

SENSOR FOR PREDNISOLONE DETECTION IN SPORTS DOPING

SENSOR PARA DETECÇÃO DE PREDNISOLONA NO DOPING ESPORTIVO

SENSOR PARA LA DETECCIÓN DE PREDNISOLONA EN EL DOPAJE DEPORTIVO



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ABSTRACT

Introduction: Prednisolone causes pro-inflammatory impulses to be inhibited and anti-inflammatory signals to be promoted. As a result, it alters how the body's immune system reacts to certain diseases. The World Anti-Doping Agency, however, has banned SNP and other glucocorticosteroids. An electrochemical sensor can be developed using a gold nanocomposite, polypyrrole nanoparticles and synthesized carbon nanotubes (Au-PPy NPs@CNTs). **Objective:** Develop an electrochemical sensor to detect prednisolone. **Method:** Au-PPy NPs@CNTs nanocomposite was chemically synthesized with a modified glassy carbon electrode (GCE) surface. **Results:** According to SEM data, the nanocomposite was composed of amorphous Au NPs, and PPy NPs deposited in tubes strongly entangled in a CNTs network. The wide linear range and low detection limit of the Au-PPy NPs@CNTs/GCE as prednisolone sensors were attributed to the combined catalytic performance of the Au and PPy NPs@CNTs nanostructures. **Conclusion:** The results of prednisolone detection in each specimen using the amperometric method indicated good accuracy. The accuracy and precision of Au-PPy NPs@CNTs/GCE for prednisolone detection were explored in blood samples from 5 young athletes aged 20-24 years who used prednisolone tablets (RSD less than 4.25%). In addition to monitoring prednisolone concentrations in athletes' serum, Au-PPy NPs@CNTs/GCE can be used as a reliable prednisolone sensor. **Level of evidence II; Therapeutic studies - investigating treatment outcomes.**

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

RESUMO

Introdução: A prednisolona faz com que os impulsos pró-inflamatórios sejam inibidos e os sinais anti-inflamatórios sejam promovidos. Como resultado, ela altera a forma como o sistema imunológico do corpo reage a certas doenças. A Agência Mundial Antidoping, no entanto, proibiu o SNP e outros glucocorticoesteroides. Usando um nanocomposto de ouro, nanopartículas de polipirrol e nanotubos de carbono sintetizados (Au-PPy NPs@CNTs), um sensor eletroquímico pode ser desenvolvido. **Objetivo:** Desenvolver um sensor eletroquímico para detectar a prednisolona. **Método:** O nanocomposto Au-PPy NPs@CNTs foi sintetizado quimicamente com uma superfície de eletrodo de carbono vítreo modificado (GCE). **Resultados:** De acordo com dados da SEM, o nanocomposto foi descoberto como sendo composto de Au NPs e NPs de PPy amorfo, depositados em tubos fortemente emaranhados em uma rede de CNTs. O amplo alcance linear e o baixo limite de detecção do Au-PPy NPs@CNTs/GCE como sensores de prednisolona foram atribuídos ao desempenho catalítico combinado das nanoestruturas de Au e PPy NPs@CNTs. **Conclusão:** Os resultados da detecção de prednisolona em cada espécime usando o método de amperometria indicaram boa precisão. A precisão e a acurácia de Au-PPy NPs@CNTs/GCE para a detecção de prednisolona foram exploradas em amostras de sangue preparadas de 5 atletas jovens de 20 a 24 anos que usaram comprimidos de prednisolona (RSD inferior a 4,25%). Além de monitorar as concentrações de prednisolona no soro dos atletas, Au-PPy NPs@CNTs/GCE pode ser usado como um sensor confiável de prednisolona. **Nível de evidência II; Estudos terapêuticos - investigação dos resultados do tratamento.**

Descritores: Técnicas Biossensoriais; Nanopartículas; Prednisolona; Doping nos Esportes.

