

ELECTROCHEMICAL SENSOR FOR DOPING CORTICOSTEROIDS IN SPORTS



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SENSOR ELETROQUÍMICO PARA CORTICOSTERÓIDES DOPANTES NOS ESPORTES

SENSOR ELECTROQUÍMICO PARA EL DOPAJE DE CORTICOESTEROIDES EN LOS DEPORTES

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ABSTRACT

Introduction: Identifying and measuring the concentration of Triamcinolone (TA) in biological fluids is essential, especially for patients receiving intensive antibiotic medication. **Objective:** Make a sensor for electrochemical detection of Triamcinolone (TA) as an anabolic steroid in sports using copper oxide nanomaterials-reduced graphene oxide nanoparticles (CuNPs/rGO). **Method:** After preparing rGO nanoparticles on a glassy carbon electrode (GCE) using the modified Hummers technique, Cu NPs were deposited on rGO/GCE. The applicability of Cu NPs/rGO/GCE was investigated to determine the TA concentration in a real sample that had been prepared. **Results:** The deposited irregular Cu NPs evidenced a diameter of about 80 nm, in agreement with SEM morphological investigations. Amperometric studies revealed that the linear range, detection limits, and sensitivity of CuNPs/rGO/GCE as a TA sensor were 10 to 80 μM , 10nM, and 0.06584 $\mu\text{A}/\mu\text{M}$, respectively. **Conclusion:** The results revealed that the RSD and recovery values were valid, providing adequate quality and reliability for practical analysis of real samples using Cu NPs/rGO/GCE. **Level of evidence II; Therapeutic studies - investigation of treatment outcomes.**

Keywords: Triamcinolone; Doping in Sports; Biosensing Techniques.

RESUMO

Introdução: É essencial identificar e medir a concentração de triamcinolona (TA) em fluidos biológicos, especialmente para pacientes que recebem medicação antibiótica intensiva. **Objetivo:** Confeccionar um sensor para detecção eletroquímica da triamcinolona como esteróides anabolizantes em esportes utilizando nanomateriais de óxido de cobre-nanopartículas de óxido de grafite reduzido (CuNPs/rGO). **Métodos:** Após a elaboração das nanopartículas rGO em eletrodo de carbono vítreo (GCE) utilizando a técnica Hummers modificada, os NPs Cu foram depositados no rGO/GCE. A fim de determinar a concentração de TA em uma amostra real que tinha sido preparada, foi investigada a aplicabilidade de Cu NPs/rGO/GCE. **Resultados:** Os Cu NPs irregulares depositados evidenciaram um diâmetro de cerca de 80 nm, de acordo com as investigações morfológicas do SEM. Estudos de amperometria revelaram que a faixa linear, limites de detecção e sensibilidade do CuNPs/rGO/GCE como sensor TA foram de 10 a 80 μM , 10nM e 0,06584 $\mu\text{A}/\mu\text{M}$, respectivamente. **Conclusão:** Os resultados revelaram que os valores de RSD e recuperação eram válidas, fornecendo qualidade e confiabilidade adequadas para análises práticas de amostras reais usando Cu NPs/rGO/GCE. **Nível de evidência II; Estudos terapêuticos - investigação dos resultados de tratamento.**

Descritores: Triamcinolona; Doping nos Esportes; Técnicas Biossensoriais.

