ELECTROCHEMICAL BIOSENSOR FOR ERYTHROPOIETIN DETECTION IN ATHLETES

BIOSSENSOR ELETROQUÍMICO PARA DETECÇÃO DE ERITROPOIETINA EM ATLETAS

BIOSENSOR ELECTROQUÍMICO PARA DETECCIÓN DE ERITROPOYETINA EN DEPORTISTAS

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ABSTRACT

Introduction: The cytokine erythropoietin (EPO) is a crucial hormone for producing RBCs, which carry oxygenated blood to the rest of the body. Objective: This paper aimed to create an electrochemical detection based on Fe2O3-NiO nanoparticles and graphene oxide to measure EPO levels in athletes' blood. Methods: On a glassy carbon electrode, Fe2O3-NiO@GO was synthesized using the electrochemical deposition method. Results: The Fe2O3-NiO@GO/GCE was validated by structural characterizations using scanning electron microscopy (SEM). The Fe2O3-NiO@GO/GCE was found to be a suitable and stable erythropoietin biosensor with a linear range of 0-500 ng/l and a detection limit of 0.03ng/l in electrochemical tests using the DPV technique. Fe2O3-NiO@GO/erythropoietin was investigated as a biosensor for erythropoietin in athlete's plasma. Conclusion: The results showed that the values obtained for recovery (94.56% to 98.40) and RSD (2.01% to 3.22%) were acceptable, indicating that the suggested technique can be used as a practical erythropoietin biosensor in blood samples. ***Level of evidence II; Therapeutic studies - investigation of treatment outcomes.***

Keywords: Biosensing Techniques; Erythropoietin; Athletes; Sports.

RESUMO

Introdução: A citocina eritropoietina (EPO), é referida como um hormônio crucial para a produção de hemácias, que transportam o sangue oxigenado para o resto dos corpos. Objetivos: O objetivo deste trabalho foi criar uma detecção eletroquímica baseada em nanopartículas de Fe2O3-NiO e óxido de grafeno para medir os níveis de EPO no sangue dos atletas. Métodos: Em um eletrodo de carbono vítreo, o Fe2O3-NiO@GO foi sintetizado usando o método de deposição eletroquímica. Resultados: O Fe2O3-NiO@GO/GCE foi validado por caracterizações estruturais utilizando microscopia eletrônica de varredura (SEM). O Fe2O3-NiO@GO/GCE foi considerado um biosensor eritropoietina adequado e estável com uma faixa linear de 0-500 ng/l e um limite de detecção de 0,03ng/l em testes eletroquímicos utilizando a técnica DPV. O Fe2O3-NiO@GO/extropoietina foi investigado como um biosensor de eritropoietina no plasma do atleta. Conclusão: Os resultados mostraram que os valores obtidos para recuperação (94,56% a 98,40) e RSD (2,01% a 3,22%) foram aceitáveis, indicando que a técnica sugerida pode ser usada como um prático biosensor de eritropoietina em amostras de sangue. ***Nível de evidência II; Estudos terapêuticos - investigação dos resultados do tratamento.***

***Descritores*:** *Técnicas Biossensoriais; Eritropoetina; Atletas; Esportes.*

RESUMEN

Introducción: La citoquina eritropoyetina (EPO), se conoce como una hormona crucial para la producción de glóbulos rojos, que transportan la sangre oxigenada al resto del cuerpo. Objetivos: El objetivo de este trabajo fue crear una detección electroquímica basada en nanopartículas de Fe2O3-NiO y óxido de grafeno para medir los niveles de EPO en la sangre de los deportistas. Métodos: Sobre un electrodo de carbono vítreo, se sintetizó Fe2O3-NiO@GO mediante el método de deposición electroquímica. Resultados: El Fe2O3-NiO@GO/GCE fue validado por caracterizaciones estructurales mediante microscopía electrónica de barrido (SEM). El Fe2O3-NiO@GO/GCE resultó ser un biosensor de eritropoyetina adecuado y estable con un rango lineal de 0-500 ng/l y un límite de detección de 0,03ng/l en ensayos electroquímicos mediante la técnica DPV. Se investigó el uso de Fe2O3-NiO@GO/extropoietina como biosensor de eritropoyetina en el plasma de atletas. Conclusión: Los resultados mostraron que los valores obtenidos para la recuperación (94,56% a 98,40) y la RSD (2,01% a 3,22%) fueron aceptables, lo que indica que la técnica sugerida puede ser utilizada como un biosensor práctico de eritropoyetina en muestras de sangre. ***Nivel de evidencia II; Estudios terapéuticos - investigación de los resultados del tratamiento.***

