APPLICATION OF PRUSSIAN BLUE IN THE DEVELOPMENT OF ENZYMATIC BIOSENSORS

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Introduction

Prussian blue (PB) is an unique material which is widely used as a signal transducer in optical and electrochemical biosensors. In most cases PB is used in biosensors due to its optical and electrochemical sensitivity to hydrogen peroxide, which is common product of enzymes oxidases. Moreover, PB also exhibits dependensy of its reduction potential on concentration of several ions (K^+, NH_4^+, Cs^+ and Rb^+), that promotes electrochemical reduction of PB into Prussian white (PW). Finaly, PB exhibits optical and electrochemical sensitivity to pH. Therefore, all these sensitivities of PB can be used in developement of optical and electrochemical biosensors. The aim of this work is to analyze the performance of PB as a signal transducer in enzymatic biosensors, to highlight the common problems which occurs during PB-based sensing and to discuss possible ways to improve reliability and overall performance of PB-based biosensors. The applicability of structural analogues of PB (PBsa) in biosensors and comparison between analytical performance of PB and PBsa are also discussed in this work.

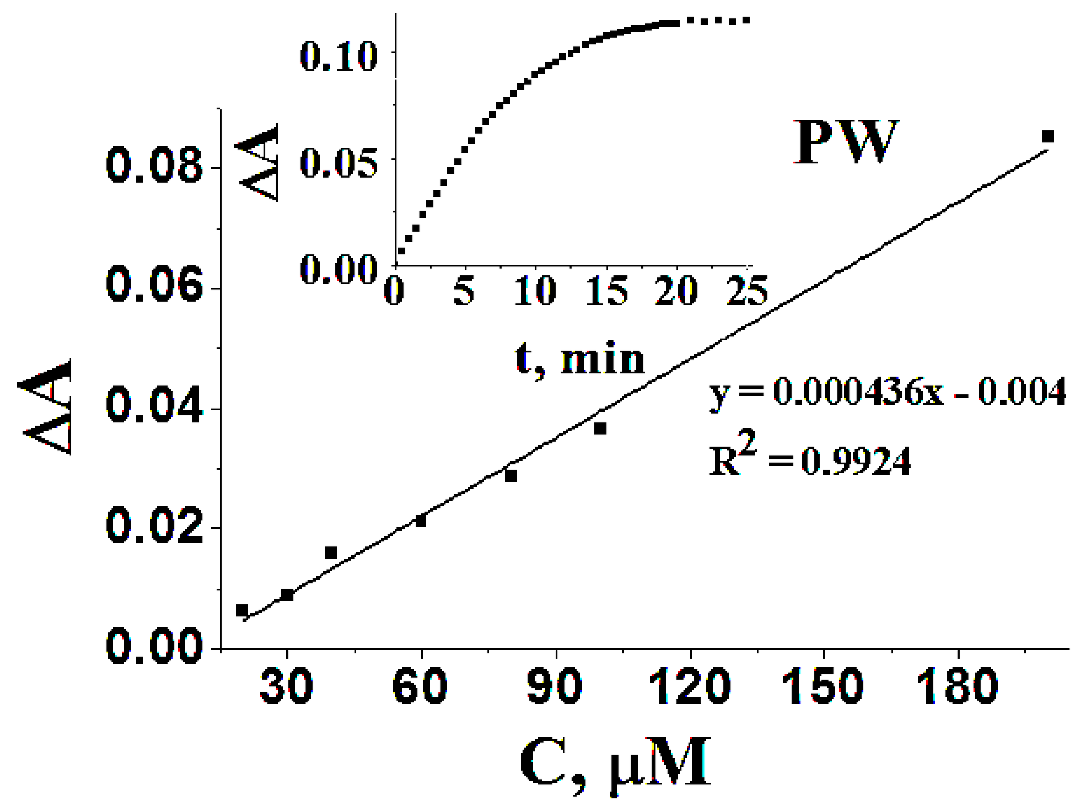

Fig. 1. Calibration curve of optical PW-based glucose biosensor. The PW-based optical glucose biosensor exhibited linear range from 0.020 mM to 0.200 mM. However, optical investigation of PW layer reveled that PW undergoes spontaneous oxidation after its deposition on electrode, thus, only freshly prepared PW can be used in development of PW-based biosensors. Moreover, long term storage of these biosensors may induce the decrease in their sensitivity. Data from "P. Virbickas et al. *J. Electrochem. Soc.*, 2019, 166, B927–B932".

