TESTOSTERONE BIOSENSOR IN SPORTS DOPING

BIOSSENSOR DE TESTOSTERONA NO DOPING ESPORTIVO

BIOSENSOR DE TESTOSTERONA EN EL DOPAJE DEPORTIVO

ORIGINAL ARTICLE
ARTIGO ORIGINAL
ARTÍCULO ORIGINAL

Zhiwei Ni[1]
(Physical Education Professional).

1. College of Physical Education, Yangzhou University, Yangzhou, Jiangsu, 225000, China.

Correspondence:
Zhiwei Ni
Yangzhou, Jiangsu, China. 225000.
zwni1987@163.com

ABSTRACT

Introduction: Testosterone is a steroid that can help with blood disorders, sexual dysfunctions, connective tissue diseases, some malignancies, intractable pain, and other serious diseases. However, it must be prescribed under medical supervision because of the risk of major adverse effects such as liver disease, heart disease, stroke, blood clots, and cancer. There is an urgent need for research on developing an electrochemical sensor to detect testosterone as a doping substance in sports. Objective: Develop an electrochemical sensor of poly(ionic liquid)-graphene oxide molecularly printed polymers (PIL/MIs/GO) to detect testosterone as a doping substance in sports. Methods: Morphological characterization of modified electrodes was performed by field emission scanning electron microscopy (FESEM), allowing the GO to be surface-mounted with fragments and apertures. Due to the holes generated by the agglomeration of PIL and MIs molecules on the wavy edges of the GO nanosheets, the surface morphology of PIL/MIs/GO/GCE also revealed a high porosity structure. Results: Compared to other synergistic influences of GO nanosheets with PIL and MIs molecules, electrochemical investigations using a differential pulse voltammetry approach indicated high selectivity, good stability, appropriate linear range, lower detection limit, and higher selectivity. Conclusion: In pharmaceutical samples and human biological fluids, the validity and accuracy of PIL/MIs/GO/GCE for the determination of testosterone demonstrated practical application. PIL/MIs/GO/GCE can thus be used as an accurate and reliable sensor for detecting testosterone as a doping agent in sports. ***Level of evidence II; Therapeutic studies - investigation of treatment outcomes.***

Keywords: Biosensing Techniques; Nanoparticles; Testosterone; Doping in Sports.

RESUMO

Introdução: A testosterona é um esteróide que pode ajudar com distúrbios sanguíneos, disfunções sexuais, doenças do tecido conjuntivo, algumas malignidades, dores intratáveis e outras doenças graves. No entanto, devido ao risco de grandes efeitos adversos como doenças hepáticas, doenças cardíacas, derrames, coágulos sanguíneos e câncer, ela deve ser prescrita sob supervisão médica. Há uma necessidade urgente da pesquisa sobre o desenvolvimento de um sensor eletroquímico para detectar a testosterona como substância dopante nos esportes. Objetivo: Desenvolver um sensor eletroquímico de poli-(líquido iônico)-polímeros impressos molecularmente em óxido de grafeno (PIL/MIs/GO) para detectar a testosterona como substância dopante nos esportes. Métodos: Efetuou-se a caracterização morfológica de eletrodos modificados por microscopia eletrônica de varredura de emissão de campo (FESEM) permitindo que o GO fosse em superfície com fragmentos e aberturas. Devido aos orifícios gerados pela aglomeração das moléculas de PIL e MIs nas bordas onduladas das nano folhas de GO, a morfologia da superfície de PIL/MIs/GO/GCE também revelou uma estrutura de alta porosidade. Resultados: Em comparação com outras influências sinergéticas das nanoquetas GO com as moléculas PIL e MIs, os resultados das investigações eletroquímicas utilizando a abordagem de voltametria de pulso diferencial indicaram alta seletividade, boa estabilidade, faixa linear apropriada, limite de detecção mais baixo e seletividade mais alta. Conclusão: Em amostras farmacêuticas e fluidos biológicos humanos, a validade e a precisão do PIL/MIs/GO/GCE para a determinação de testosterona demonstraram aplicação prática. O PIL/MIs/GO/GCE pode assim ser utilizado como um sensor preciso e confiável para a detecção de testosterona como agente dopante no esporte. ***Nível de evidência II; Estudos terapêuticos - investigação dos resultados do tratamento.***