***Descriptores*:** *Técnicas Biosensibles; Eritropoyetina; Atletas; Deportes.*

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INTRODUCTION

The hormone and glycoprotein cytokine erythropoietin (EPO), sometimes referred to as haematopoietin and haemopoietin, is crucial for the production of red blood cells, which transport oxygenated blood to rest of bodies.[1,2] When the cells' oxygen concentrations are low and also in response to applied hypoxia, the kidneys and lungs create EPOs. The hormone then increases the production of red blood cells in the bone marrow.[3] Hypoxemia produced by chronic lung disease that produces elevated amounts of EPO and constant exposure to low levels of oxygen both result in excess EPO.[4] It results in polycythaemia, which is a disorder marked by a higher red cell count. EPO production can also be caused by high altitude or right-to-left heart shunts. There are, however, certain symptoms that are broad and non-specific, such as weakness, exhaustion, headache, itching, joint discomfort, and disorientation.

Exogenous erythropoietin and recombinant human erythropoietin are biopharmaceuticals made in cell philosophy by recombinant DNA technology and are referred to as erythropoiesis-stimulating agents. These medications are utilized to treat anemia caused by chronic kidney disease, anemia from myelodysplasia, and anemia from chemotherapy for cancer[1]. Because raising hemoglobin concentrations above 11 g/dL to 12 g/dL can result in death, infarction, strokes, deep vein thrombosis, and tumor recurrence, hemoglobin level control is crucial in the EPO therapy.[5] The red blood cells provide oxygen to cells, especially muscle cells, permitting them to function more effectively, sportsmen frequently utilize erythropoietin. The substance is prohibited from usage in sports meanwhile it is supposed to advance an athlete's act and providing users an unfair advantage over unaffected rivals. EPO also causes a person's blood to become thicker, which may raise the risk of blood clots.[6]

As a result, numerous studies using techniques like high-performance liquid chromatography,[7] immunochromatography,[8] affinity probe separation technique,[9] fluorescence, Raman,[10] radioimmunoassay,[11] and electrochemical methods[12-14] have been devoted to the production, identification, and detection of the level of rhEPO into human blood. Due to their affordability, sensitivity, and selectivity, electrochemical approaches have garnered the most interest among these methods of determination.[15-17] Studies have also shown that it is possible to modify the electrodes in these approaches by employing hybrid and composite, which can increase repeatability, constancy, and sensitivity.[12-14] In order to easily determine the presence of erythropoietin in human blood plasma, this study was conducted on the production of a sensor based on Fe_2O_3-NiO@GO/GCE.

MATERIALS AND METHODS

The GCE was modified with a magnetic GO nanocomposite using the electrochemical deposition approach.[18,19] 50 mg of GO nanosheets were distributed in 50 ml of 0.1M phosphate-buffered solutions (PBS) to prepare the electrochemical electrolyte. PBS is made from a handle all aspects of Na_2HPO_4 and NaH_2PO_4. During the 50 minute sonication phase, the scattered GO was exfoliated. GO in scattered suspension received 2 mM $FeCl_3$, 2mM $NiCl_2.6H_2O$, and 0.1 M H_2O_2 additions. Prior to modification, the GCE was periodically washed in alumina powder and subsequently sonicated in a solution of ethanol and DI water for 20 minutes. The electrochemical preparation and observations were performed by a potentiostat/galvanostat into a compartmentalized, three-electrode electrochemical system with just an Ag/AgCl/3M KCl electrode material, Pt mesh as the counter electrode, and a pure GCE as that of the working electrode. The arranged solution was gently stirred throughout electro deposition. Nanocomposites of Fe_2O_3 and NiO were potentiodynamically electrodeposited using the CV method at 10mV/s scanning rate.

