Use of ferrocene-modified graphene oxide for fabrication of bienzymatic sarcosine biosensor

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Introduction

Enzyme biosensors are beneficial tools in pharmacology, clinical practice, agriculture, food quality control, monitoring of infectious disease pathogens and the spread of environmental pollution factors[1–4].

The biosensor consists of a biological recognition element (enzyme or another biomolecule) and a signal transducer[4]. The signal transducer is responsible for the conversion of recognition signal to physical or chemical processes that can be recorded by specialized detectors.

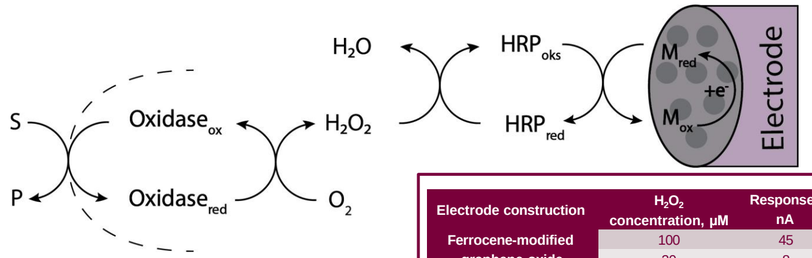
Ferrocene and its derivatives are commonly employed as mediators due to their advantageous qualities for electron transfer, including reversibility, low-potential regeneration, and stable redox states[5]. However, the use of free mediators can pose challenges such as mediator leakage or sample contamination. Therefore, reagentless devices can be developed, where all components are secured on the electrode surface.

Sarcosine is typically present in trace amounts in human serum or urine, elevated levels may signal various diseases including Alzheimer's, dementia, sarcosinemia, and prostate cancer, making it a promising biomarker[6]. Additionally, sarcosine is found in a variety of food sources such as egg yolks, legumes, nuts, vegetables, and meats. Therefore, the accurate quantification of sarcosine holds significant importance in clinical chemistry, as well as in the food and fermentation industries. The development of detection devices for sarcosine is an appealing area of research.[7]

The aim of the study

The objective of this work was to prepare ferrocene-modified graphene oxide and to utilize the prepared material in the development of a biosensor for detecting low concentrations of oxidase substrate, in this case sarcosine.

Results



The constructed bi-enzyme biosensor responded to the sarcosine (substrate of the SOx) and to the hydrogen peroxide (substrate of the HRP) (Fig. 1). This response to the substrates at 0 V of electrode potential results in the increase of the reduction current.

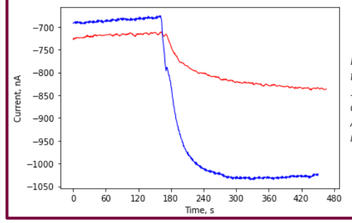


Fig. 1. The response of the biosensor to 100 µM of sarcosine (red curve) or 100 µM of hydrogen peroxide (blue curve) at electrode potential of 0 V vs Ag/AgCl in 50 mM PBS, containing 100 mM KCl, pH 7.0.

The acquirement of a low working potential for the biosensor is a beneficial quality, serving to avoid signal interference from other electroactive compounds present in samples. Employing electrodes containing ferrocene-modified graphene oxide and immobilized HRP and sarcosine oxidase our findings revealed the highest response at negative working potentials, with a slight decline as the working potential increased.(Fig. 2.) Since observed decline was only about 3 nA per 50 mV of potential change and response at -0.15 V was not significantly lower than at 0 V. Working potential of 0 V vs Ag/AgCl was selected for further analysis.

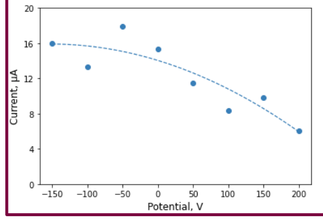


Fig. 2. The dependence of biosensor response to 20 µM of sarcosine on electrode potential. Measurements were carried out in 50 mM PBS, containing 100 mM KCl, pH 7.0.

Electrode construction	H_2O_2 concentration, µM	Response, nA
Ferrocene-modified graphene oxide	100	45
	20	8
Ferrocene-modified graphene oxide + HRP	100	549
	20	123
Ferrocene-modified graphene oxide + HRP + SOx	100	134

Table 1. Responses of differently constructed biosensors to hydrogen peroxide.

Constructing electrodes with solely ferrocene-modified graphene oxide and comparing them with those containing immobilized HRP, we measured their responses to 20 µM and 100 µM concentrations of hydrogen peroxide. Electrodes with HRP immobilization exhibited approximately a 14-fold increase in response current (Table 1).

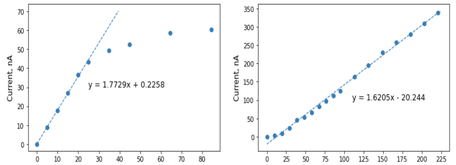


Fig. 3 . Calibration curves of biosensors constructed with different amounts of ferrocene modified graphene oxide (0.1 mg left and 0.45 mg right).. Electrode potential 0 V vs Ag/AgCl, 50 mM PBS, containing 100 mM KCl, pH 7.0. In displayed equations of linear approximation slope value is equivalent to sensitivity of electrode

We constructed electrodes containing two different quantities of ferrocene-modified graphene oxide alongside both HRP and sarcosine oxidase enzymes and calibrated them using sarcosine concentrations up to 225 µM. The tested electrode contained 0.1 mg of ferrocene-modified graphene oxide and demonstrated a sensitivity of 1.8 nA/µM within a linear range extending up to 25 µM. Afterwards, constructing an electrode with an increased quantity of ferrocene-modified graphene oxide (0.45 mg), it maintained a similar sensitivity of 1.62 nA/µM while expanding the linear range to 220 µM (Fig. 3).

Conclusions

The research shows that by employing ferrocene-modified graphene oxide, HRP and oxidase enzyme a bi-enzymatic biosensor for oxidase substrate can be created. Even though solely ferrocene-modified graphene oxide coated GCE responds to hydrogen peroxide, addition of HRP greatly increases response. Created biosensor works at low potential 0 V vs Ag/AgCl and can detect micromolar concentrations of sarcosine. By varying quantity of ferrocene-modified graphene oxide linear range can be changed.

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