***Descritores*:** *Técnicas Biossensoriais; Nanopartículas; Testosterona; Doping nos Esportes.*

RESUMEN

Introducción: La testosterona es un esteroide que puede ayudar en los trastornos sanguíneos, la disfunción sexual, las enfermedades del tejido conectivo, algunos tumores malignos, el dolor intratable y otras enfermedades graves. Sin embargo, debido al riesgo de que se produzcan efectos adversos importantes, como enfermedades hepáticas, cardíacas, accidentes cerebrovasculares, coágulos sanguíneos y cáncer, debe prescribirse bajo supervisión médica. Es urgente investigar el desarrollo de un sensor electroquímico para detectar la testosterona como sustancia dopante en el deporte. Objetivo: Desarrollar un sensor electroquímico de polímeros impresos molecularmente de poli(líquido iónico)-óxido de grafeno (PIL/MIs/GO) para detectar la testosterona como sustancia dopante en el deporte. Métodos: La caracterización morfológica de los electrodos modificados se llevó a cabo mediante microscopía electrónica de barrido de emisión de campo (FESEM) permitiendo que el GO estuviera en la superficie con fragmentos y aberturas. Debido a los agujeros generados por la aglomeración de moléculas de PIL y MIs en los bordes ondulados de las

nanohojas de GO, la morfología superficial de PIL/MIs/GO/GCE también reveló una estructura de alta porosidad. Resultados: En comparación con otras influencias sinérgicas de las nanohojas de GO con las moléculas PIL y MIs, los resultados de las investigaciones electroquímicas utilizando el enfoque de la voltamperometría diferencial de impulsos indicaron una alta selectividad, una buena estabilidad, un rango lineal apropiado, un límite de detección más bajo y una mayor selectividad. Conclusión: En muestras farmacéuticas y fluidos biológicos humanos, la validez y precisión de PIL/MIs/GO/GCE para la determinación de testosterona demostró su aplicación práctica. Así pues, PIL/MIs/GO/GCE puede utilizarse como un sensor preciso y fiable para la detección de la testosterona como agente dopante en el deporte. ***Nivel de evidencia II; Estudios terapéuticos - investigación de los resultados del tratamiento.***

Descriptores: *Técnicas Biosensibles; Nanopartículas; Testosterona; Doping en los Deportes.*

DOI: http://dx.doi.org/10.1590/1517-8692202329012022_0442 Article received on 08/09/2022 accepted on 10/05/2022

INTRODUCTION

Steroids are medications like androstenedione, testosterone, methyl testosterone, and danazol, that can help with blood disorders, sexual dysfunctions, connective tissue illness, some malignancies, intractable pain, and other serious diseases.[1] However, due to the risk of major adverse effects like liver disease, heart disease, stroke, blood clots, and cancer, they must only be set and used in medical supervision. As a result, numerous studies have been carried out on gas chromatography, enzyme-linked immunosorbent assay, liquid chromatography, fluorimetry, mass spectrometry, spectrophotometry, radio immunoassay, photodiode-array, yeast estrogen screen assess, and electrochemical detection approaches in order to find a quick and easy way to detect steroid abuse.[2-6] Electrochemical techniques, for example, have revealed a varied range of applicability in environmental and food investigations, as well as clinical diagnostics, thanks to their simplicity of usage, low-cost, and capacity to improve and optimize during the last decade. The use of nanomaterials and different composites to modify and miniaturize electrochemical sensors improves selectivity and sensitivity.[7]

Anabolic steroids are testosterone derivatives that are synthesized (man-made). In men, testosterone is the primary sex hormone. It's required for the development and maintenance of male sex features like facial hair, a deep voice, and muscle growth. Because testosterone induces increases in muscular mass, which is a positive adaptation for many power-based sports, both naturally and synthetically raised testosterone levels may improve strength and sports performance. For testosterone sensors, just a few electrochemical investigations have been conducted.[8-14] As a result, our research focused on the development of a PIL/MI/GO/GCE electrochemical sensor for detecting testosterone as a doping substance in sports.

MATERIALS AND METHODS

The GO was prepared from graphite powder using a modified Hummers process.[15] 2 g graphite powder was introduced to an ice water bath with a combination of 70 mL sulfuric acid and 30 mL nitric acid. The suspension was then gradually supplemented with 10 g potassium permanganate. The mixture was then swirled for 70 minutes at 30°C. Following that, 100 mL DI water was slowly added to the mixture while stirring at 40°C for 50 minutes, and then 10 mL H_2O_2 was added while stirring at 35°C for 45 minutes to create a bright yellow suspension. For 15 minutes, suspension was centrifuged in 1500 rpm/minutes. To separate/exfoliate stacked graphene oxide sheets, supernatant was detached and the resulting GO was ultrasonically disseminated in 3mL of 0.1M PBS for 90 minutes. The 0.1M PBS was made using 0.1M sodium phosphate at a 1:1 volume ratio.