RESUMEN

Introducción: La prednisolona hace que se inhiban los impulsos proinflamatorios y se promuevan las señales antiinflamatorias. Como resultado, altera la forma en que el sistema inmunológico del cuerpo reacciona a ciertas enfermedades. Sin embargo, la Agencia Mundial Antidopaje ha prohibido el SNP y otros glucocorticosteroides. Utilizando un nanocompuesto de oro, nanopartículas de polipirrol y nanotubos de carbono sintetizados (Au-PPy NPs@CNTs), se puede desarrollar un sensor electroquímico. **Objetivo:** Desarrollar un sensor electroquímico para detectar la prednisolona. **Método:** Se sintetizó químicamente el nanocompuesto Au-PPy NPs@CNTs con una superficie de electrodo de carbono vítreo (GCE) modificada. **Resultados:** Según los datos del SEM, se comprobó que el nanocompuesto estaba compuesto de Au NPs y NPs de PP amorfo y depositados en tubos fuertemente enredados en una red de CNTs. El amplio rango lineal y el bajo límite de detección de Au-PPy NPs@CNTs/GCE como sensores de prednisolona se atribuyeron al rendimiento catalítico combinado de las nanoestructuras de Au y PPy NPs@CNTs. **Conclusión:** Los resultados de la detección de prednisolona en cada muestra mediante el método amperométrico indicaron una buena precisión. Se exploró la exactitud y precisión de las Au-PPy NPs@CNTs/GCE para la detección de prednisolona



en muestras de sangre preparadas a partir de 5 jóvenes atletas de entre 20 y 24 años de edad que utilizaban tabletas de prednisolona (RSD inferior al 4,25%). Además de controlar las concentraciones de prednisolona en el suero de los deportistas, Au-PPy NPs@CNTs/GCE puede utilizarse como un sensor fiable de prednisolona. **Nivel de evidencia II; Estudios terapéuticos - investigación de los resultados del tratamiento.**

Descriptores: Técnicas Biosensibles; Nanopartículas; Prednisolona; Doping en los Deportes.

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INTRODUCTION

Prednisolone (PNS) is a steroid that is used to treat a wide range of ailments, including arthritis, hormone imbalances, ulcerative colitis, psoriasis, allergic disorders, and kidney and blood cell issues¹. In order for receptor-glucocorticoid developments to migrate into cell nucleus, somewhere they advantages of this method by the Binding area and link to glucocorticoid reaction basics, PNS molecules diffuse through cell membranes and link to glucocorticoid receptors.² It causes pro-inflammatory impulses to be inhibited and anti-inflammatory signals to be promoted. As a result, PNS alters how the body's immune system reacts to certain diseases. Prednisolone's most frequent adverse effects include excessive perspiration, weight gain, indigestion, and insomnia.

The World Anti-Doping Agency, however, has prohibited PNS and other glucocorticosteroids. PNS can be administered orally or intravenously to muscles to lessen the discomfort and inflammation that are frequently brought on by vigorous exercise. Athletes claim that glucocorticosteroids assist them endure the discomfort of intense exercise and enable them to recuperate more quickly for the following event.³ PNS use for a brief period of time can significantly boost performance in submaximal exercise. As a result, it might make it impossible for there to be fair competition. As a result, a lot of research has been done using techniques like liquid chromatography,⁴ mass spectrometry,⁵ gas chromatography,⁶ ultraviolet detection,⁷ fluorogenic DNA Probes,⁸ Raman spectroscopy,⁹ and electrochemical methods¹⁰⁻¹² to determine PNS in clinical specimens and body fluid. Between these techniques, electrochemical methods have been established as a straightforward, inexpensive, and accurate solution for the food and medical industries, as well as for environmental monitoring. By modifying electrodes with a variety of nanostructures, bio-recognition components, and compositions, electrochemical sensors' durability, selectivity, and sensitivity can be improved. In order to detect PNS as just doping agents in sport, this study absorbed on the development of electrochemical sensors are based on an electrode modified with Au-PPyNPs@CNTs.

METHOD

Exprimental details

The precursor was ultrasonically added to 1 ml of L-cysteine hydrochloride monohydrate and 1 ml of cysteamine aqueous solution for the production of Au NPs.¹³ 1 ml of 10 mM NaBH₄ aqueous solution was gradually added to the solution after 20 minutes of ultrasonication. The resulting mixture was then allowed to react for 120 minutes. Six grams of polyvinyl alcohol were added to the solution and stirred magnetically for 30 minutes in order to create the Au-PPy NPs.¹⁴ Au NPs and PPy NPs have a constant mass ratio of 1:1. The polymerization reaction was then started by adding 3 g of FeCl₃ to the product while magnetic stirring it at 6°C. After 10 hours, the liquid phase was filtered to remove the sediments, and the sediments were then washed with deionized water. The generated Au-PPy NPs were disseminated in ethanol using ultrasonography.