RESUMEN

Introducción: Es esencial identificar y medir la concentración de triamcinolona (TA) en los fluidos biológicos, especialmente en los pacientes que reciben medicación antibiótica intensiva. **Objetivo:** Fabricar un sensor para la detección electroquímica de triamcinolona como esteroides anabólicos en el deporte utilizando nanomateriales de óxido de cobre-nanopartículas de óxido de grafeno reducido (CuNPs/rGO). **Método:** Tras la preparación de las nanopartículas de rGO sobre el electrodo de carbono vítreo (GCE) utilizando la técnica de Hummers modificada, se depositaron los NPs de Cu sobre el rGO/GCE. Para determinar la concentración de TA en una muestra real que había sido preparada, se investigó la aplicabilidad de los NPs de Cu/rGO/GCE. **Resultados:** Los NPs de Cu irregulares depositados presentaban un diámetro de unos 80 nm, de acuerdo con las investigaciones morfológicas del SEM. Los estudios amperométricos revelaron que el rango lineal, los límites de detección y la sensibilidad de Cu NPs/rGO/GCE como sensor de TA era de 10 a 80 μM , 10nM y 0,06584 $\mu\text{A}/\mu\text{M}$, respectivamente. **Conclusión:** Los resultados revelaron que los valores de RSD y recuperación eran válidos, proporcionando una calidad y fiabilidad adecuadas para el análisis práctico de muestras reales utilizando Cu NPs/rGO/GCE. **Nivel de evidencia II; Estudios terapéuticos - investigación de los resultados del tratamiento.**

Descriptor: Triamcinolona; Doping en los Deportes; Técnicas Biosensibles.



INTRODUCTION

Triamcinolone (TA) is a member of the corticosteroid medication family, which includes artificial testosterone analogues used to increase muscle mass.¹ As a glucocorticoid, TA also stops the body from releasing chemicals that induce inflammation.² When used topically, TA relieves the itching, redness, drying, crusting, scale, pain, and inflammation of a number of skin disorders, including eczema, psoriasis, and allergic responses.³ Additionally, TA is utilized to treat some cancers.⁴ An upset stomach, stomach discomfort, vomiting, restlessness, dizziness, sleeplessness, headache, melancholy, anxiety, acne, excessive hair growth, simple bruising, and irregular or no menstrual periods are just a few of the negative effects that TA may produce. Once intramuscularly, intravenously, injected orally, or rectally, TA aids athletes in losing weight without incurring a severe decrease in power, which it leads to be forbidden in-competition by the World Anti-Doping Agency in 2014.⁵ Therefore, it is crucial to identify and measure the TA concentration in biological fluids, clinical specimens, and athletes, especially for patients receiving intensive antibiotic medication.⁶

Through the use of micro- and nanostructured materials, electrochemical analyses, a very affordable technology, can be optimized and downsized. By increasing the surface area and facilitating electron transmission on electrode surfaces, nanofabrication methods can also be employed to boost the effectiveness of electrochemical sensors. This study uses Cu NPs/rGO/GCE to electrochemically identify TA as anabolic steroids for urine samples.

MATERIALS AND METHODS

The rGO was created using a modified version of Hummers' approach in the first phase.⁷ The rGO was created using a modified version of Hummers' approach in the first phase.³ In a nutshell, 8 grams of 98 percent pure graphite powder were ball milling for 100 minutes in order to separate grapheme nanomaterials from graphite and decrease the van-der Waals connectivity between the films. The powder was then added to a beaker containing 200 ml of 97 percent H₂SO₄ and stirred in an ice bath for 30 minutes. 30g of KMnO₄ (98%) was then added to glass to finish the oxidation reactions and create the homogenous dark brown solution. The solution was then ultrasonically sonicated over 6 hours at 35°C with 100 ml of deionized water added. The mixture was then heated at 80°C for 50 minutes. After that, 30 ml of H₂O₂ was added to stop the reaction (35 percent). The resulting suspension was then rinsed with deionized water and 35 percent HCl, respectively. Centrifuging was done for 30 minutes at 2000 rpm with the acquired GO suspension. Centrifuged GO was mixed with a 150g/l L-ascorbic acid (98%) solution as a decreasing agent. The mixture was then heated for 60 minutes at 80 degrees Celsius before being instantly sonicated for 10min at room temperature. Resulting rGO was then rinsed with 2M HCl solution and DI water, respectively, before being centrifuged at 2000 rpm for 20 minutes. The resulting rGO suspension was then dropped-coated onto the GCE surfaces and allowed to air dry.