Prior to the alteration, the surface of GCE was prepared by polishing it by alumina powder onto a polishing material, then ultrasonically washing it with DI water and ethanol aimed at 10 min each, then drying it at room temperature. The electrodeposition was carried out on a potentiostat-galvanostat in a traditional three electrode-electrochemical cell that featured GCE as the working electrode, Ag/AgCl and Pt wire as the reference and counter electrodes, respectively.

A homogenous mix of 0.2g tetrabutylammonium perchlorate and 100 ml acetonitrile was placed on the electrode surface to modify the surface of GCE and GO/GCE with MI [16]. The electrode was then dried at room temperature with a moderate flow of dry nitrogen. To make PIL/MI/GO/GCE, PIL/MI/GCE, and PIL/GCE, poly(4-vinylpyridine) was deposited in an electrochemical cell with bare or modified GCE as working electrodes. The testosterone-free plasma sample is used for the creation of the real specimen of human plasma. With 0.1 M PBS, the plasma specimens (10 mL) were watery to 100mL. Analytical investigations of genuine samples were conducted using the conventional addition method. On the Autolab system, differential pulse voltammetry (DPV) investigations were performed in a three-electrode electrochemical cell with improved GCE as the working electrode in 0.1M PBS as the electrochemical electrolyte. Field emission scanning electron microscopy was used to investigate the morphology of electrode surfaces.

This work was conducted based on the Declaration of Helsinki principle. The participants signed the Free and Informed Consent Form (EHIC).

RESULTS AND DISCUSSION

SEMs of GO and PIL/MI/GO/GCE are shown in Figure 1. Figure 1a illustrates the overlying of flake coatings onto the surface in a FESEM picture of GO. The holey and rough appearance of GO is observed, with cracks and breaks that can arise due to carbon atom rearrangement through the graphitization procedure.[17] Figure 1b demonstrates that PIL/MI/GO/GCE has a porous structure due to holes formed by aggregation of PILand MI molecules on the crenelated edge of GO nanosheets. The use of a co-functional monomer made up of MI and PILas could improve adsorption and conductivity.[18]

Figure 2 depicts the DPV curves and PIL/MI/GO/GCE calibration plots for serial injections of 10M testosterone solution in 0.1M PBS at a 10 mV/s scan rate. The electrocatalytic current increases linearly as the testosterone concentration increases from 1 to 60 µM, with a deviation from linearity aimed at higher concentrations because of saturation of dynamic sites

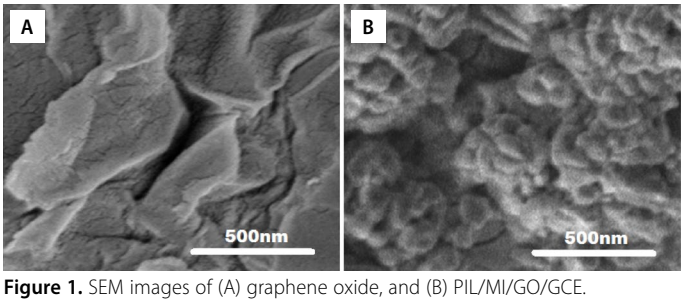

Figure 1. SEM images of (A) graphene oxide, and (B) PIL/MI/GO/GCE.

on improved electrodes.[19] PIL/MI/GO/GCE as a testosterone sensor has a sensitivity of 0.7395 A/M and a detection limit of 0.003 M, respectively.

Table 1 shows the results of a PIL/MI/GO/GCE interference study to determine testosterone in the existence of chief metabolites in fluids of body using DPV analysis in 0.1M PBS at 10 mV/s scan rate for successive injections of 1M testosterone and 10M hypoxanthine, ascorbic acid, uric acid, dopamine, albumin, nitrite, glucose, and xanthine as interferents.[20] The modified electrode exhibits a spectacular peak current in response to testosterone solution additions, but injections of other interference substrates produce no appreciable electrocatalytic peak current.[21] As a result, the interference substrates listed in Table 2 have no effect on testosterone detection, and the PIL/MI/GO/GCE can be employed as a selective testosterone sensor into biological fluid specimens like blood plasma and human urine.

