BIOSENSOR DEVELOPMENT IN SPORTS DOPING WITH DEXAMETHASONE

DESENVOLVIMENTO DE BIOSSENSOR NO DOPING ESPORTIVO COM DEXAMETASONA

DESARROLLO DE UN BIOSENSOR EN EL DOPING DEPORTIVO CON DEXAMETASONA

ORIGINAL ARTICLE
ARTIGO ORIGINAL
ARTÍCULO ORIGINAL

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ABSTRACT

Introduction: Dexamethasone is a type of drug that is considered a steroid. It belongs to a class of drugs known as corticosteroids. Objective: Develop an electrochemical sensor of dexamethasone in a pharmaceutical sample using electrodes modified with nanostructures of MnO2 and reduced graphene oxide (MnO2/rGO). The glassy carbon electrodes (GCE) used to make the GO nanostructures were first modified using a modified Hummers technique before electrochemically reduced. Methods: MnO2 nanomaterials were electrochemically deposited on rGO/GCE. SEM structural investigation indicated vertical tetragonal crystal development of -MnO2 nanostructures in sprayed rGO nanostructures. Results: Because of the high composite surface area, multiple exposed active sites, and the synergistic effect of MnO2 and rGO, the electrocatalytic reaction to dexamethasone of MnO2/rGO/CPE was shown to be broad, selective, stable, and sensitive in electrochemical tests using amperometry. It was established that the linear range, sensitivities, and detection limit of the sensor are 0 to 260 μM, 4.6153μA/μM and 0.005 μM, respectively. The MnO2/rGO/CPE was tested for accuracy and applicability in determining dexamethasone in pharmacological and human urine samples. Conclusion: The results revealed that the sensor could prepare acceptable recovery (96.34%) and RSD (3.58%), suggesting that it could be used as a reliable dexamethasone sensor in clinical samples. ***Level of evidence II; Therapeutic studies - Investigation of treatment outcomes.***

Keywords: Biosensing Techniques; Doping in Sports; Dexamethasone; Sports.

RESUMO

Introdução: A dexametasona é um tipo de medicamento considerado um esteróide. Pertence a uma classe de medicamentos conhecida como corticosteróides. Objetivo: Este estudo teve como objetivo desenvolver um sensor eletroquímico de dexametasona em uma amostra farmacêutica utilizando eletrodos modificados com nanoestruturas de MnO2 e óxido grafeno reduzido (MnO2/rGO). Os eletrodos de carbono vítreo (GCE), que foram utilizados para fazer as nanoestruturas GO, foram primeiramente alterados através de uma técnica Hummers modificada antes de serem reduzidos eletroquimicamente. Métodos: Os nanomateriais de MnO2 foram depositados eletroquimicamente no rGO/GCE. A investigação estrutural do SEM indicou o desenvolvimento vertical do cristal tetragonal de -MnO2 nanoestruturas em nanoestruturas de rGO pulverizadas. Resultados: Em virtude da alta área de superfície composta, dos múltiplos locais ativos expostos e do efeito sinérgico de MnO2 e rGO, a reação eletrocatalítica à dexametasona de MnO2/rGO/CPE mostrou ser ampla, seletiva, estável e sensível nos testes eletroquímicos utilizando a amperometria. Foi estabelecido que o alcance linear, sensibilidades e limite de detecção do sensor são de 0 a 260 μM, 4,6153μA/μM e 0,005 μM, respectivamente. O MnO2/rGO/CPE foi testado para precisão e aplicabilidade na determinação de dexametasona em amostras farmacológicas e de urina humana. Conclusão: Os resultados revelaram que o sensor é capaz de preparar uma recuperação aceitável (96,34%) e RSD (3,58%), sugerindo que ele poderia ser usado como um sensor de dexametasona confiável em amostras clínicas. ***Nível de evidência II; Estudos terapêuticos - Investigação dos resultados do tratamento.***

Descritores: *Técnicas Biossensoriais; Doping nos Esportes; Dexametasona; Esportes.*

RESUMEN

Introducción: La dexametasona es un tipo de fármaco considerado como un esteroide. Pertenece a una clase de medicamentos conocidos como corticosteroides. Objetivo: Este estudio tiene como objetivo desarrollar un sensor electroquímico de dexametasona en una muestra farmacéutica utilizando electrodos modificados con nanoestructuras de MnO2 y óxido de grafeno reducido (MnO2/rGO). Los electrodos de carbono vítreo (GCE), que se utilizaron para fabricar las nanoestructuras de GO, se modificaron primero mediante una técnica de Hummers modificada antes de ser reducidos electroquímicamente. Métodos: Los nanomateriales de MnO2 se depositaron electroquímicamente sobre rGO/GCE. La investigación estructural por SEM indicó el desarrollo vertical del cristal tetragonal de las nanoestructuras de -MnO2 en las nanoestructuras de rGO pulverizadas. Resultados: En virtud de la elevada área superficial del compuesto, los múltiples sitios activos expuestos y el efecto sinérgico del MnO2 y el rGO, la reacción electrocatalítica a la dexametasona del MnO2/rGO/CPE demostró ser amplia, selectiva, estable y sensible en pruebas electroquímicas mediante amperometría. Se estableció que el rango lineal, las sensibilidades y el límite de detección del sensor son de 0 a 260 μM, 4,6153μA/μM y 0,005 μM, respectivamente. Se probó la precisión y aplicabilidad del MnO2/rGO/CPE

