ENZYME ACTIVITY CHARACTERIZATION BY ELECTROCHEMICAL SENSOR

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ABSTRACT

The electrochemical sensors enable effective measurement of enzyme activity. The measurement consists of two steps. The first one is calibration of electrochemical sensor current to the product of enzymatic reaction. The rate of product evolution is measured in the second step. The methods have been implemented into the program module. Main properties of measurement are controlled, analyzed and stored automatically by software.

1 INTRODUCTION

Enzyme is a biological catalyst composed from proteins, are specific to a particular reaction. In live organisms they are participating in many important processes for surviving.

The measurement of in vivo activity and functionality of enzymes is an important tool for their study. It can elucidate the origin of many diseases, such as neurodegenerative Alzheimer disease. The electrochemical sensors enable easy and effective way of measurement enzymes characteristics.

The features of controlling software are presented on the example of glucose oxidase enzyme.

2 THEORETICAL PRINCIPLES

Glucose oxidase is ideal enzyme for testing due to its stability. Hydrogen peroxide as product of the reaction of glucose oxidase with β-D-glucose is measured.

$$\beta\text{-D-glucose} + O_2 + H_2O \xrightarrow[\text{glucose oxidase}]{E} \text{glucon acid} + H_2O_2 .$$

It enables an easy calibration of the results of measurement by addition of product into the solution. Exact amount of product causes current response which can be easily measured.

The initial reaction rate is calculated by extrapolation of experimental data by second order polynomial fitting to time zero.

3 MEASUREMENT, CONTROLLING SOFTWARE AND AUTOMATION

The common measurement process consists of few steps (Fig. 1:). After the start of measurement, the pump of microflow system is switched on. The steering of solution begins. It effect initial polarization of electrochemical sensor and current stabilization in sequence.

When current is stabilized enough, then follow addition of β-D-glucose, H_2O_2 for calibration purposes and glucose oxidase enzyme, which will start the reaction we are mainly concerned in.

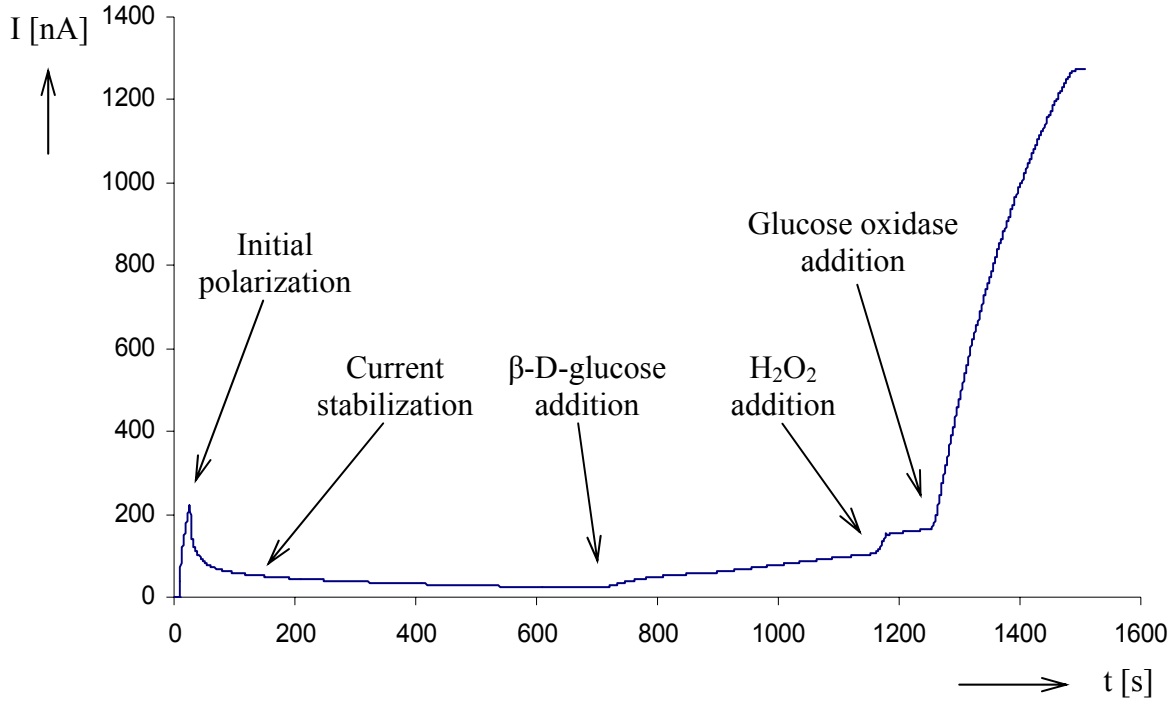

Fig. 1: *Measurement signal record.*

Fig. 2: is showing measurement progress, which will be displayed by controlling software.