Into the pharmaceutical specimens and biological fluids, the validity, precision, and applicability of PIL/MI/GO/GCE for testosterone assessment were assessed. The DPV measurements were performed using PIL/MI/GO/GCE in a prepared genuine pharmaceutical sample of testosterone boosters with 0.1M PBS at a 10mV/s scan rate in sequential additions of testosterone solution in order to analyze prepared real specimen of testosterone boosters. Figures 3a and 3b show the DPV results and calibration plots, demonstrating that the testosterone level in prepared specimen is 0.96 mg/ml, which is consistent with the prepared genuine specimen testosterone solution concentration of boosters.[22] Table 2 further shows that for prepared genuine samples of testosterone boosters, satisfactory recovery values (97.52 to 99.02%) and RSD values (2.97 to 3.87%) were obtained. There were no real specimens of blood plasma aimed at individuals undergoing testosterone behavior in a local health center or hospitals to examine the applicability of the sensor in human biological fluids. As a result, a typical addition procedure was used to study the testosterone-free plasma sample. For prepared genuine specimens of human plasma, DPV measurements were performed on PIL/MI/GO/GCE into 0.1M PBS at a 10mV/s scan rate. Table 2 also shows the testosterone levels in a genuine plasma sample as well as the results of the analysis. Acceptable recovery (96.05 to 99.41%) and RSD values are provided (2.56 to 3.52%). As a result, PIL/MI/GO/GCE can be employed as a perfect and consistent testosterone sensor in clinical specimens.

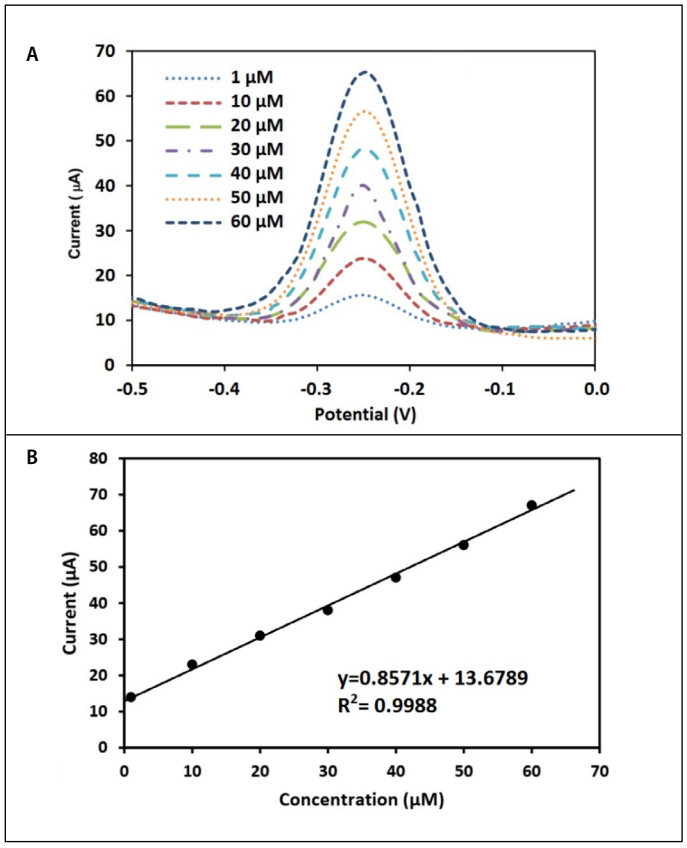

Figure 2. (A) The DPV plots and (B) calibration curve of PIL/MI/GO/GCE in 0.1M PBS at 10mV/s scan rate under successive adding of testosterone solution.

