by the fouling of the electrode surface due to the accumulation of the oxidation products on its surface. The electrochemical reaction occurs at an applied potential between +600 and +800 mV (*vs*.Ag/AgCl) and NADH detection is characterized by a low sensitivity.

The Ph.D. thesis has been structured into two parts: state of the art (I) and the original contributions (II) in the field of electrochemical sensors for detection of NADH.

The main objective of the thesis was the development of sensitive and selective sensors for detection of NADH, in order to eliminate the disadvantages of the conventional electrodes, such as high oxidation potential, passivation of surface electrodes by accumulation of reaction products and low selectivity. In this sense, the surface of the screen-printed electrode (SPE) was modified with polyelectrolytes, electrochemically reduced graphene oxide (ERGO), gold nanoparticles (AuNPs)

and composite materials that contribute independently or synergistically to improve the electron transfer.

The thesis reports the preparation of the sixth composite materials GO-PAH, ERGO-PAH, AuNPs-GO, AuNPs-ERGO, AuNPs-GO-PAH and AuNPs-ERGO-PAH. They were characterized by FT-IR and Raman spectroscopy and by scanning electron microscopy (SEM). The FT-IR spectroscopy confirmed the presence of the quaternary nitrogen in the structure of polyallylamine hydrochloride (PAH). The presence of positive charge is essential to explain the electrochemical properties of NADH sensors based on this polyelectrolyte. The Raman spectroscopy was used for characterization of materials based on graphene oxide (GO) and electrochemically reduced graphene oxide in order to confirm the electrochemical reduction of GO. The SEM images revealed that electrochemical reduction of graphene oxide did not modify the specific texture of GO and that PAH acts like a stabilizer for AuNPs in ternary composites AuNPs-GO-PAH and AuNPs-ERGO-PAH.

Chapter II.1 describes the construction, optimization and characterization of a PAH modified SPE sensor for sensitive and selective detection of NADH. It was demonstrated that PAH film deposited on the surface of screen-printed electrode allows the rapid detection of NADH at a lower applied potential comparative with bare electrodes, with a high sensitivity, on a large response range and with a detection limit of 0.22 μM.

PAH deposition was realized by o simple method that does not need laborious purification and/or functionalization procedures. PAH/SPE sensor presents electrocatalytic activity for oxidation of NADH and can be used for development of biosensors based on NAD-dependent dehydrogenases.

Chapter II.2 presents the realization and characterization of a sensor for the detection of NADH modified with polyallylamine hydrochloride and electrochemically reduced graphene oxide. The electrochemical reduction of graphene oxide was performed directly on the surface of the working electrode using cyclic voltammetry. The use of ERGO-PAH led to an improvement of the response characteristics of the NADH sensor, such as: extended linear range, lower detection limit, higher stability and selectivity.

Chapter II.3 describes the use of a composite based on gold nanoparticles (AuNPs), ERGO and PAH as electrode material. This composite material led to an increase of amperometric response of the NADH sensor. AuNPs-ERGO-PAH/SPE sensor presents outstanding analytical performances: rapid detection, high sensibility and reproducibility and a good selectivity comparative with other similar NADH sensors reported in the literature.

From our knowledge, the presented results were the first analytical applications of PAH, ERGO-PAH and AuNPs-ERGO-PAH in NADH sensing, reported in the literature. Besides their

electrochemical properties, these materials can be used as matrices for enzymes immobilization. These properties will be exploited for developing biosensors based on NAD-dependent enzymes with applications in food quality control.

The results reported in this Ph.D. thesis have been published in ISI quoted journals, as follows:

1. Poly(allylamine hydrochloride) modified screen-printed carbon electrode for sensitive and selective detection of NADH
Lucian Rotariu, Oana Maria Istrate, Camelia Bala
Sensors and Actuators B: Chemical, **2014**, 191, 491-497 (IF: 4.097)
2. NADH sensing platform based on electrochemically generated reduced graphene oxide–gold nanoparticles composite stabilized with poly(allylamine hydrochloride)
Oana Maria Istrate, Lucian Rotariu, Virgil Emanuel Marinescu, Camelia Bala
Sensors and Actuators B: Chemical, **2016**, 223, 697-704 (IF: 4.097)
3. Electrochemical determination of NADH using screen printed carbon electrodes modified with reduced graphene oxide and poly(allylamine hydrochloride)
Oana Maria Istrate, Lucian Rotariu, Camelia Bala
Microchimica Acta, **2015**, doi:10.1007/s00604-015-1595-4, online from 11 august (IF: 3.741)