Differential pulse voltammetry (DPV) tests were carried for the electrochemical extraction of the reduction protein from the adapted electrode surface into 0.1M PBS using an AUTOLAB electrochemical mechanism inside a three-electrode electrochemical cell at a scan rate of 10 mV/s. Equal volumes of 0.2 M NaH_2PO_4 and 0.2 M Na_2HPO_4 were combined to create the 0.1 M PBS. The erythropoietin concentration range of enzyme-linked immunosorbent analysis kit (ELISA) calibration graph was in ranges of 10-335 ng/l when it was used to analyze erythropoietin in blood serum. Using a scanning electron microscope, the morphology of the nanostructures was examined.

Erythropoietin was selectively extracted from human blood plasma using the Ab-conjugated magnetic beads. After that, 0.2 ml of Ab-conjugated magnetic beads were added while magnetic swirling to the athlete's blood plasma. The sample was then kept at 25°C for 10 minutes. Finally, the sample and magnetic beads are magnetically separated. 0.2M glycine and 0.2M KOH were combined in an equal volume for 10 minutes at 25°C in order to eliminate the absorbing erythropoietin as from conjugated magnetic beads. The glycine buffer was then removed from the produced erythropoietin solution using a gravity flow size exclusion technique.

The authors state that the research was conducted in accordance with the principles embodied in the Declaration of Helsinki. The authors state that the participants participated in this study voluntarily and were informed consent to participate in the study. The authors confirming that consent was given for publication by all participants.

RESULTS AND DISCUSSIONS

The morphological properties of GO/GCE and Fe_2O_3-NiO@GO/GCE are depicted in Figure 1. Figure 1a's SEM picture of GO/GCE reveals a stack of impossibly thin GO nanosheet with such a pore structure just on GCE surfaces that is heavily rippled, crumpled, and wrinkled. According to Figure 1b, Fe-Ni nanoparticles with spherical shapes are uniformly decorated on two dimensional nanostructure of GO without agglomeration, showing a high pores and wide electro active surfaces for analytical ions diffusion and capability to speed up the redox reaction.[20]

The amperometry technique was the subject of additional electrochemical experiments. Into 0.1M PBS, at 0.25V, 0.19V, 0.24V, 0.19V, and 0.19V potentials, respectively, Figure 2 shows the amperometric observations of GCE,Fe_2O_3/GCE,GO/GCE,Fe_2O_3@GO/GCE, and Fe_2O_3-NiO@GO/GCE. As can be seen, observed amperometric currents of any and all electrodes rose after the injection of the 50 µM DA solution in 100s.Additionally, the amperometric reactions of the GCE, Fe_2O_3/GCE,GO/GCE, Fe_2O_3@GO/GCE, and Fe_2O_3-NiO@GO/GCE after 350 seconds of adding the 50µM DA solution into electrochemical cell indicate 25 percent, 16 percent, 18 percent, 13 percent, and 6 percent reduce in electrocatalytic current, proving to stable reaction of Fe_2O_3-NiO@GO/GCE to determine DA since flexible properties, excellent adhesion, good electronic and mechanical characteristics of GO which may improve the electrocatalytic efficiency of Fe_2O_3-NiO-based electrode. The many functional groups on its surface

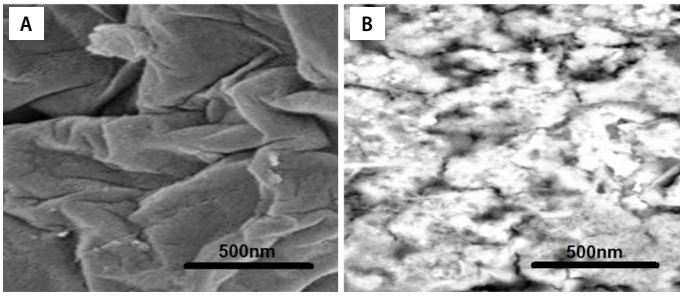

Figure 1. FESEM images of (A)GO/GCE (B)Fe2O3-NiO@GO/GCE.

of GO nanosheets make them advantageous for modifying the shape of electrocatalysts and for interacting more powerfully with magnetic particles.[21] Additionally, active surfaces catalytic units can be stabilized and enabled to have synergistic effects at interface between Fe-Ni nanostructures and GO nanosheets.[22] Therefore, utilizing Fe_2O_3-NiO@GO/GCE, the following amperometric investigations were carried out to investigate the sensitivities, limit of detection, linear-range, selectivity, and precision of DA sensors.