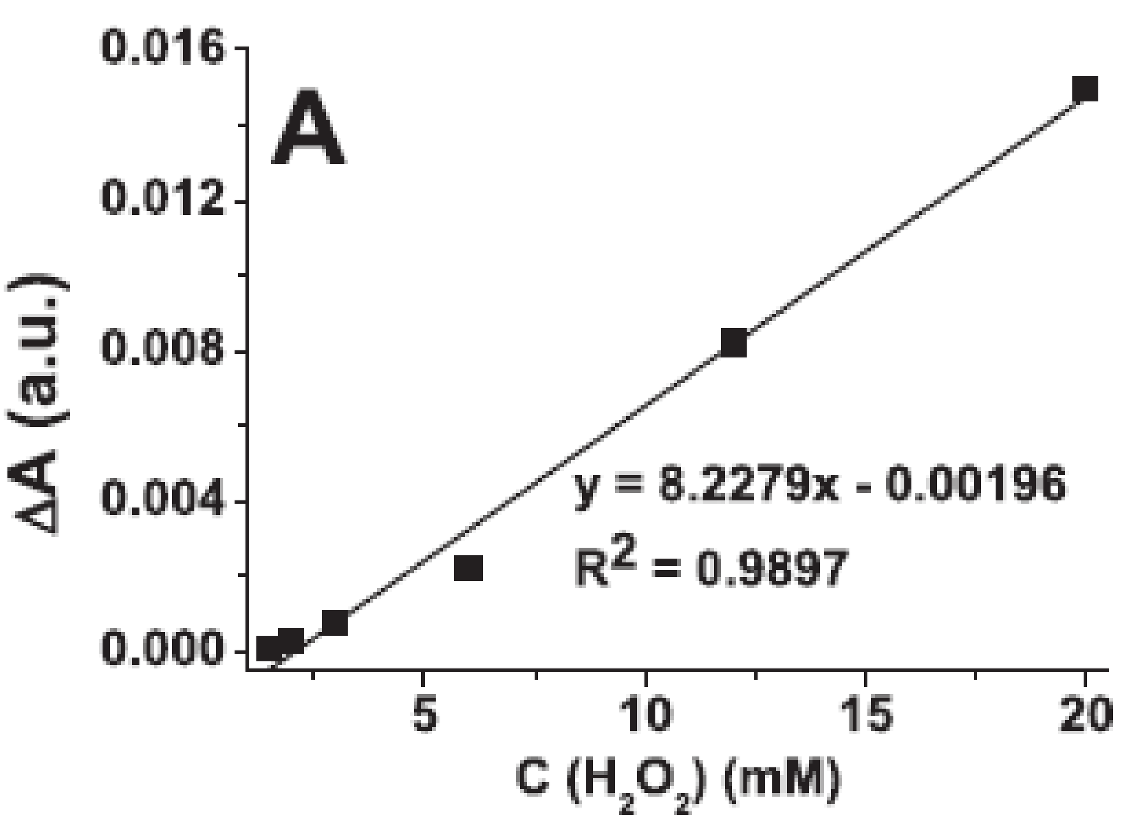

Fig. 2. Calibration curve of optical cobalt hexacyanoferrate (CoHCF)-based H_2O_2 sensor. Sensor was able to detect H_2O_2 concentration in linear detection range from 1.5 mM to 20 mM. Furthermore, optical investigations of CoHCF layer indicated that CoHCF is resistant to the oxidative impact of air. Data from "P. Virbickas et al., *Electrochimica Acta*, 2020, 362, 137202".

