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Novel Electrochemical Lactate Biosensors Based on Prussian Blue Nanoparticles

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Introduction

In electrochemical catalysis, a well-known compound is Prussian blue. It is used to detect hydrogen peroxide, amino acids, and DNA with success. Prussian blue nanoparticles are frequently referred to as "artificial peroxidase" due to their superior operational stability and catalytic properties to natural peroxidase enzymes. In oxidase-based biosensors, which are based on the oxidation of the substrate and the subsequent formation of hydrogen peroxide as a product, artificial peroxidase can be utilized. An electric current is then produced when hydrogen peroxide is reduced or oxidized on an electrode. Hydrogen peroxide can be reduced by Prussian blue, lowering the electrochemical reaction's potential and increasing biosensor selectivity. Prussian blue was likewise utilized in the planning of the glucose biosensors reasonable for harmless continuous glucose location in human perspiration [1].

Lactate is a significant halfway of numerous biochemical cycles. Because cancer, multiple sclerosis and brain injuries can all lead to the accumulation of lactate in the human body, the detection of lactate in biological fluids is useful for both biological and medical research. The oxidation of lactate to pyruvate by lactate oxidase results in the formation of hydrogen peroxide, which can be detected electrochemically. Polymers with ionic-exchanging properties are commonly used to immobilize the enzyme on an electrode's surface. One of the monomers that is used for the immobilization of enzymes is 3-(aminopropyl) triethoxysilane. This compound forms a stable membrane of 3-aminopropyltriethoxysiloxane when it is polycondensed. Using lactate dehydrogenase in place of lactate oxidase, immobilizing the enzyme with a different polymer, and employing a variety of transducers are some examples of alternative designs that can serve as the foundation for other electrochemical biosensors [2,3].

Description

Helicon (Russia) supplied the K_2HPO_4 and KH_2PO_4 that were needed. Chimmed (Moscow, Russia) supplied the KCl and $NiCl_26H_2O$. K_3 [Fe(CN)6] and $FeCl_36H2O$ were purchased from Sigma Aldrich (Burlington, MA, USA). Reachim (Moscow, Russia) provided the 30% H_2O_2 solution. Isopropyl liquor was acquired from Plastopolymer (St. Petersburg, Russia). Sorachim (Lausanne, Switzerland) supplied the lactate oxidase enzyme (EC 1.1.3.2) from Pediococcus species in lyophilized powder with 72 IU of activity. Sigma Aldrich (Burlington, MA, USA) supplied the water-based solution of 40% sodium L-lactic acid. The three-electrode planar screen-printed biosensors had a silver reference electrode, a carbon working electrode with a diameter of 1.8 mm, and a carbon auxiliary electrode. Rusens, located in Moscow, Russia, supplied these electrodes that were screen-printed [4,5].

The potentiostat Palmsens3 (Palm Instruments BV, Houten, The Netherlands) was used for electrochemical measurements. Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK) was used for dynamic light scattering particle size distribution analysis. Lambda 950 (Perkin-Elmer, Waltham, MA, USA) was used for spectroscopic measurements. The response blend comprised of 75 mM $K_3[Fe(CN)_6]$ and 75 mM $FeCl_3$ in an answer of 100 mM KCl and 100 mM HCl. A 50 mM H_2O_2 solution was added to initiate sedimentation. Ultrasound was used to mix and distribute the mixture, and centrifugation and sediment separation followed. The dynamic light scattering method was used to measure the nanoparticle sizes (refraction index of 1.56 and absorption coefficient of 0.9). The nanoparticles were kept in a 100 mM KCl and 100 mM HCl solution. The grouping of the nanoparticles in a suspension was estimated utilizing spectrophotometry, with ε700 nm (per PB unit cell)=4.85 × 104 M–10cm^{-1} [6].

Conclusion

Prussian Blue nanoparticles were made by ultrasonically reducing the mixture of $K_3[Fe(CN)_6]$ and $FeCl_3$ with hydrogen peroxide. The size conveyance of the nanoparticles was estimated with dynamic light dispersing. According to, nanoparticles with an average diameter of 35 nm were utilized for subsequent steps because this size falls within the range in which the electrocatalyst is distributed across the working electrode's entire surface. After the nanoparticles were suspended and dropped onto the electrode's surface, a cyclic voltammogram was recorded to activate the coating. A cyclic voltammogram of the cathode changed with nanoparticles displays a couple of pinnacles that relate to the progress from Prussian blue to its diminished structure, Prussian White, as well as the other way around. According to, it is necessary for the intercalation of K^+ cations in the Prussian blue layer to activate an electroactive coating. Additionally, the voltammogram can be utilized to calculate the concentration of Prussian blue on the biosensor's surface and, consequently, to regulate the amount of Prussian blue deposited on the working electrode. As indicated by the voltammogram, the typical measure of kept Prussian blue was 14 nmolcm$^{-2.}$

It is possible to compare the analytical characteristics of biosensors and the catalytical properties of an immobilized enzyme by using the apparent Michaelis constant, which is an essential parameter in biosensors. Its value is determined by the transducer's effects, the enzyme's membrane accessibility, and the enzyme's Michaelis constant. As a result, developing biosensors of a particular design can benefit from the calculation of the apparent Michaelis constant. Various groupings of lactate were added to the cell with the lactate biosensor in a cluster mode. One of the chronoamperograms that was recorded during this procedure. Plotting Michaelis–Menten curves with the obtained data was used to calculate the apparent Michaelis constant. The curve that corresponds to this one as can be seen, the linear range for lactate detection is 5 × 10^{-6}–1 × 104 M; sensitivity is 134 ± 12 mA cm^{-2} M^{-1} in this range.

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Conflict of Interest

There are no conflicts of interest by author.

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