The durability effect of the Fe2O3-NiO@GO/GCE response to the 20 ng/l erythropoietin into 0.1M PBS at 10 mV/s scan rate is shown in Figure 3. Because Ni NPs were electrodeposited on the wall surfaces and tips of the empty nanotubes, trying to form mesoporous metal-carbon interference, it can be seen that the difference between first and 40th DPV respondents has shown an 6 percent decrease, which is connected with sufficient certainty of response of the suggested electrode for erythropoietin detection. This mesoporous metal-carbon interfering and more stable biomolecule stabilization inside the pore structure of electrode material can be facilitated by this tip-growth model.[23] During the 25-minute electrochemical reduction of erythropoietin, the trapped active molecules in the pores bundle can stop dynamic material loss and stop the shuttle process.[24]

Figure 4 shows the calibration plot of the electrode and the DPV responses to the addition of various protein concentrations in 0.1M PBS at a 10 mV/s scan rate. As can be shown, the electrochemical adsorption current increases linearly as protein content rises between 0 and 500 ng/l. The calibration plot displays the departure from of the linear relation for the increasing protein content. The detection threshold was 0.02ng/l. Additionally, the study's sensing results are contrasted with those of

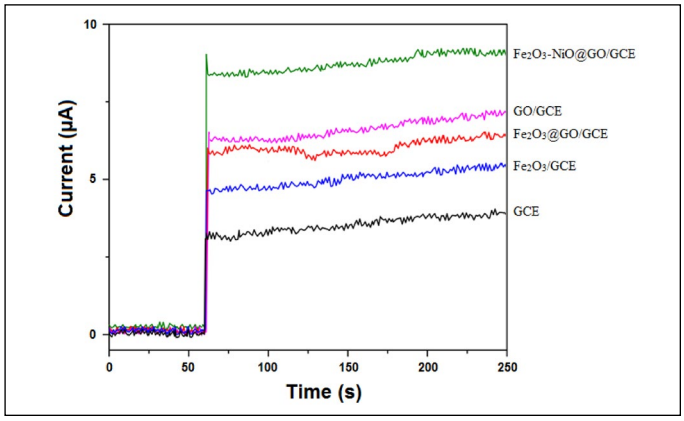

Figure 2. Amperometric of GCE, GO/GCE,Fe2O3/GCE,Fe2O3@GO/GCE and Fe2O3--NiO@GO/GCE with adding 50μM DA into 0.1M PBS.

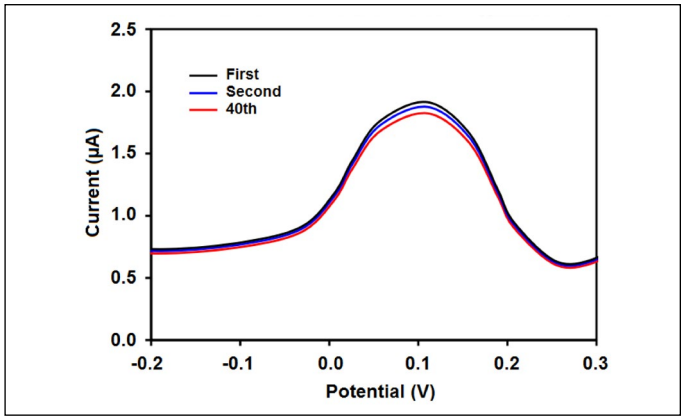

Figure 3. Stability effects of the Fe2O3-NiO@GO/GCE reaction to the 20ng/l erythropoietin into 0.1M PBS at 10 mV/s scan rate, with the first, second, and 40th DPV responses.

previously published erythropoietin sensors. It can be seen that the sensor through Fe_2O_3-NiO@GO/GCE displays is superior to or on par with that of other erythropoietin sensors that have been reported. This is due to the Fe_2O_3-NiO@GO frameworks' good catalytic performance, which includes their ability to provide biomolecules with a advanced specific surface region, anchoring places, and charge-transfer channels.[25]