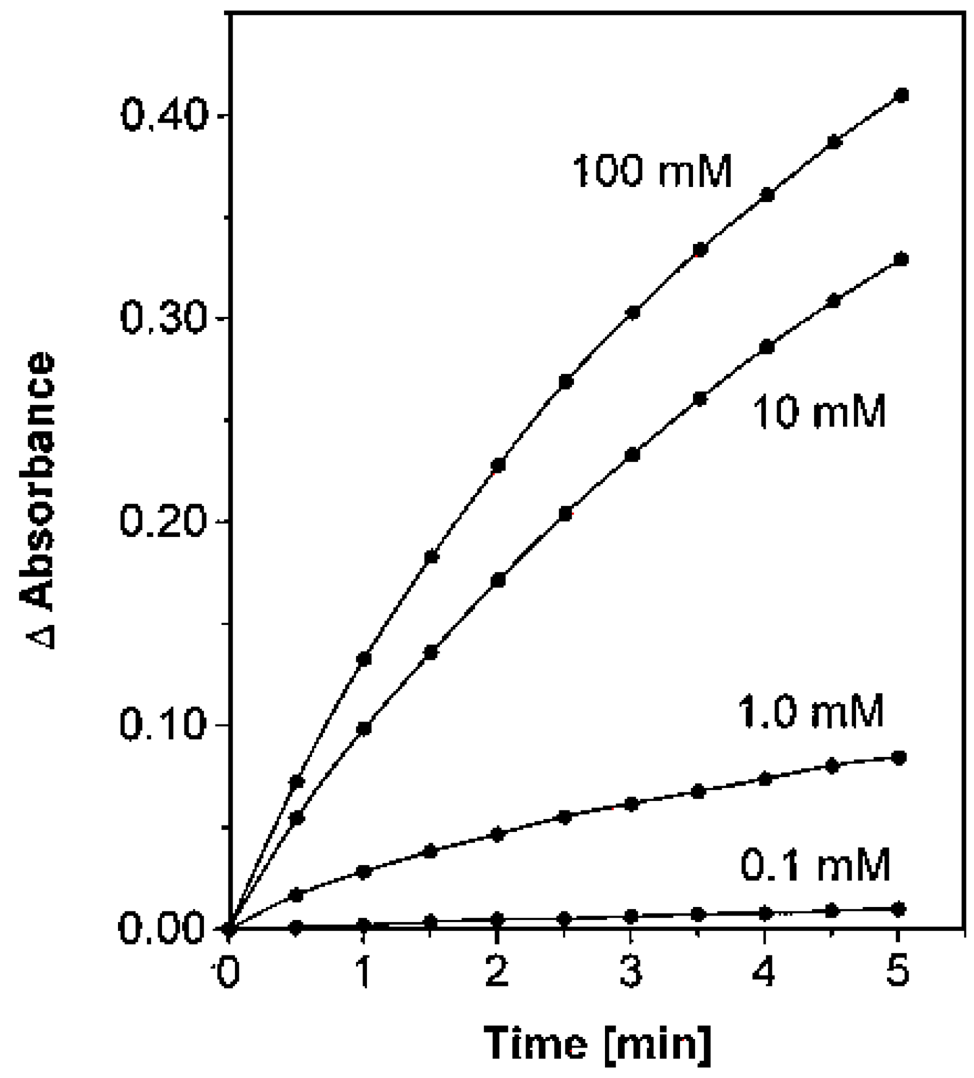

Fig. 3. Optical investigation of PB-based urea biosensor. "Insoluble" form of PB ($(Fe_4^{3+}[Fe^{2+}(CN)_6]_3)$) was applied in this biosensor. "Insoluble" form of PB exhibits reversible optical sensitivity to pH in range from 3 to 9. This sensitivity of "insoluble" PB to pH is caused by alkaline media, which initiates reversible hydrolysis of PB. However, at pH values greater than 9 irreversible hydrolysis of PB occurs. Data from "R. Koncki, O. S. Wolfbeis, Anal. Chem. 1998, 70, 2544–2550".

