ELECTROCHEMICAL SENSOR FOR ANTI-DOPING IN ATHLETES

SENSOR ELETROQUÍMICO PARA ANTI-DOPING EM ATLETAS

SENSOR ELECTROQUÍMICO PARA ANTIDOPING EN DEPORTISTAS

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ABSTRACT

Introduction: Several athletes use steroids such as nandrolone aiming at muscle hypertrophy and performance gain. The current research focused on developing a GO-TiO2 nanostructure as an electrochemical sensor for detecting Nandrolone (ND) like doping agents. Objective: Develop a graphene oxide and carbon paste-modified TiO2 nanocomposite electrode (TiO2-GO/CPE) as an electrochemical biosensor for the detection of anabolic steroids in the urine of athletes. Methods: The hydrothermal approach was employed to make GO-TiO2 nanocomposites, while the modified Hummers approach was used to make GO nanofilaments. Results: The interaction of TiO2 nanostructures with GOES resulted in the anchoring of TiO2 nanoparticles on the surface of GO nanowires, as demonstrated by structural investigations of the generated nanocomposite using SEM. The DPV approach was used to investigate the electrochemical properties of an anabolic steroid sensor, which revealed a stable and selective response to anabolic steroids and superior performance to previously reported anabolic steroid sensors. Conclusion: RSD values ranged from 3.20% to 4.45%, indicating that the developed electrochemical anabolic steroid sensor can be used as a viable detection technique to identify anabolic steroids in human biological fluids. ***Level of evidence II; Therapeutic studies - investigation of treatment outcomes.***

Keywords: Steroids, Anabolic; Doping in Sports; Biosensing Techniques; Nanocomposites.

RESUMO

Introdução: Vários atletas fazem uso de esteróides como nandrolone visando a hipertrofia muscular e ganho de performance. A pesquisa atual se concentrou no desenvolvimento de uma nanoestrutura GO--TiO2 como um sensor eletroquímico para detecção de Nandrolone (ND) como agente dopante. Objetivo: Desenvolver um eletrodo de nanocomposto de óxido de grafite e pasta de carbono modificado (TiO2-GO/CPE) como um biossensor eletroquímico para a detecção de esteróides anabólicos na urina de atletas. Métodos: A abordagem hidrotérmica foi empregada para fazer nanocompósitos de GO-TiO2, enquanto a abordagem Hummers modificada foi usada para fazer nanofilamentos de GO. Resultados: A interação das nanoestruturas de TiO2 com GOES resultou na ancoragem de nanopartículas de TiO2 na superfície dos nanofilamentos de GO, como demonstrado pelas investigações estruturais do nanocomposto gerado usando SEM. A abordagem DPV foi utilizada para investigar as propriedades eletroquímicas de um sensor de esteróides anabólicos, que revelou uma resposta estável e seletiva aos esteróides anabólicos, bem como um desempenho superior ao dos sensores de esteróides anabólicos anteriormente relatados. Conclusão: Os valores de RSD variaram de 3,20% a 4,45%, indicando que o sensor de esteróides anabolizantes eletroquímicos desenvolvido pode ser usado como uma técnica de detecção viável para identificar esteróides anabolizantes em fluidos biológicos humanos. **Nível de evidência II; Estudos terapêuticos - investigação dos resultados do tratamento.**

Descritores: *Esteroide Anabolizante; Doping nos Esportes; Técnicas Biossensoriais; Nanocompostos.*

RESUMEN

Introducción: Varios atletas hacen uso de esteroides como la nandrolona con el objetivo de hipertrofia muscular y aumento de rendimiento. La presente investigación se centró en el desarrollo de una nanoestructura de GO-TiO2 como sensor electroquímico para la detección de nandrolona (ND) como agente dopante. Objetivo: Desarrollar un electrodo de nanocompuesto de óxido de grafito y pasta de carbono modificado (TiO2-GO/CPE) como biosensor electroquímico para la detección de esteroides anabólicos en la orina de atletas. Métodos: Se empleó el enfoque hidrotérmico para hacer nanocompuestos de GO-TiO2, mientras que el enfoque de Hummers modificado se utilizó para hacer nanofilamentos de GO. Resultados: La interacción de las nanoestructuras de TiO2 con el GOES dio lugar al anclaje de las nanopartículas de TiO2 en la superficie de los nanofilamentos de GO, tal y como demostraron las investigaciones estructurales del nanocompuesto generado mediante SEM. El enfoque de DPV se utilizó para investigar las propiedades electroquímicas de un sensor de esteroides anabólicos, que reveló una respuesta estable y selectiva a los esteroides anabólicos, así como un rendimiento superior a los sensores de esteroides anabólicos reportados anteriormente. Conclusión: Los valores de RSD oscilaron entre el 3,20% y el 4,45%, lo que indica que el sensor electroquímico de esteroides *anabólicos desarrollado puede utilizarse como una técnica de detección viable para identificar esteroides anabólicos en fluidos biológicos humanos.* ***Nivel de evidencia II; Estudios terapéuticos - investigación de los resultados del tratamiento.***