CNTs were refluxed for an entire night at 90 °C in 3 M HNO₃, which led to minor oxidation and functionalization of the side walls of the CNTs.¹⁵ The treated CNTs were then filtered, washed with ultrasonic energy, and dried. After that, the CNTs were dissolved using ultrasonic technology in ethanol and introduced to the suspension of Au-PPy NPs. The resulting mixture was then poured onto GCE and allowed to dry to create the Au-PPy NPs@CNTs nanocomposites. The mass ratio of CNTs to Au-PPy NPs was set at 3:1.

Five young athletes, aged 20 to 24, whose blood serum was provided, used PNS pills, which each contain 10 mg of PNS. After 3 days of taking the tablets, plasma serum samples were given, and they were centrifuged at 2000 rpm for 10 min. The supernatants that were produced were harvested and used to make 0.1M PBS that was used as actual samples. Au-PPy NPs@CNTs were used in produced genuine samples from blood samples at -0.95V to use the amperometric to detect the PNS concentration in serum samples. PNS levels in blood serum samples were also examined using enzyme-linked immunosorbent test kit (ELISA). Scanning electron microscopy (SEM) was used to describe the morphologies of changed electrodes. All the procedures and the investigation in human subjects were conducted based on the Malaysian Guideline for Independent Ethics Committee Registration and Inspection, and National Statement on Ethical Conduct in Human Research with approval H21REA1103/HREC.

RESULTS AND DISCUSSIONS

The SEM images of the CNTs and Au-PPyNPs@CNTs materials are displayed in Figure 1. Figure 1a's SEM picture of the CNTs film shows a network of densely tangled tubes with sizes between 50-100 nm that are dispersed across the GCE during in the drop casting. Figure 1b showed that the Au-PPy NPs@CNTs nanocomposite was made up of Au-PPy NPs that had been deposited on the f-CNT network. The Au-PPy NPs are attributed to the adsorption of CNTs by chemical complex interaction as a result of the abundant functional groups of CNTs. As can be seen, pure Au NPs are covered by the PPy layer because the surfaces of Au-PPy NPs is uniform.¹⁶ The electrocatalytic activity may be improved by the formation of additional active sites on Au-PPyNPs@CNTs. Additionally, the polymer matrix makes it easier for CNTs to effectively transmit load from a polymer matrix.¹⁷

1µm The DPV curves for GCE,CNTs/GCE, Au@CNTs/GCE, and Au-PPy NPs@CNTs with 0.1M PBS at a scanning speed of 10mV/s in the absence and presence of 5µM PNS are shown in Figure 2 for potential ranges

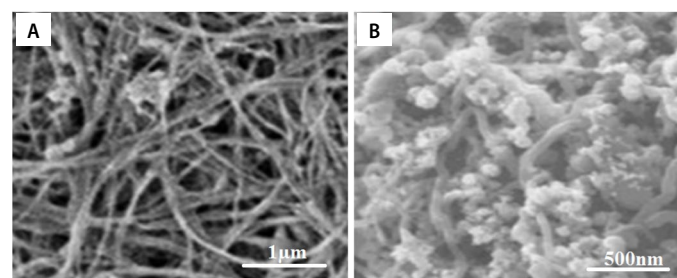


Figure 1. SEMs of (a)CNTs, (b)Au-PPyNPs@CNTs nanocomposites.

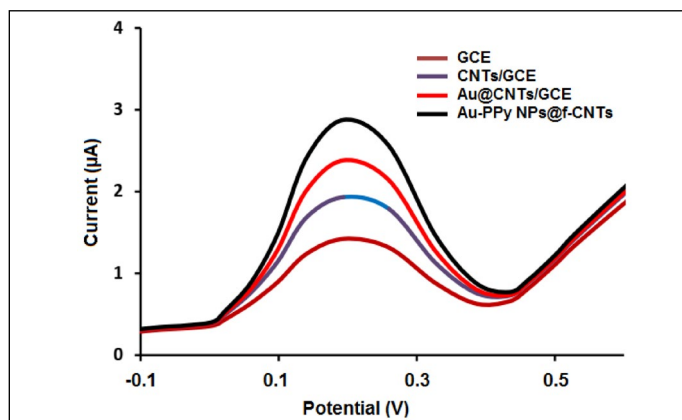


Figure 2. DPV curves of GCE, CNTs/GCE, Au@CNTs/GCE and Au-PPy NPs@CNTs in 0.1M PBS at 10mV/s scan rate into 5µM PNS.