en la determinación de dexametasona en muestras farmacológicas y de orina humana. Conclusión: Los resultados revelaron que el sensor es capaz de preparar una recuperación aceptable (96,34%) y una RSD (3,58%), lo que sugiere que podría utilizarse como un sensor fiable de dexametasona en muestras clínicas. ***Nivel de evidencia II; Estudios terapéuticos - Investigación de los resultados del tratamiento.***

Descriptores: *Técnicas Biosensibles; Doping en los Deportes; Dexametasona; Deportes.*

DOI: http://dx.doi.org/10.1590/1517-8692202329012022_0416 Article received on 07/02/2022 accepted on 10/05/2022

INTRODUCTION

Doping refers to use of illicit substances in team sports and is a significant issue with all athletes, coaching staff, managers, sports federations, and fans of all sports because it detracts from the attempts of clean athletes, prevents others from competing fairly, and distorts the true competitive spirit[1]. It is also known as performance-enhancing medications and is used to enhance sports ability.[2] It can have negative and long-lasting side properties, including cancer, infertility, sudden death, high blood pressure, nosebleeds, heart attacks, psychosis, sinusitis, and tremors[3].

A number of performance-enhancing substances, including street drugs, stimulant, anabolic steroids, peptidases, alcohol, beta blocker, diuretic, gene manipulation, β2 agonists, anabolic steroids, and anti-estrogens, have been outlawed by the World Anti-Doping Agency (WADA).[4] As a result, testing on and off the field of play occurs accidentally on athletes who compete in sports. Depending on the drugs being tested, test can be performed on urine and blood samples.[5]

Dexamethasone (DXM), the glucocorticoid used to treat inflammation in various body areas, is included as a performance-enhancing steroid on WADA-2019 list of prohibited substances.[6] The potency of DXM, a long-acting systemic corticosteroid, is approximately 25 times greater than that of short-acting products. It might improve athletic performance to support athletes competing at high elevations.[7] As a result, numerous studies have been carried out employing liquid chromatography, chemometry, chemical ionization-mass spectrometry, capillary electrophoresis, and gas chromatography to determine DXM.[8-12] Among these techniques, electrochemical procedures are quick and inexpensive and may be able to enhance sensor qualities.[13-15] Rapid movement of samples via nanomaterials based sensors has been shown to improve the absorption, catalytic, sensing response, and interaction.[16] As a result, this study was conducted to create MnO_2/rGO/CPE for the purpose of identifying DXM as a doping element in sport.

METHODS

Experimental details

The modified Hummers method was used to manufacture GO.[17] 200 mL of sulfuric acid and 5g of graphite powder were combined in an ice bucket for 20 minutes, then 20g of $KMnO_4$ and 200 mL of distilled water were added, and the mixture was stirred magnetically for 50 minutes at 30°C. The finished product has a three-day setting. Following that, the suspension received 20mL H_2O_2 and 400mL of distilled water. The resulting mixture was filtered and cleaned with 1M HCl solution and DI water, respectively, after undergoing a 50-minute ultrasonication treatment. The resulting suspension was then dried in an oven for 20 hours at 70°C after being centrifuged for 20 minutes at 2000 rpm.

For the purpose of making rGO/GCE, 10 mg of the acquired GO was ultrasonic assisted exfoliated for 50 minutes in 20 mL of distilled water. On GO nanosheets, the major functional groups that contain oxygen are dispersed.[18] The GCE surface was coated with 5 L of the GO suspension. The produced sample, Ag/AgCl electrode, and Pt plate electrode were used as the working electrode, reference electrode, and auxiliary electrode, respectively, during the electrooxidation of the GO nanostructure on GCE in a potentiostat. Three potential cycles between -0.80 V and 0.70 V were performed on the GO/GCE with a scan rate of 10mV/s in a 0.2 phosphate buffer solutions (PBS).

Through an electrodepositon technique, MnO_2 nanostructures were added towards rGO/GCE and GCE surface.[19] On an Autolab with a potentiostat, electrodeposition was carried out for 10 min at room temperature. The 0.2M $KMnO_4$ and 3mM $NaNO_2$ aqueous solution mixture were made into the electrodeposition electrolyte in an equal volume ratio.