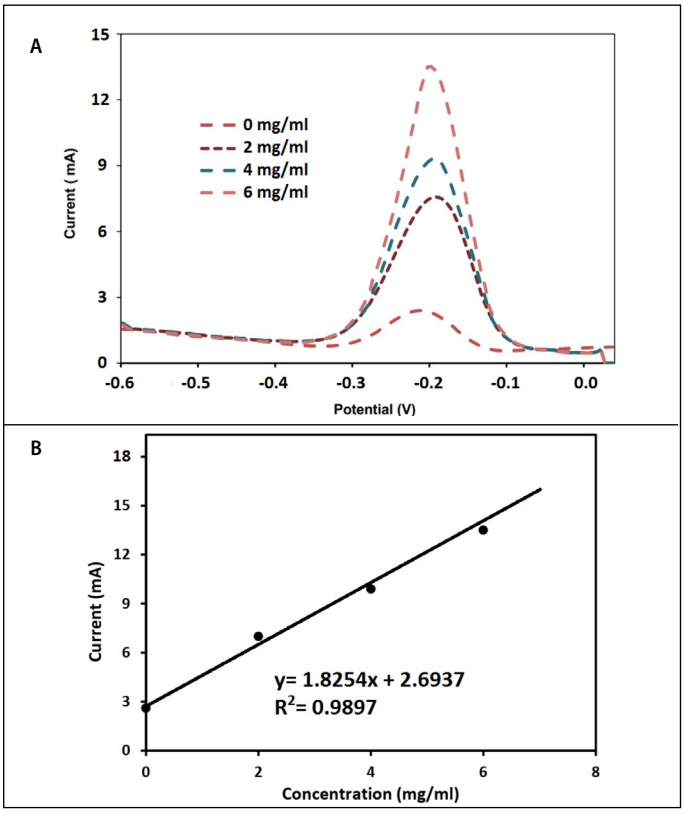

Figure 3. (A) DPV responding and (B) calibration curve of PIL/MI/GO/GCE in a real sample of testosterone boosters made with 0.1M PBS at a 10mV/s scan rate with successive adding of testosterone solution.

Table 1. The electrocatalyst peak value of DPV measures of PIL/MI/GO/GCE at a 10mV/s scan rate for repeated injections of 1µM testosterone and 10M interferents in 0.1M PBS.

Substances	Added(µM)	Peak current(µA)	RSD*(%)
Testosterone	1	0.741	±0.0122
Uric acid	10	0.029	±0.0026
Ascorbic acid	10	0.022	±0.0037
Albumin	10	0.012	±0.0024
Xanthine	10	0.031	±0.0031
Glucose	10	0.037	±0.0073
Hypoxanthine	10	0.009	±0.0026
Dopamine	10	0.022	±0.0074
Nitrite	10	0.021	±0.0113

*Relative standard deviation.

Table 2. The findings of the PIL/MI/GO/GCE for determining testosterone in actual testosterone booster specimens and human plasma.

Samples	Added (mg/ml)	Obtained (mg/ml)	Recovery (%)	RSD (%)
Testosterone boosters	0.00	0.97	-	-
	2.00	1.93	97.52	2.97
	4.00	3.84	96.75	3.13
	6.00	5.93	98.33	2.74
	8.00	7.95	99.02	3.87
Human plasma	0.00	0.00	-	-
	2.00	1.91	96.05	2.56
	4.00	3.92	98.27	2.96
	6.00	5.94	98.48	3.39
	8.00	7.96	99.41	3.52

CONCLUSIONS

The synthesis of PIL/MI/GO/GCE for electrochemical detecting testosterone as a doping substance in sports was presented in this paper. The modified Hummers technique was used to make GO nanosheets that were then electrodeposited onto GCE. PIL was electrodeposited on MI/GO/GCE after MI was changed on GO/GCE. The results revealed cracks and breaks on surface of GO nanosheets, and the morphology of PIL/MI/GO/GCE indicated a high porosity structure because of cavities formed by the aggregation of PIL and MI molecules on the crenelated edges of GO nanostructures. In comparison to other synergetic influence of GO nanosheets with PIL and MI molecules, electrochemical experiments revealed high selectivity, good stability, appropriate linear range, lowest limit of detection, and highest selectivity. In pharmaceutical samples and human biological fluids, the power and accuracy of PIL/MI/GO/GCE for testosterone determination were investigated, and the findings showed suitable recovery and RSD values. As a result, PIL/MI/GO/GCE can be employed as a precise and dependable testosterone sensor in clinical samples.