of -0.1 to 0.6V. Prior to the addition of 5µM PNS, no redox peak could be seen on the DPV graphs of GCE, CNTs/GCE, Au@CNTs/GCE and Au-PPy NPs@CNTs. Anodic peak at 0.25V after adding 5µM PNS to the electrochemical cell was attributed to the decrease of PNS.¹¹ The keto group at position 3 of prednisolone, which provide a carbonyl group coupled with the double bond and produce matching hydroxyl into 2H⁺, 2e reaction, are the sites suitable for reduction.^{10,11} A Au-PPy NPs@CNTs exhibits the larger electrocatalytic current when the DPV curves of the electrodes are compared, showing a sensitive reaction because of the synergic catalytic action of the nanocomposite Au-PPy NPs@CNTs. Additionally, the CNTs/GCE and Au@CNTs/GCE comparisons demonstrate how Au nanostructures enhance catalytic activity, and the mixture of Au NPs and CNTs has produced a new category of hybrid nanostructures with effective ionic diffusion, that also led to an increase in charge transport due to the formation of globular diffusion regions from around electrode materials.¹⁸

Additional research was done to assess the stability of the electrodes' response to the additional PNS. First and 40th DPV curve for the GCE, CNTs/GCE, Au@CNTs/GCE, and Au-PPy NPs@CNTs within 0.1M PBS containing 5µM PNS are shown in Figure 3 at scan rates of 10mV/s and potential ranges of -0.1 to 0.5V, respectively. After 40 further scans, it can be seen that the maximum peak density of electrodes at -0.95V decreases. The peak current change for GCE, CNTs/GCE, Au@CNTs/GCE, and Au-PPy NPs@CNTs has been observed to be 24 percent, 13 percent, 14 percent, and 3 percent, respectively, after 40 continuous scans. This shows the advanced stability of the electrochemical reactions of Au-PPy NPs@CNTs due to the mixture of -ATP molecule with the very stable nanostructure of CNTs and AuNPs.

Additionally, self-assembled of -ATP molecule was generated on the CNTs and Au nanostructures by thiol groups during the electropolymerization, which pointed to the material's high stability.^{19,20} For the following electrochemical investigations on PNS, Au-PPy NPs@CNTs were employed as a extremely sensitive and improved electrode.

Au-PPy NPs@CNTs were successively injected with 20M PNS solution with 0.1M PBS, and Figure 4 shows the amperometric reaction and subsequent calibration curve. With each PNS injection into the electrochemical cell, I can see a linear improvement in the amperometric current. The suggested sensor's sensitivity, detection limit, and linear-range are determined to be 0.1984 µA/µM, 0.005 µM, and 0-200 µM, respectively. The wide-linear range and reduced limit of detection of Au-PPy NPs@CNTs as a PNS sensor, which are due to the nanocomposite's synergistic catalytic action, were demonstrated when these sensing properties are compared to those of other PNS sensors. Due to their strong conductivity and substantial specific surface area, CNTs and Au nanostructures improve sensitivity.²¹ In order to produce stable

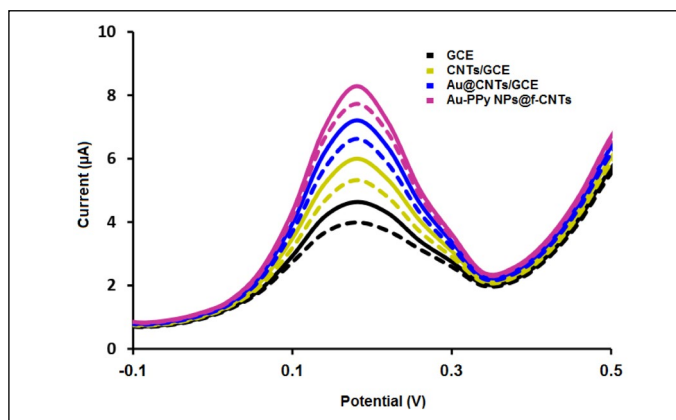


Figure 3. First (solid-line) and 40th (dashed-line) DPV curves of GCE, CNTs/GCE, Au@CNTs/GCE and Au-PPy NPs@CNTs into 0.1M PBS having 5µM PNS at 10mV/s scan rate.