On an AUTOLAB Potentiostat in a three-electrode electrochemical cell with Ag/AgCl, Pt-wire, and improved CPE as that of reference-, counter-, and working-electrodes, respectively, amperometry and cyclic voltammetry (CV) studies were performed. In electrochemical experiments, 0.2M PBS, which was made from 0.2M NaH_2PO_4 and 0.2M Na_2HPO_4, was employed as the electrolyte. With the use of scanning electron microscopy, the morphology of changed electrodes was investigated (SEM).

This study was conducted in accordance with the Declaration of Helsinki principle. The participants signed the Free and Informed Consent Form (EHIC) for this work.

RESULTS AND DISCUSSION

In 0.1 M PBS pH 7.0 with 1 mM [Fe(CN)6]3/4- as the reactive site in equal volume fraction at 10mV/s scan rate, Figure 1 shows the CV plots of GCE, rGO/GCE, MnO2/GCE, and MnO2/rGO/GCE. As can be seen, CV curves display two redox peaks having peak potential separations of 0.06, 0.09, 0.08, and 0.06 V in GCE, rGO/GCE, MnO2/GCE, and MnO_2/rGO/GCE, respectively (ΔEp=|(Ep,c -Ep,a)|). As is evident from the weak peaks and wide Ep, the GCE surface has been modified with rGO and MnO2 nanostructures, enhancing peak currents and lowering Ep values. As a result of its high conductivity and high electron mobility, it is also noted that rGO plays a role in enhancing the electrochemical current.[20] When compared to MnO2 nanostructures, the rGO exhibits a higher electron--transfer rates and a larger effective surface area because of its numerous surface functional groups and residual sp3-bonded carbon to oxygen.[21] For MnO2/rGO/GCE, we see a greater peak current and a lower Ep, which is consistent with the synergistic impact of nanocomposite and high-porous, sharp MnO2 nanostructure tips.

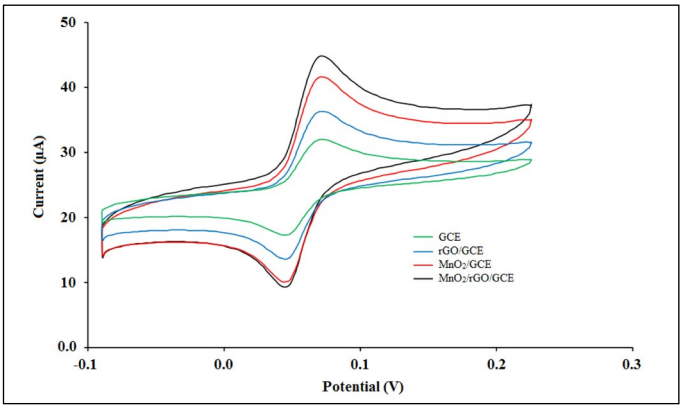

Figure 1. CV plots of GCE,rGO/GCE,MnO2/GCEand MnO2/rGO/GCE into 0.2M PBS having 1mM [Fe(CN)6]3−/4 in equivalent volume ratio.

For the purpose of determining the sensing characteristics of MnO_2/rGO/CPE as a DXM sensor, additional electrochemical tests using the amperometry technique were carried out. Figure 2 shows the proposed sensor's amperometry response and the calibration curve that was produced in 0.2M PBS at 2000 rpm after the addition of 20μM DXM. The sensor's quick response may be seen with each adding 20μM DXM solution. As can be seen, the amperometric currents increase linearly from 0 to 260μM with adding DXM solution. Estimates for the sensor's sensitivity and detection limit are 4.6153μA/μM and 0.005 μM, respectively. The described DXM sensors are used to compare the MnO_2/rGO/CPE sensing qualities that were acquired. The distribution of oxygen-containing ligands on CNTs, which creates the defect, and the nanomaterials substrate for grounding the metallic nanoparticels, which provide a large number of absorbing sites on MnO_2/rGO/CPE, are both associated with the wide linear range for DXM determination that is observed in MnO_2/rGO/CPE.

The precision and usefulness of MnO_2/rGO/CPE to DXM measurement in pharmaceutical and human bodily fluid samples were studied. Amperometry tests were carried out utilizing MnO_2/rGO/CPE in prepared actual sample with 0.2M PBS in sequential adding DXM solution in order to analyze the prepared genuine medicinal specimen of DXM tablet. The calibration plot shown in Figure 3 suggests that the produced sample's DXM content is 4.96mg/ml, which is extremely near to the real sample of tablets' prepared DXM concentration of 5 mg/ml. Additionally, it can be seen from Table 1 that the obtained recovery values for manufactured real specimens of DXM tablet are satisfactory at 95.33% and 3.61%, respectively. The analytical applicability of the sensor was investigated in preparation of human serum free of DXM, and the findings indicate that DXM was not found in the actual serum sample. The analytical data are likewise shown in Table 1, with satisfactory recovery (96.34%) and RSD (3.58%) values. MnO_2/rGO/CPE can therefore be employed as trustworthy DXM sensors in clinical samples.