To use an Auto Lab electrochemical assessment methodology in three electrode method with an Ag/AgCl (2 M KCl) as a reference-electrode, a Pt wire as a counter-electrode, and a GCE or rGO/GCE as a working-electrode over the cyclic voltammetry (CV) method, the CuNPs were successfully deposited onto GCE and rGO/GCE in the second step. In a summary, the GCE or rGO/GCE was submerged in an electrochemical electrolyte composed of deoxygenated 0.2 Na₂SO₄ and 50 mM Cu(NO₃)₂. At a scan rate of 20 mV/s for 60 cycles, CV scanning was used in the potential range of -0.2 V to 0.6 V.

Four individuals, aged 20 to 30, whose urine samples were collected had taken Tricort pills, that contain 5mg of TA each tablet. Since half-life

of TA was supposed to be 10-20 hours,⁸ urine samples were obtained 8 hours after tablets were administered. The materials were prepared for electrochemical studies by centrifuging them in 0.2 M PBS for 20 minutes at 2000 rpm. The level of TA in urine samples was also determined using the ELISA kit for TA enzyme-linked immunosorbent assay.

Using a scanning electron microscopy (SEM) and X-ray diffractometer (XRD), respectively, crystallographic and morphological studies were performed. Amperometry and differential pulse voltammetry (DPV) methods were used to conduct electrochemical experiments in a potentiostat-galvanostat under the direction of GPES 4.8 software. The electrolyte used was 0.2 M phosphate buffer solution (PBS), which was made by combining stock solutions of 0.2M NaCl (98%) and 0.2M NaH₂PO₄-Na₂HPO₄ (98%).

In this research only urine samples were used in the analysis and the work was conducted based on the Declaration of Helsinki principle. The participants signed the Free and Informed Consent Form (EHIC).

RESULTS AND DISCUSSION

SEM micrographs of rGO/GCE, CuNPs/GCE, and CuNPs/rGO/GCE are shown in Figure 1. The porosities of rGO is depicted in Figure 1a's SEM image along with the usual folds, ripples, and wrinkled nanostructures. As can be seen, there is a significant amount of local buildup at the interfaces of rGO nanosheets, which may be caused by the reduction process⁹ that can remove the majority of oxygen-containing groups and sp³ carbon. Under π - π interaction, it can lead to increased rGO nanosheet restacking.¹⁰ Figure 1b depicts a morphology of Cu nanoparticles electrodeposited on GCE, which exhibits irregular nanoparticles with an estimated diameter of 175 nm. The shape of Cu NPs, rGO, and GCE On the surface of the rGO nanosheets, CuNPs have been deposited with good dispersion, as shown in Figure 1c. CuNPs have an average diameter of 130 nm. When Figures 1b and 1c are compared, it can be seen that this same electrodeposited Copper NPs are much smaller and less aggregated when rGO is present. Large grains of nanoparticles are also visible onto surfaces of GCE, which indicates that the Cu NPs, rGO, and GCE all have more absorption and electroactive sites. This increases the effective surface area and charged particle transfer dealings in electrochemical cells. Additionally, the addition of CuNPs can lessen the aggregation of rGO nanostructures, which led to increased electrochemical activity for the trio of compounds (CuNPs/rGO/GCE).

The CV plots of GCE, rGO/GCE, CuNPs/GCE, and CuNPs/rGO/GCE in 0.2 M Na₂SO₄ are shown in Figure 2 at a scanning rate of 20 mV/s. As can be seen in Figure 2, neither the GCE nor the rGO/GCE CV curves exhibit any redox peaks. The anodic peak at 0.23 V and 0.18 V that were associated with the oxidation of Cu to Cu²⁺ and cathodic peak at 0.21 V and 0.15 V that were associated with the reduction of Cu²⁺ to Cu are visible on the CV plots of CuNPs/GCE and CuNPs/rGO/GCE.^{11,12} Comparing the CV plots of GCE and rGO/GCE, as well as the CV plots of CuNPs/GCE and CuNPs/rGO/GCE, the rGO impact is revealed. According to these findings, the peak potential and peak current gap of CuNPs/rGO/GCE toward Cu NPs/GCE both increase while the backdrop current in rGO/GCE/direction GCE's of travel decreases. This increased the number of sites accessible for quick electron transport in electrochemical reactions and was connected with the high planar