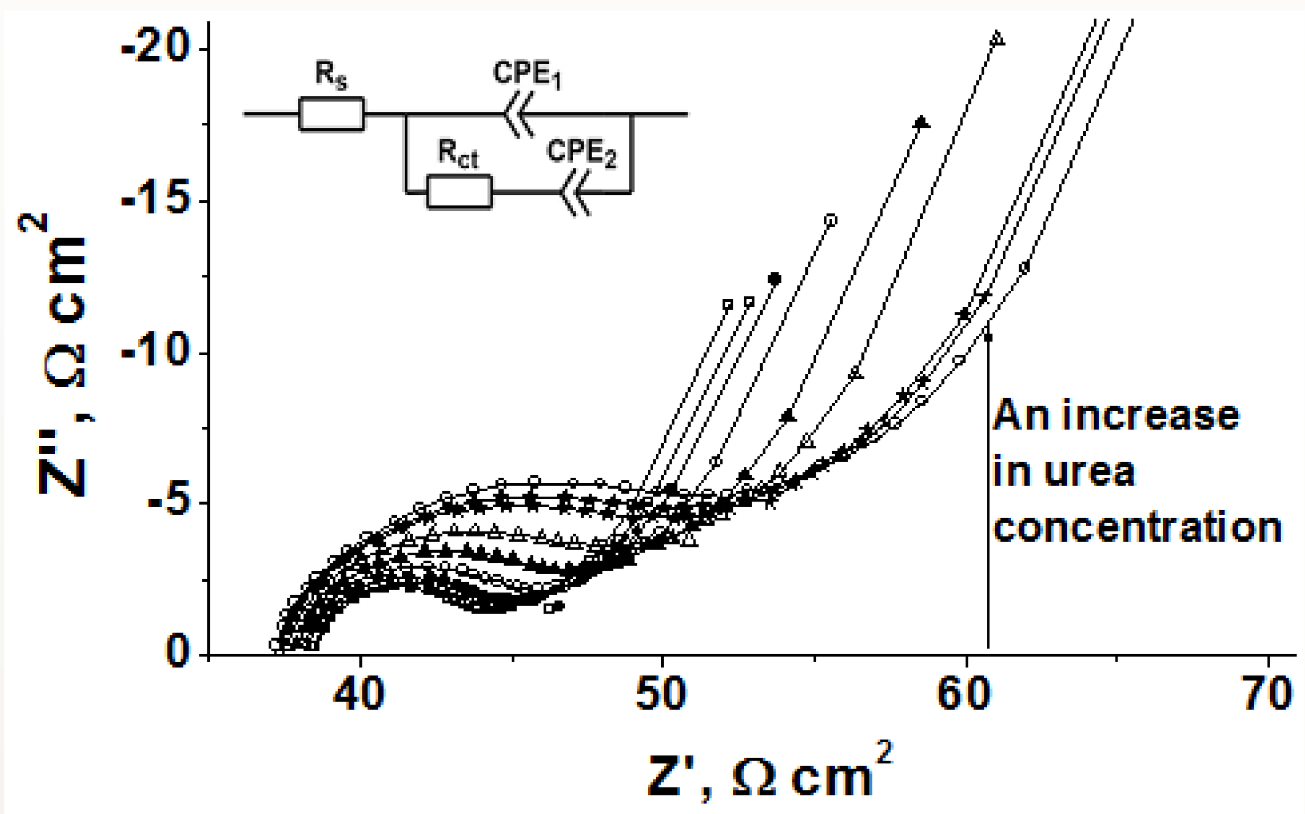

Fig. 3. Impedimetric investigation of PB-based urea biosensor. "Soluble" form of PB ($NH_4^+Fe^{3+}[Fe^{2+}(CN)_6]$,) was applied in development of this biosensor. The working principle of this biosensor is based on the role of hydrogen ion in charge transport through the PB film – it is postulated that hydrogen ion is involved in electron-hopping between neighboring active positions in the PB. Therefore, as urease-catalyzed hydrolysis of urea is accompanied by increase in pH of enzymes surroundings, this increase in pH results in increase in charge transfer resistance through the PB layer. Data from "A. Valiūnienė et al., J. Electroanal. Chem. 2021, 895, 115473".