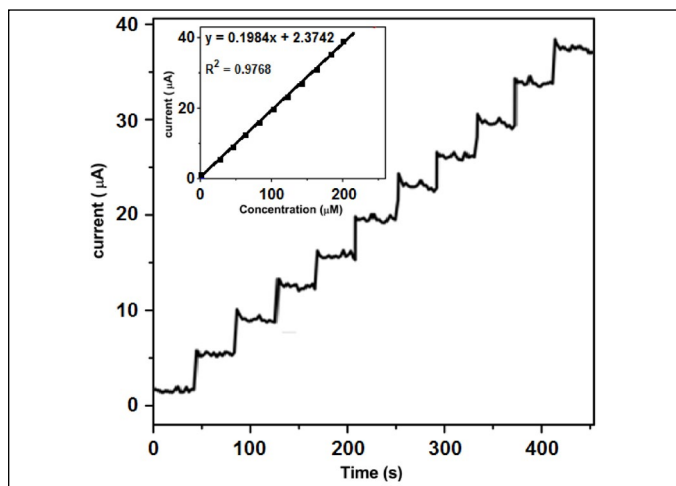


Figure 4. Amperometric and calibration plots of Au-PPy NPs@CNTs to consecutive injection of 20µM PNS solutions into 0.1M PBS.

host-guest complexes, the functional monomer p-ATP interacts strongly with the nanocomposite.^{22, 23}

Using the amperometry technique in 0.1M PBS, the sensitivity of Au-PPy NPs@CNTs as just a PNS sensor was further investigated in the presence of numerous metabolic compounds in bodily fluids and medications as interfering agents. After adding 1µM PNS and 20µM interfering agents successively, the resultant amperometric current is shown in Table 1. The amperometric reaction of Au-PPy NPs@CNTs to the addition of PNS was demonstrably higher than that of interference agents, and trying to interfere agents exhibit negligible electrocatalytic signals, according to the results. As a result, employing Au-PPy NPs@CNTs to determine PNS is not hindered by the interference agents in Table 1. The effective recognition locations inside the molecularly imprinted polymeric membrane for target molecule are related to the sensitivities of Au-PPy NPs@CNTs.²⁴ Additionally, the polymerization of polymer electrolytes onto Au@CNTs nanocomposites, that supports biocompatibility and electroactivity, improves the conductance of molecularly imprinted sensors.²⁵

CONCLUSIONS

In order to identify PNS as a sport doping agent, Au-PPy NPs@CNTs were electrochemically studied in this work. In order to create the sensors, the Au@CNTs composites was electropolymerized with ATP and TBAP on the Au@CNTs/GCE surface. According to the results of SEM and XRD investigations, the Au NPs were efficiently electropolymerized on Au@CNTs/GCE and were uniformly deposited on CNT porous networks in

Table 1. The amperometric measurement of Au-PPy NPs@CNTs into 0.1M PBS after consecutive adding of 1µM PNS and 20µM interfering agents.

Electrode	Added(µM)	Peak current responses(µA)	RSD(%)
Prednisolone	1	0.8712	±0.0117
Ascorbic Acid	20	0.0331	±0.0015
Dopamine	20	0.0162	±0.0017
Uric Acid	20	0.0128	±0.0013
Ephedrine	20	0.0228	±0.0011
Acetaminophen	20	0.0209	±0.0014
Nitrite	20	0.0241	±0.0018
Xanthine	20	0.0309	±0.0025
Chlordiazepoxide	20	0.0111	±0.0018
Albumin	20	0.0432	±0.0028
Diazepam	20	0.0643	±0.0047
Hypoxanthine	20	0.0333	±0.0029
Metolazone	20	0.0269	±0.0023
Glucose	20	0.0178	±0.0019
Naproxen	20	0.0184	±0.0016

AUTHORS' CONTRIBUTIONS: The work is conceived by Wenping Wang, its knowledge content is completed by Mohd Rozilee Wazir Norjali Wazir, investigated by Soh Kim Geok, formal analysis by Yongqi Gao, manuscript is drafted by Wei Xiong. Each authors contributed equally in execution and writing of this manuscript.

the Au-PPy NPs@f-CNT nanocomposite. The sensitivity, detection limit, and linear range of the proposed sensor were achieved at 0.1984 µA/µM, 0.005 µM, and 0-200 µM, respectively, according to the results of electrochemical investigations, indicating stable, selective, and sensitive performances of Au-PPy NPs@CNTs to detect PNS. It was found that Au-PPy NPs@CNTs had a wider linear-range and small detection limit when compared to PNS sensors that had been described in the literature. In synthesized samples of serum samples from 5 young volunteer groups who used PNS tablets, the validity and precision of Au-PPy NPs@CNTs to determine PNS were examined. The results of the assessments of PNS for every specimen using amperometry and ELISA approaches exhibit better precision and accord between both ELISA and amperometry measuring device on Au-PPy NPs@CNTs. Therefore, it is possible to monitor the level of PNS in athletes' blood serum using Au-PPy NPs@CNTs as a trustworthy PNS sensor.

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

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