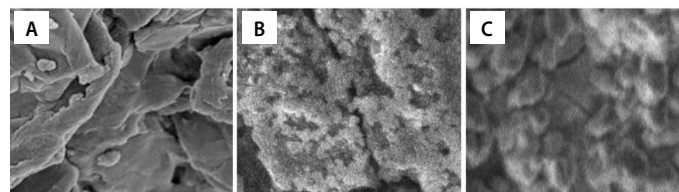


Figure 1. SEM micrographs of (A) rGO/GCE, (B) CuNPs/GCE and (C) CuNPs/rGO/GCE.

edge density of layers of graphene and its porous nature.¹³ Additionally, rGO nanosheets function as a conducting component to improve electronic conductivity.¹⁴

The amperometric observations and calibration graph for the reaction current of Cu NPs/rGO/GCE to incremental additions of 10 M TA into 0.2 M PBS are shown in Figure 3(a). Cu NPs/rGO/GCE has been found to respond quickly to the addition of TA solutions, indicating a quick transfer of electrons on the surfaces of Pt@GO that may be facilitated by the porous nanocomposite matrix's ease of adsorption of intermediates and high pore volume.¹⁵ The corresponding calibration graph shows that adding 10 μM TA solution incrementally increases the amperometric signal linearly from 0 to 80 μM, and the calculated sensing parameters for sensitivity and detection-limits are 0.06584 μA/μM and 10 nM, respectively. In Table 1, the implementation of the developed electrochemical TA sensor is contrasted with that of other electrochemical TA sensors that have been described in the literature. CuNPs/rGO/GCE is found to have a wider linear range than some other sensors. This indicates that the proposed sensor performs favorably and even better than previously reported sensors, which is attributed to the enhanced electron permeability of CuNPs/rGO and the synergic catalytic influence between Cu and GO. The subsequent reaction of Cu nanoparticles with GO functional groups that contain oxygen may help in liberation of Cu crystal lattice and rise the catalytic activity.¹⁶

In genuine samples of human urine collected from four patients receiving pharmaceutical treatment with TA, the accuracy and application of CuNPs/rGO/GCE to determine TA were examined. The TA level into prepared 0.2M PBS from urine specimens was evaluated by amperometric measurements employing CuNPs/rGO/GCE under serial injections of TA solution. The amperometry records and calibration curve from first sample (V1) are revealed in Figure 3(b), which demonstrates that the prepared sample's TA content is 0.232 μM, which is extremely close to the TA concentration that was found through the ELISA assess. The amperometric and ELISA analyses were also run on the remaining three urine specimens, and the results are summarized in Table 1 with an average of 5-times using both assays to determine TA, indicating good accordance to the two assesses. The proposed amperometric TA sensors has acceptable detection validity and accuracy for determining TA into human urine specimens, as shown by the acquired RSD values, which are also shown in Table 1 and are less than 4.03 %.

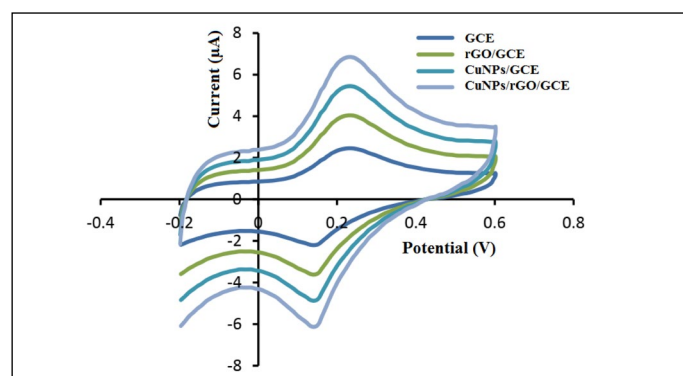


Figure 2. CV plots of GCE, rGO/GCE, CuNPs/GCE and CuNPs/rGO/GCE in 0.2M Na₂SO₄.