Descriptores: *Esteroide Anabólico; Doping en los Deportes; Técnicas Biosensibles; Nanocompuestos.*

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INTRODUCTION

Nandrolone (ND) is an androgen and anabolic steroid which has been alkylated. It is also known as 19-nortestosterone.[1] It is proposed that it enters cells by receptor-mediated endocytosis and binds with the androgen receptor as a mechanism of action.[2] The androgen receptor experiences a conformational shift after connecting with the androgen receptor, enters the nuclei, dimerizes, and can subsequently bind to Gene sequences to regulate transcription.[3]

ND is being used to treat anemia induced by renal dysfunction, as well as to treat senile and menopausal osteoporosis as an adjuvant therapy.[4] It is often used to treat burns, adverse reactions, eye injuries, especially corneal injuries, and cancer-related appetite loss, and also prostate and breast cancer.[5] The increase of muscle tissue and lean mass is favored by ND, which may lead to a reduction in cardiometabolic risk components. Furthermore, studies on ND suggests that it may be useful in the therapy of joint repair, notably in the treatment of rotator cuff injuries.[6]

Side effects of ND use include headaches, oedema, gastrointestinal discomfort, diarrhoea, stomach pain, jaundice, menstruation abnormalities, and hypertension.[7] To grow muscle and improve performance, several professional sportsmen, recreational athletes, and even kids misuse steroids like ND.[8] According to research, ND taken at doses higher than those recommended by doctors can injure the liver and cause incipient fibrosis.[9]

ND boosts muscle strength and endurance by increasing testosterone and endogenous testosterone levels, and it was most probably used as a doping medication in sports in the 1960s. Many analysis techniques for identifying and analyzing ND amounts in biofluids and clinical specimens have been explored as a result, including liquid chromatography,[10] mass spectrometry,[11] gas chromatography,[12] capillary electrophoresis,[13] spectrophotometry,[14] electrochemical methods,[15,16,17,18] Raman spectroscopy,[19] near-Infrared Spectroscopy[20] and enzyme-linked immunosorbent assay kit (ELISA).[21] When it came to detecting anabolic steroids for clinical samples, electrochemical methods surpassed the others in respect of selectivity and sensitivity. Only a few studies on the calculated from the equation of ND have indeed been undertaken to our knowledge.[17] As a result, the current research focused on the development of a GO-TiO_2 hybrids nanostructure as an electrochemical sensor for detecting ND as doping agents in blood and urine samples.

METHODS

Experimental details

To make the carbon paste electrode, a homogenous paste of produced natural graphite and paraffin oil in a 7:3 weight ratio was mixed at 30°C and transported to a hollowed polytetrafluoroethylene tube with a copper wire for electrical contact. The paste's surface was smoothed using a soft paper once it had cooled. After that, DI water was used to rinse the prepared CPE.

The following approach was used to prepare GO using the modified Hummers method:[22] In a 250 mL volumetric flask, a homogenous mixture containing 1 g graphene (99%), 25 mL sulfuric acid (99%), and 200 g sodium nitrate (99.0%) was created under mechanical stirring in an ice bath for 40 minutes. Then, at 45°C for 40 minutes, 2 g Ppotassium permanganate (90%) and 200 mL sterile distilled (DI) water were gently added to the reaction mixture during magnetic stirring. The obtained suspension was then treated with 20 mL hydrogen peroxide (30%). The unrestricted graphite particles with in resulting mixture were removed using centrifugation at 2000 rpm for 10 minutes followed by filtration. he completed mixture was cleaned before being placed in the oven to dry for 10 hours at 80 °C.

Hydrothermal technique was used to make TiO_2-GO nanocomposite. A homogenous mixture of 30 mg graphene powder, 10mL of 2 M sulfuric acid (99.99%), and 0.2 mL titanium isopropoxide (98%) for 15 minutes ultrasonically created. The mixture was then transferred to just a Teflon-lined steel autoclave with a capacity of 20 mL. It was sealed and baked for 20 hours at 170 °C. To get a homogeneous suspension, 10 mg of the TiO_2-GO nanocomposite were ultrasonic assisted dispersed in 20mL of dimethylformamide (DMF) for 2 hour. The completed mixture was then slowly poured onto the ready CPE surface and allowed to dry at room temperature.

