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Towards Polyamine Biosensors: Stabilizing Prussian Blue Electrodes with Polymers for Sensitive H_2O_2 Detection

Neža Sodnik[1,2], Giulia Della Pelle[1,2] and Kristina Žagar Soderžnik[1,2]
[1]Department for Nanostructured Materials, Jožef Stefan Institute, Jamova cesta 39, SI-1000 Ljubljana, Slovenia,
[2]Jožef Stefan International Postgraduate School, Jamova cesta 39, SI-1000 Ljubljana, Slovenia

Corresponding Author's e-mail address: neza.sodnik@ijs.si

Summary:
Polyamines (PAs) are critical biomolecules linked to various diseases, yet their detection remains challenging due to limited electrochemical activity. Enzymatic biosensors enable indirect detection via hydrogen peroxide (H_2O_2), measured using Prussian Blue (PB) electrodes. However, PB's poor stability in neutral media limits performance. This study investigates the use of polymers (chitosan and PDDA) to enhance the stability and sensitivity of PB-modified screen-printed electrodes for H_2O_2 detection, laying the groundwork for future polyamine biosensors.

Keywords: Prussian blue, hydrogen peroxide, polyamines, screen-printed electrodes, enzymatic biosensors.

Background, Motivation and Objective

Polyamines (PAs), such as spermine and spermidine, are essential biomolecules involved in various physiological processes, including cell cycle regulation, gene expression, and membrane stability. Their concentrations in biological fluids like urine and blood are tightly regulated, and deviations from normal levels are associated with various health conditions, including neurodegenerative diseases and multiple types of cancer. Therefore, the ability to monitor PAs in biological samples is critical for the future development of diagnostic and therapeutic tools. [1]

Despite their known biological importance, real-time detection of PAs remains challenging. Conventional chromatography-based methods often lack portability and are cost- and labour-intensive. In response, electrochemical sensors have emerged as a promising alternative, offering rapid detection with minimal sample preparation and without the need for highly trained personnel. These sensors utilise bio- and nanomaterials to sensitively and selectively detect PAs in complex matrices such as urine and saliva. [1, 2]

Because PAs possess limited electrochemical activity, only oxidising at high working potentials around 1 V vs. Ag/AgCl, indirect detection using enzymatic biosensors is a more viable approach. These sensors utilise enzymes (polyamine oxidase) to catalyse PA oxidation, generating hydrogen peroxide (H_2O_2) as a by-product, which is then detected electrochemically. To enhance selectivity and minimize interferences, redox mediators like Prussian blue (PB) are used to detect H_2O_2 at lower potentials (~0 V vs. Ag/AgCl). However, the main limitation of PB lies in its reduced electrochemical stability in neutral media, which is essential for maintaining enzyme activity. [2, 3]

Different approaches have been proposed to stabilize the PB layer. Numerous studies have shown that combining PB NPs with polymers and nanomaterials generally improves the stability of the layer. [3] In this work, we investigate the binding interactions of common polymers with PB and evaluate their effect on the sensor's sensitivity toward H_2O_2. The sensors are based on screen-printed electrodes (SPEs) for enhanced portability and ease of use.

Materials and Methods

PB nanoparticles were synthesized via a polyvinylpyrrolidone-assisted crystallization method, following the procedure reported in the literature. [4]

Suspensions were prepared containing 1 mg/mL PB and 10 mg/mL of the selected polymer. For electrode modification, 4 µL of the PB/polymer suspension was drop-cast onto the carbon working electrode of an SPE (DRP-150, Dropsens, Metrohm). The modified electrodes were dried at 70 °C for 2 hours.

Chronoamperometric measurements were performed using a PalmSens potentiostat at a fixed potential of 0 V vs. Ag/AgCl in phosphate-buffered saline (PBS, pH 7.4).

Results and Discussion

The electrochemical performance of PB-modified electrodes with or without polymer (PDDA or chitosan) integration was evaluated for H_2O_2 detection. As shown in Fig. 1, all three sensors responded to stepwise additions of H_2O_2 with a proportional increase in cathodic current, confirming successful detection. The electrodes modified with chitosan (SPEC-PB/CHIT) and Poly(diallyldimethylammonium chloride) (SPEC-PB/PDDA) showed significantly higher current response than the unmodified PB electrode (SPEC-PB). However, while the PDDA-modified sensor maintained suitable stability, the addition of chitosan led to increased signal noise and reduced stability, indicating the need for further optimization of this formulation.

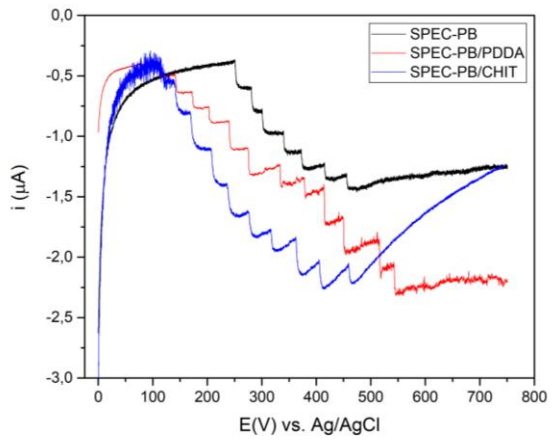

Fig. 1: Chronoamperometric response SPEC-PB electrodes to stepwise additions of hydrogen peroxide (H_2O_2) in PBS (pH 7.4) at 0 V vs. Ag/AgCl. Sensors were modified with either no polymer (black), PDDA (red), or chitosan (blue).

Fig. 2. shows the calibration curves for all three sensors, with the slope reflecting the sensitivity towards H_2O_2. The unmodified PB sensor showed the lowest sensitivity but maintained the widest linear range. In contrast, both polymer-modified sensors demonstrated significantly higher sensitivity in the low to mid-micromolar range, making them more suitable for real-life applications. Overall, these preliminary results suggest that incorporating polymers into the PB layer enhances the sensing performance. This lays the groundwork for further investigations using alternative polymers, such as polydopamine, poly(caffeic acid), and Nafion, to further optimize sensor characteristics, and serve as a support for enzyme immobilization.

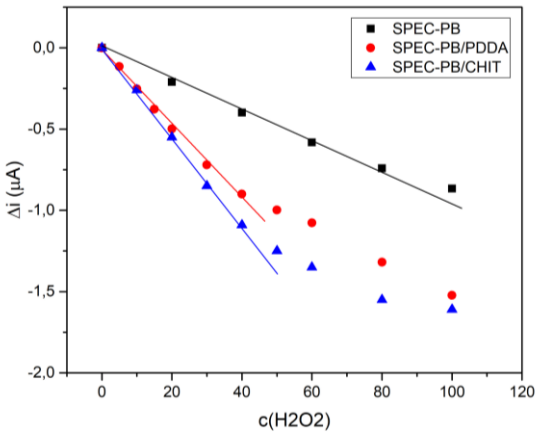

Fig. 2: Calibration curves showing the change in current (Δi) as a function of H_2O_2concentration for SPEC-PB, SPEC-PB/PDDA, and SPEC-PB/CHIT sensors.

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