Analyze of the measurement consists of two steps. The first one is calibration of electrochemical sensor current to the product of enzymatic reaction (Fig. 3:). The rate of product evolution is measured in the second step (Fig. 4:).

The measured concentration is corrected to change of volume due to additions of chemicals. The sensor signal correction to the background current is made (Fig. 5:). The initial reaction rate is calculated by extrapolation to time zero of second order polynomial fitting by experimental data.

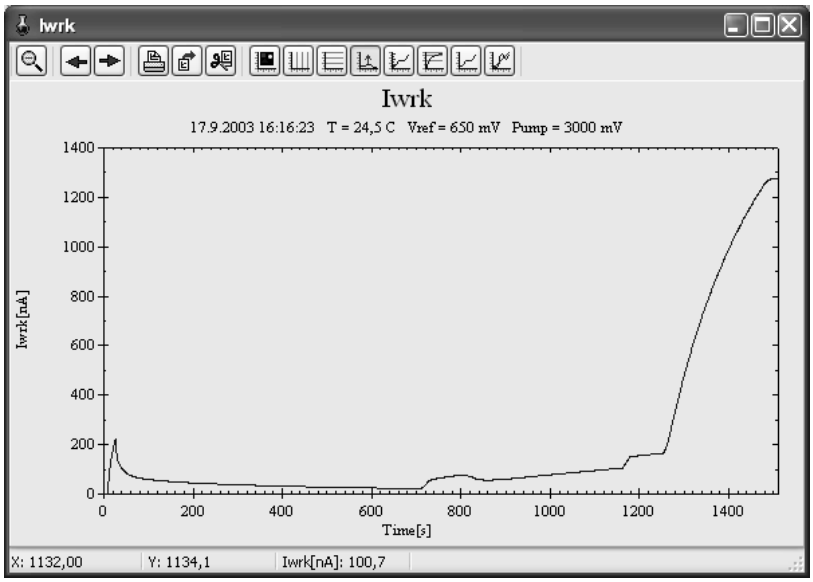

Fig. 2: *Measurement signal record in controlling software.*

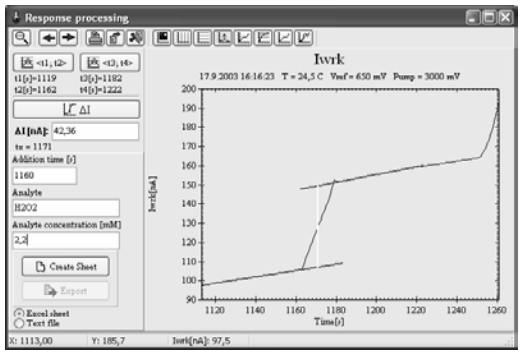

Fig. 3: *Tool for calibration.*

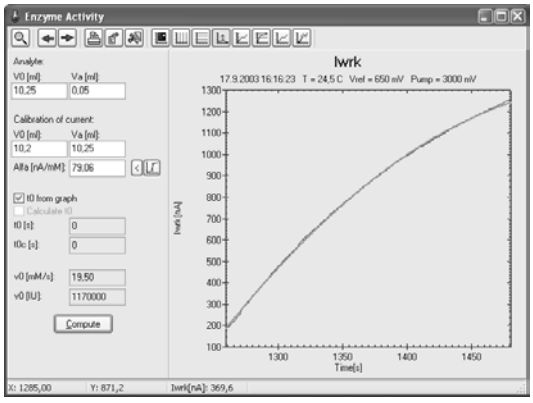

Fig. 4: *Initial rate evaluation.*

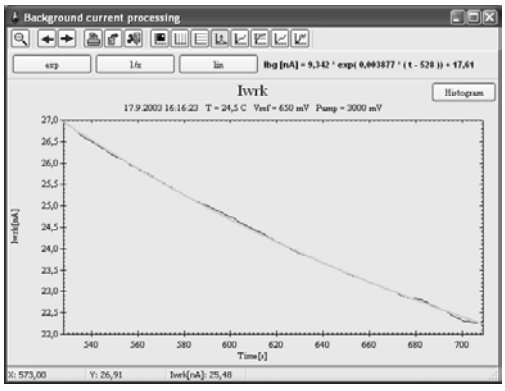

Fig. 5: *Background current correction.*

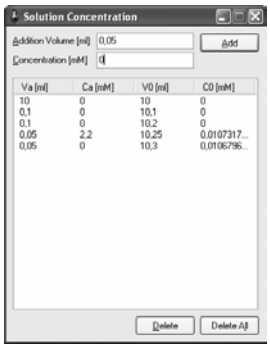

Fig. 6: *Integrated tool for concentration evaluation.*

The methods have been implemented into the program module. Main properties of measurement are controlled, analyzed and stored automatically by software. The tool for easy solution concentration evaluation and tracking is included (Fig. 6:).

