Ph.D. Thesis

Electroconducting Conjugated Polymer Based Biocatalytic Uric Acid Sensor

Róbert Dobay

Consultants: Dr. Gábor Harsányi

Technical University of Budapest
Department of Electronic Technology

Dr. Csaba Visy

University of Szeged
Department of Physical Chemistry

Budapest, 2000.

Thesis 1 The feasibility of the poly-N-Methylpyrrole layer – in virtue of its structure – for immobilizing uricase enzyme with retained catalytic activity was confirmed. The one step electropolymerization took place in 0.1 M Sodium Dodecyl Sulfate solution containing the monomer (Methylpyrrole, 0.1 $\frac{\text{mmol}}{\text{dm}^3}$) and 60 $\frac{\text{mg}}{\text{dm}^3}$ uricase enzymes. The principle conclusion was exploited that by means of the enzyme's negative charge in this type of agents it can be absorbed in the polymer film in consequence of electrostatic interactions.

Thesis 2 a) However, the uirc acid itself showed electroactivity at the polymer electrode, the repeated calibrations proved that the current originated from the H_2O_2 oxidation is possible by means of a selective measurement method. The bipotentiostatic measurement technique, never used for biosensor calibration before, created opportunity for selectively registering the current of the enzyme and the polymer electrode thus determining the current difference. The stationary currents were measured instead of fast transients with this method.

b) The investigation of adding glucose (2 ill. 4 $\frac{\text{mmol}}{\text{dm}^3}$) and ascorbic acid (1 $\frac{\text{mmol}}{\text{dm}^3}$) to the basic solution demonstrated that the bipotentiostatic technique compensated the interferences much more effectively than other solutions so far.

Thesis 3 It was concluded that the DS^- anion, never used for biosensors before, is suitable to ensure the duality of unperturbed polymer layer deployment and appropriate pH (pH 6.5) for enzyme immobilization. The immobilization of the enzyme molecules and the uniformity of the two electrodes were proved with registering absorbance spectra. During the detection (reduction) the immobilized DS^- anions were blocked to be detached from the polymer matrix due to their large size, thus the polymer film showed cation-changing properties exclusively that inhibited the negatively charged urate ions to penetrate in the structure.

Thesis 4 The operation parameters of the biosensors were optimized as follows:

a) The calibration at different electrode potentials proved that the biosensor was safely practicable at relatively low values (E < 225 mV) with appropriate signal to noise ratio.

b) The temperature calibration measurements showed stable detection characteristic in the 20-40 °C range, enabling the sensor to operate at room temperature.

c) The detection process, repeated at different pH values, concluded that the maximum current difference is obtained around the isoelectric point of the enzyme (pH_i=6.3). The solution pH had effect only on the enzyme electrode, while it left the polymer electrode invariable.

Thesis 5 Combining the galvanostatic polymerization (A = 0.3 cm^3, I = 0.9 mA, t = 150 s) and the cyclic voltammetric conditioning a stable structure was prepared, with more than 40 days operation stability, exceeding the previously reported ones prepared with similar technology.