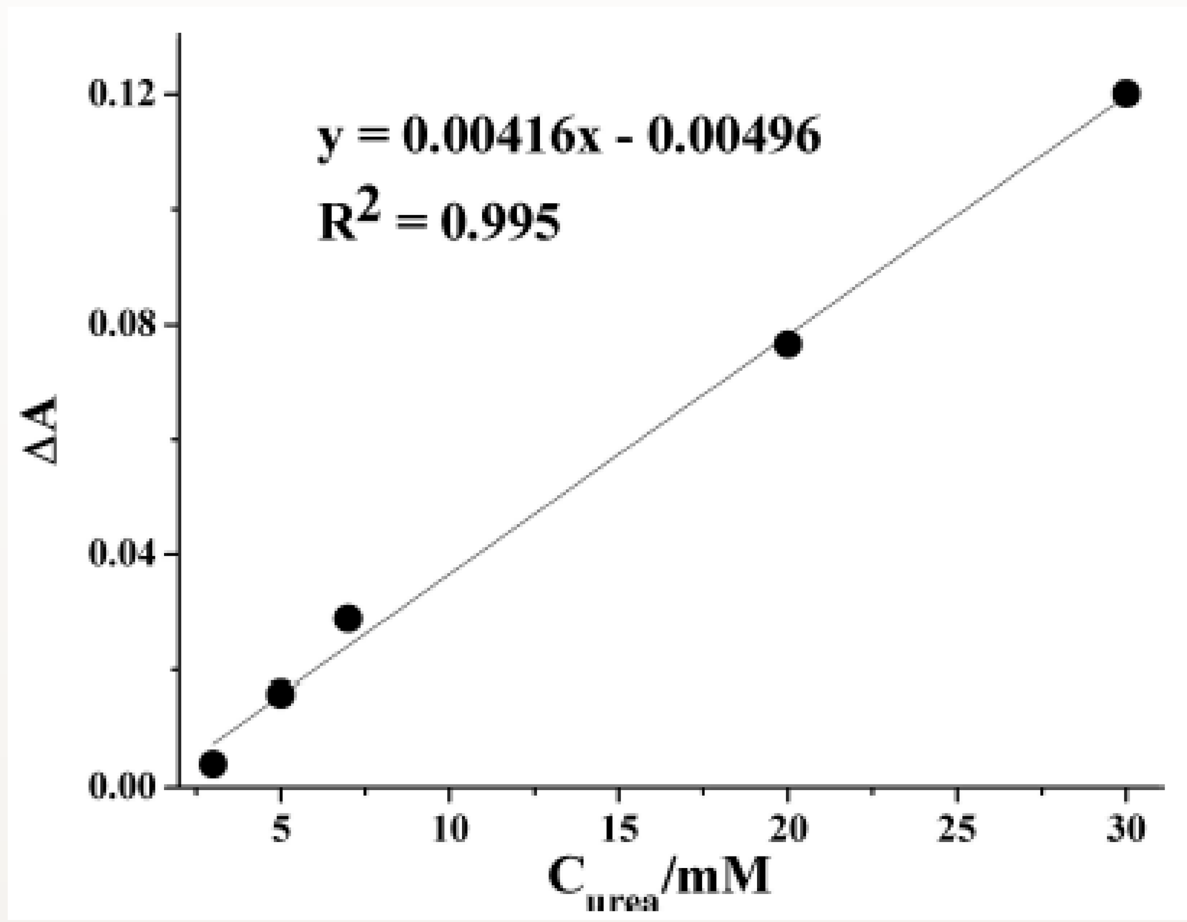

Fig. 3. Calibration curve of PB-based electrochromic urea biosensor. "Soluble" form of PB ($K^+Fe^{3+}[Fe^{2+}(CN)_6]$,) was applied in this biosensor. It is known that an increase in NH_4^+ ions concentration causes an increase in PB's reduction potential, thus, urease-catalyzed hydrolysis of urea results in fading of PB layer and that enables to quantify concentration of urea. Data from "A. Valiūnienė et al., Electroanal. 2020, 32, 503–509".

Conclusions

PB has many applications in development of biosensors. However, its is important to chose appropriate form of PB for the development of certain biosensor. "Soluble" for of PB exhibits better electrochemical characteristics and higher resistance to pH than "insoluble" form of PB. Therefore, "soluble" PB is typically used in electrochemical PB-based biosensor. However, only "insoluble" form of PB exhibits optical sensitivity to pH.

In sensing of hydrogen peroxide PB exhibits greater sensitivity than its structural analogues (e.g. CoHCF). However, PB-based optical hydrogen peroxide sensors are not resistant to the oxidative impact of air as it can reduce their sensitivity to hydrogen peroxide.