The nonlinear numerical methods are used in evaluation. The conditions of numerical stability have been involved into the experimental procedure and they are automatically tested during measurement procedure.

4 REAGENT, CHEMICALS AND MATERIALS

- Microflow system with integrated three-electrode amperometric sensor AC1.W2.RS (BVT Technologies, a.s., www.bvt.cz).
- 10 ml of phosphate buffer, pH=8, 100 µl of KCl, polarizing voltage +650 mV.
- β-D-glucose 1M, hydrogen peroxide 2,2 mM, glucose oxidase enzyme.

5 RESULTS, DISCUSSION AND CONCLUSION

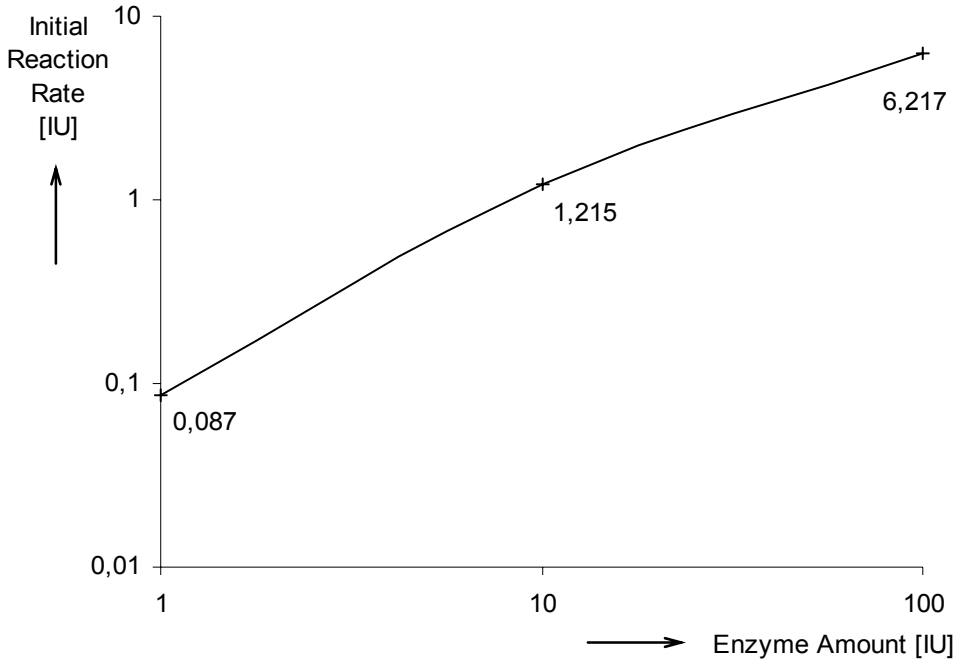

Fig. 7: *Dependency of reaction rate on enzyme activity.*

Electrochemical evaluating of enzyme activity is well described in literature. The aim of this work wasn't reinventing this method, but converts it to the formal method suitable for daily routine usage. It involves correction of background current, module for response calibration processing and initial reaction rate evaluation by extrapolation to time zero of second order polynomial fitting. All this processing is done in background and transparently to user. Resulting application is capable to get enzyme activity just in 15 minutes.

ACKNOWLEDGEMENT

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