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

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

REFERENCES

1. Tauchen J, Jurášek M, Huml L, Rimpelová S. Medicinal Use of Testosterone and Related Steroids Revisited. Molecules. 2021;26(4):1032.
2. Aufartová J, Mahugo-Santana C, Sosa-Ferrera Z, Santana-Rodríguez JJ, Nováková L, Solich P. Determination of steroid hormones in biological and environmental samples using green microextraction techniques: An overview. Anal Chim Acta. 2011;704(1-2):33-46.
3. Fang TY, Praveena SM, deBurbure C, Aris AZ, Ismail SNS, Rasdi I. Analytical techniques for steroid estrogens in water samples - A review. Chemosphere. 2016;165:358-68.
4. Homo-Delarche F. Glucocorticoid receptors and steroid sensitivity in normal and neoplastic human lymphoid tissues: a review. Cancer Res. 1984;44(2):431-7.
5. Vedhi C, Eswar R, Gurumallesh Prabu H, Manisankar P. Determination of triamcinolone acetonide steroid on glassy carbon electrode by stripping voltammetric methods. Int J Electrochem Sci. 2008;3:509-18.
6. Tahir S, Yasmeen K, Hanif M, Khaliq O, Muhammad H, Tahiri IA, et al. Electrochemical Methodology for NSAID's Determination and its Interaction with Steroid Dexamethasone. Int J Electrochem Sci. 2019;14:5748-62.
7. Sun Z, An Y, Li H, Zhu H, Lu M. Electrochemical investigation of testosterone using a AuNPs modified electrode. Int J Electrochem Sci. 2017;12:11224-34.
8. Primpray V, Chailapakul O, Tokeshi M, Rojanarata T, Laiwattanapaisal W. A paper-based analytical device coupled with electrochemical detection for the determination of dexamethasone and prednisolone in adulterated traditional medicines. Anal Chimica Acta. 2019;1078:16-23.
9. Balaji K, Reddy TM, Reddy SJ. Determination of prednisolone, dexamethasone and hydrocortisone in pharmaceutical formulations and biological fluid samples by voltammetric techniques using b-cyclodextrin modified carbon paste electrode. Afr J Pharm Pharmacol. 2008;2(8):157-66.
10. Munyentwali A, Zhu L. Electrochemical determination of prednisolone at ordered mesoporous carbon modified electrode: application to doping monitoring. J. Electrochem. Soc. 2015;162(4):H278.
11. Goyal RN, Bishnoi S. Simultaneous voltammetric determination of prednisone and prednisolone in human body fluids. Talanta. 2009;79(3):768-74.
12. Yilmaz S, Skrzypek, Dilgin Y, Yagmur S, Coskun M. Electrochemical oxidation of prednisolone at glassy carbon electrode and its quantitative determination in human serum and tablets by Osteryoung square wave voltammetry. Curr Anal Chem. 2007;3(1):41-6.
13. Smajdor J, Piech R, Paczosa-Bator B. A Novel Method of High Sensitive Determination of Prednisolone on Renewable Mercury Film Silver Based Electrode. Electroanalysis. 2016;28(2):394-400.
14. Zayed SI. Cathodic adsorptive stripping voltammetric determination of prednisolone in pharmaceutical preparation and human urine. Acta Chim Slov. 2011;58(1):75-80.
15. Hummers WS, Offeman RE. Preparation of Graphene oxide. J Am Chem Soc. 1958;80:1339.
16. Mazzotta E, Picca RA, Malitesta C, Piletsky SA, Piletska EV. Development of a sensor prepared by entrapment of MIP particles in electrosynthesised polymer films for electrochemical detection of ephedrine. Biosens Bioelectron. 2008;23(7):1152-6.
17. Li H, Dai S, Miao J, Wu X, Chandrasekharan N, Qiu H, et al. Enhanced thermal conductivity of graphene/polyimide hybrid film via a novel "molecular welding" strategy. Carbon. 2018;126:319-27.
18. Gui R, Guo H, Jin H. Preparation and applications of electrochemical chemosensors based on carbon--nanomaterial-modified molecularly imprinted polymers. Nanoscale Adv. 2019;1(9):3325-63.
19. Fourou H, Braiek M, Bonhomme A, Lagarde F, Zazoua A, Jaffrezic-Renault N. Voltammetric sensor based on a double-layered molecularly imprinted polymer for testosterone. Anal Lett. 2018;51(3):312-22.
20. Alam M, Rahman MM, Asiri AM, Fazal MA. A reliable electrochemical approach for detection of testosterone with CuO-doped CeO2 nanocomposites-coated glassy carbon electrode. J Mater Sci: Mater Electron. 2021;32(4):5259-73.
21. Hussain MM, Asiri AM, Uddin J, Rahman MM. An enzyme free simultaneous detection of γ-amino-butyric acid and testosterone based on copper oxide nanoparticles. RSC Adv. 2021;11(34):20794-805.
22. Çimen D. Testosterone Imprinted poly (HEMA-MAA) Nanoparticles Based Surface Plasmon Resonance Sensor for Detection of Testosterone. ChemistrySelect. 2022;7(5):e202103949.