After receiving intramuscular injections of steroid decanoate, urine and serum samples were taken from four young athletes aged 18 to 32. Because ND has a half-life of 7–14 days,[23] urine and blood measurements were collected two days after injection. Before being utilized to prepare 0.1M PBS as genuine samples for electrochemical experiments, the samples were then centrifuged separately for 15 minutes at 2000 rpm. The Steroid Racing ELISA kit has also been used to find out how much ND had been in urine and serum specimens.

Field emission scanning electron microscopy was used to investigate the morphology of electrode surfaces. The electrodeposition was carried out on a potentiostat-galvanostat in a traditional three electrode-electrochemical cell that featured CPE as the working electrode, Ag/AgCl and Pt wire as the reference and counter electrodes, respectively. The electrolyte used for the electrochemical experiments was 0.1M phosphate buffer solution (PBS), which has been created by mixing standard solution of 0.1 M NaCl (99%) and 0.1M NaH_2PO_4–Na_2HPO_4 (98%,).

Footnote

The authors are accountable for all aspects of the work related to the accuracy or integrity of any part of the work are appropriately investigated. The study was conducted in accordance with the Declaration of Helsinki. The participants signed the Free and Informed Consent Form (EHIC).

RESULTS AND DISCUSSION

Figure 1 illustrates the GO-TiO_2/CPE DPV responses and calibration curve after injecting 10 M ND solutions with 0.1 M PBS in the potential range of 0.1V to 0.9V at a 20mV/s scan rate. With 0.999 correlation coefficient, the electrocatalytic reaction of GO-TiO_2/CPE is demonstrated to increase linearly for each addition of 10mM ND solutions in the range of 1 to 60mM. The following is how the linear relation is realized:

$$Y(\mu A) = 0.4567\ [ND]\ (\mu A/\mu M) + 0.1437 \quad (1)$$

Where [ND] specifies the amount of ND present. 0.4547A/M and 3nM, respectively, are the computed sensitivity and limit of detection (LOD).

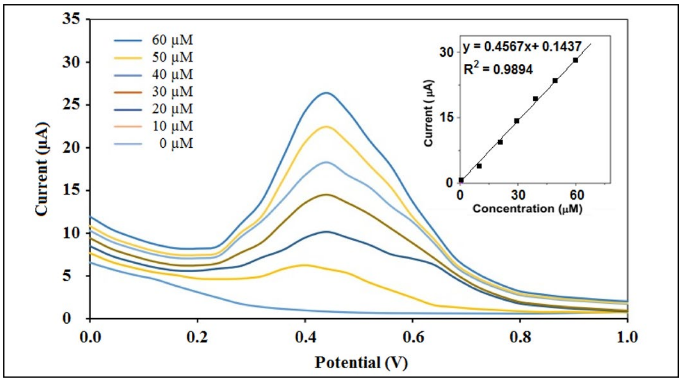

Figure 1. DPV results and calibration diagram of GO-TiO2/CPE after adding 10μM ND solutions to 0.1M PBS.

The advanced ND sensor shows a wider linear range reaction to ND and positively and even connectivity features of GO-TiO2/CPE than the many reported ND sensors, that can be attributed to the higher conductivity and outstanding catalytic performance of TiO_2 nanoparticles with the GO-TiO_2 nanocomposites.[24]

Figure 2 displays the effects of DPV electrocatalytic current flow of GO-TiO2/CPE at 0.50V through 0.1M PBS under addition of ND and 4-fold surpluses interfering additives at a 20 mV/s scan rate, demonstrating that the sensor to 1M ND solvent is substantially larger than that of the interferents, and indicating no major changes in electrocatalyst signal of ND at 0.50V, suggesting that the addition These results demonstrate that the proposed sensor has a good selectivity for measuring ND in pharmaceutical and biological urine specimens.

Figure 3 shows the DPV measurement results and calibrations diagram of a generated genuine specimen of plasma from one of athletes (V1) that used GO-TiO2/CPE through 0.1M PBS and ND at a 20mV/s scan rate. The ND concentration in a made authentic sample of blood is 0.095μM, which is very close to the ND level determined by an ELISA analysis (Table 1). The DPV and ELISA studies were also carried out on all prepared authentic samples from blood and urine specimens of additional athletes, with the findings presented in Table 1, indicating that the two assays are reasonably consistent. Furthermore, the RSD values presented in Table 1 range from 3.07 to 4.06%, suggesting that the proposed electrochemical ND sensor may be used to determine ND in human biofluids with high accuracy.