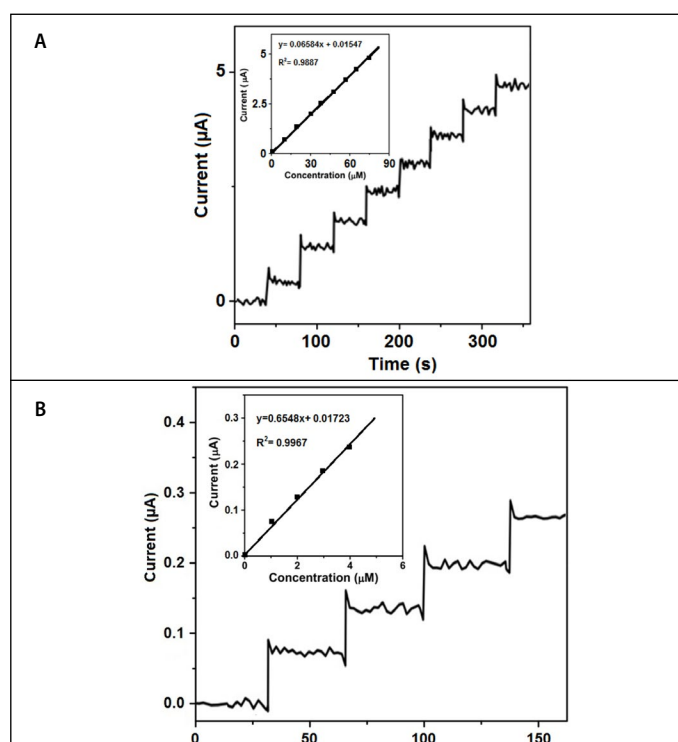


Figure 3. (A): Amperometric analysis of CuNPs/rGO/GCE to successive adding 10 μM TA into 0.2M PBS. Inset figure shows calibration graph (B): Amperometric analysis of CuNPs/rGO/GCE to successive adding of 1 μM TA into 0.2M PBS produced from urine specimen of first volunteer. Inset figure shows calibration graph.

Table 1. The findings of amperometry and ELISA assess to determine of TA in produced real specimens from human urine specimens making from four volunteers.

Samples	TA content in produced urine specimens (μM)			
	Amperometry		ELISA	
	CuNPs/rGO/GCE	RSD(%)	ELISA	RSD(%)
V1	0.232	±3.11	0.239	±3.51
V2	0.160	±3.18	0.166	±4.01
V3	0.198	±4.03	0.199	±3.98
V4	0.175	±4.01	0.181	±3.65

CONCLUSIONS

This effort was done to create CuNPs, rGO, and GCE for the electrochemical analysis of TA in samples. CuNPs were successfully deposited on rGO/GCE after rGO nanosheets were created using a modified Hummers process. The pore structures of rGO nanostructures and CuNPs into cubic structure were generated, according to studies on the structural and morphological properties of nanostructures. For the CuNPs/rGO/GCE TA sensor, electrochemical tests revealed that the linear range, detection limits, and sensitivity were reached at 0 to 80 μM, 10 nM, and 0.06584 μA/μM, respectively. This approach gave adequate quality and reliability for practical analyses in actual samples utilizing CuNPs/rGO/GCE, according to a study of the application of CuNPs/rGO/GCE to determination of TA concentration into prepared real sample, which indicated RSD and recovery values were acceptable.

The author declare no potential conflict of interest related to this article

AUTHORS' CONTRIBUTIONS: The work is conceived and executed by Yudan Xu. The author is fully responsible for execution and writing of this manuscript.

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