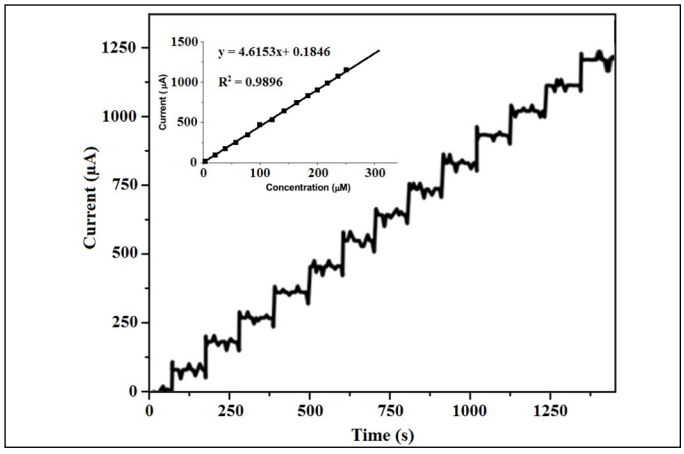

Figure 2. Amperometry of MnO2/rGO/CPE into 0.2M PBS and 2000 rpm rotating speed toward adding of 20μM DXM. Inset figure shows the calibration curve.

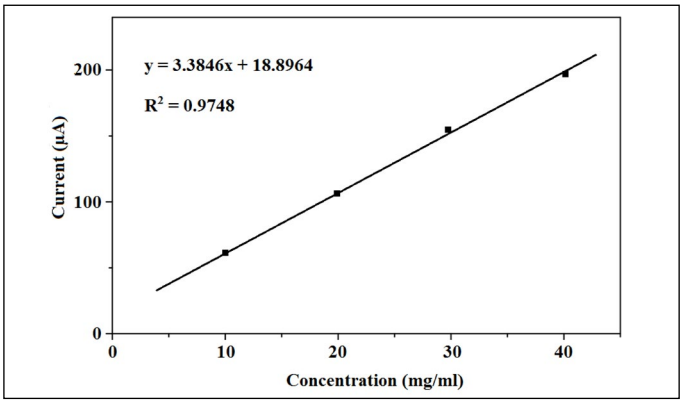

Figure 3. The calibration curve of MnO2/rGO/CPE into prepared real specimen of DXM tablet with 0.2M PBS in successive adding DXM solution.

Table 1. Analytical results of MnO2/rGO/CPE to detect DXM into purposed real specimen of DXM tablet and human blood.

Samples	Add(mg/ml)	Found(mg/ml)	RSD(%)	Recovery(%)
DXM tablet	0	3.67	-	-
	10	13.65	3.06	98.53
	20	22.57	3.32	95.33
	30	33.59	2.77	98.62
	40	42.62	3.61	96.28
Human blood	0	0.00	-	-
	10	9.84	2.72	98.18
	20	19.82	3.16	98.48
	30	28.83	3.58	96.34
	40	39.09	3.27	96.93

CONCLUSIONS

In this study, GO that had been changed by GCE was created using the modified Hummers method, and it was subsequently reduced to use the electrochemical approach. Then, on rGO/GCE, MnO_2 nanostructures were electrodeposited. The vertical development of tetragonal crystalline -MnO_2 nanoplates over crushed rGO nanosheets was demonstrated by morphological and structural data. Results of electrochemical tests revealed that MnO_2/rGO/CPE demonstrated a broad-spectrum, sustained, and selective electrocatalytic reaction to DXM. The sensor's linear range, sensitivity, and detection limit, which are 0 to 260 μM, 4.6153μA/μM and 0.005 μM, respectively, were determined. Medical and human body fluid specimens were utilized to test the application of MnO_2/rGO/CPE to DXM determination. The findings indicated acceptable values for RSD and recovery, indicating that the constructed sensor may be utilized as a dependable DXM biosensor for clinical specimens.

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

AUTHORS' CONTRIBUTIONS: The work is conceived by Sapta Kunta Purnama, knowledge content by Rumi Iqbal Doewes, methodology and execution done by Gunathevan Elumalai, Investigation and formal analysis is by Siti Hartini Azmi, project administration and resources is by Islahuzzaman Nuryadin, supervision, validation and drafting of the manuscript is completed by Manshuralhudlori. All authors contributed equally in execution and writing of this manuscript.

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