CONCLUSIONS

Finally, the goal of this research was to create GO-TiO_2 hybrid nanomaterials designed GCE as biosensors for detecting ND as anabolic steroids in blood and urine specimens. Crystallographic and morphological studies revealed that a well-crystallized GO-TiO_2 nanocomposites structure with multiple active sites, a roughness and porosity surface, and a high effective surface area was produced. The electrochemical characteristics of GO-TiO_2/CPE as such an ND sensor indicated a stable, precise, and sensitive reaction to ND, as well as positively and even better achievement than other previously reported ND sensors, that can be credited to a higher conductivity and outstanding catalytic performance of TiO_2 nanoparticles for the GO-TiO2 nanocomposites. GO-TiO_2/CPE was tested for linear range (1 to 60μM), sensitivity (0.3547 μA/μM), and limit of detection (3nM). The clinical implementation of a newly designed ND sensor was examined in order to quantify the amount of ND in the serum and urine of four youth sports aged 18 to 32 who were given nandrolone decanoate subcutaneous injection. The results demonstrated that the DPV and ELISA studies agreed quite well, and that the created electrochemical ND sensor can be employed as a feasible sensing strategy for assessing ND in human bodily fluids due to the acceptable RSD values.

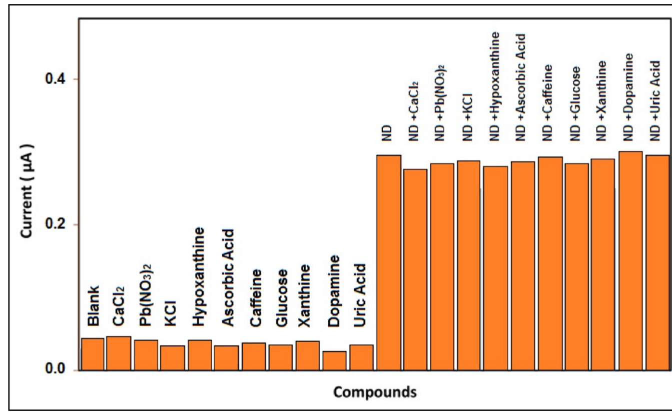

Figure 2. DPV electrocatalytic currents of GO-TiO2/CPE at 0.5V with successive additions of 0.1M PBS and 1μM ND, and 4-fold excesses interfering chemicals; (Blank specimen is stated to the 0.1M PBS without analytes).

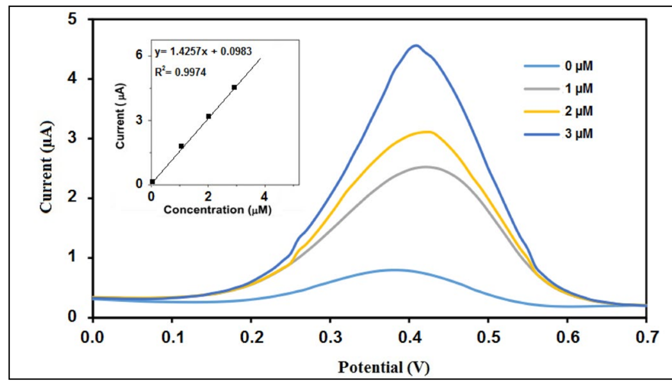

Figure 3. DPV results and calibration diagram of GO-TiO2/CPE to sequential injections of ND in 0.1 M PBS obtained from the first volunteer's serum sample.

Table 1. The results of DPV and ELISA studies to detect ND in ready real samples from blood and urine samples obtaining from young athletes aged 18 to 32 years who controlled nandrolone decanoate through subcutaneous injections.

ND level in prepared serum specimens (μM)				
Case No.	Amperometry		ELISA-kit	
	GO-TiO_2/CPE	RSD(%)	ELISA	RSD(%)
C1	0.094	±3.39	0.096	±3.58
C2	0.098	±3.47	0.097	±3.65
C3	0.095	±4.22	0.095	±3.98
C4	0.087	±4.18	0.089	±3.83
ND level in prepared urine specimens (μM)				
C1	0.074	±3.58	0.083	±3.59
C2	0.070	±3.07	0.078	±4.17
C3	0.069	±3.06	0.078	±4.14
C4	0.072	±4.06	0.091	±3.89

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

AUTHORS' CONTRIBUTIONS: The work is conceived and executed by Changxiang Chen and Chen Xiangwei. The authors are fully responsible for execution and writing of this manuscript.

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