Erythropoietin was isolated using polymer-functionalized magnetic beads and reduced on a Fe_2O_3-NiO@GO/GCE utilizing chronoamperometry in order to investigate the application of the Fe_2O_3-NiO@GO/GCE for erythropoietin sensing in athlete's plasma proteins as real sample. Electrochemical sensing relies on the efficient isolation of erythropoietin in human serum, one of the most intricate biological matrices. EDAC, acting as a mediator, can react with an antibody's carboxyl group to create an intermediate that is amine-reactive and can covalently conjugate the amide bond of magnetic beads.[26] The main amines (HN2 groups) in functionalized magnetic beads are simultaneously activated by the adding of EDAC/NHS carbodiimide binding reagent to polyvinyl pyrrolidone-functionalized magnetic nanoparticles.[27] In order to extract erythropoietin of human plasma, these activated groups may form a covalent link of amide with antibody onto surface of the beads.[28,29] The results of the quantification through DPV and ELISA performances in the real sample preparations are shown in Table 1. These results implied that the average level of erythropoietin inside this plasma sample was estimated to be 8.06 ng/l and 8.48 ng/l through DPV and ELISA methods, respectively, indicating excellent correlation and precision between the two methods. Additionally, Table 1 displays the outcomes of analytical experiments using the DPV methodology and the usual addition method. Relative standard deviation (RSD) values of 2.01 % to 3.22 % and recovery values of 94.56 % to 98.40 % are found to be acceptable, allowing the suggested technology to be used as a practicable erythropoietin biosensor in blood serum samples.

CONCLUSIONS

This research involved the easy detection of erythropoietin in blood plasma using an electrochemical sensor based on Fe_2O_3-NiO@

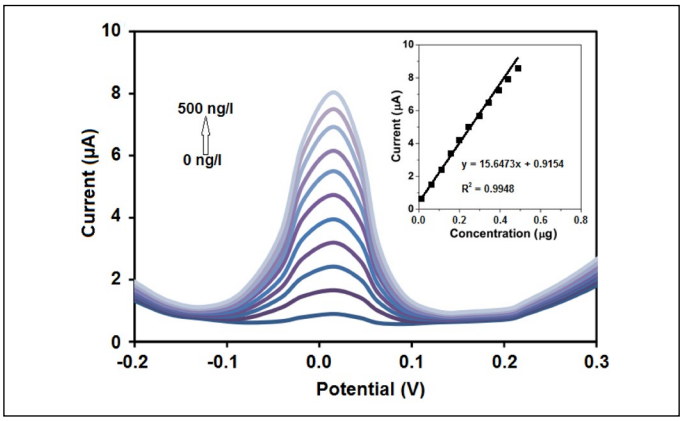

Figure 4. DPV and calibration curves of electrodes to add different level of protein into 0.1M PBS at 10 mV/s scan rate.

Table 1. Results of detection of erythropoietin into real plasma specimen by ELISA and DPV methods.

ELISA		DPV				
Content (ng/l)	RSD (%)	Content (ng/l)	Added (ng/l)	Measured (ng/l)	Recovery (%)	RSD (%)
9.52	2.18	9.12	10.0	9.2	95.00	2.01
			20.0	19.8	98.40	2.57
			30.0	27.4	94.56	3.13
			40.0	38.5	97.02	3.22

GO/GCE. The Fe_2O_3-NiO@GO onto GCE surface was modified using the electrodeposition method, and erythropoietin was reduced on the Fe_2O_3-NiO@GO/GCE surface using chronoamperometry. Fe_2O_3 and NiO nanoparticles were highly heterogeneous deposited onto surface of GO/GCE, according to the findings of structural studies. According to electrochemical experiments, the Fe_2O_3-NiO@GO/GCE erythropoietin biosensor had a linear range of 50–350 ng/l and 0.03 ng/l limit of detection. It was also stable and responsive. Results showed that the values obtained for recovering and RSD were appropriate, and the proposed approach can be used as a practical erythropoietin biosensing for blood serum samples. The suitability of a Fe_2O_3-NiO@GO/GCE as erythropoietin biosensing in athlete's blood serum as a real specimen was examined.

All authors declare no potential conflict of interest related to this article

AUTHORS' CONTRIBUTIONS: The work is conceived and its knowledge content of the manuscript is completed by Long Jin. The methodology and execution is done by Juan Wang. Investigation, formal analysis and drafting is done by Lei Wu. All the authors equally contributed in execution and writing of this manuscript.

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