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Review

A Review: Electrochemical Biosensors for Testosterone Detection

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Abstract- Male development is greatly influenced by testosterone, which also helps to build bone and muscle, as well as the testis, penis, and prostate. Endocrinology relies heavily on hormone and related biomarkers measurements. Chromatographic techniques are commonly used to analyze hormones because they offer excellent separation and quantification capabilities. Due to their usability, portability, simplicity, and sensitivity, electrochemical biosensors have grown more and more in popularity over time for hormone detection. Electrochemical biosensors are divided into four groups: impedimetric, potentiometric, amperometric, and conductometric. Biosensors based on electrochemistry are ideal for early clinical analysis, but their commercialization is hindered by poor reproducibility. An overview of electrochemical biosensors that detect testosterone hormones is presented in our review. After an introduction to biosensors and electrochemical biosensors, testosterone hormone, the immobilization process and analytical performance of the electrochemical biosensors will be discussed. The linear ranges, the limits of detection, reproducibilities, and regenerations of developed biosensors are discussed in this paper.

Keywords- Testosterone; Electrochemical sensor; Biosensors; Bioreceptor; Nanomaterial

1. INTRODUCTION

In medical diagnostics, laboratory measurements have become increasingly important. A modern endocrinologist measures the hormone concentration in target tissues and the output of endocrine glands [1]. Any increase or decrease in hormone levels can have significant effects on the environment and human health, so monitoring them continuously is essential [2]. Usually, all hormones are secreted by glands and travel via diffusion or blood circulation to their target cells. Hormones can bind to plasma membrane receptors and stimulate cell responses without entering the cell. It is also possible for other hormones to enter the target cell and interact with its receptors [3]. The most important androgen male hormone is testosterone. Male spermatogenesis, sex characteristics, and fertility are controlled by testosterone. Testosterone has a critical function in the first development of sexual characteristics, including enlargement of the penis and testes, spermatogenesis, and an increase in libido, all of which is mediated by testosterone. Testosterone regulates secondary masculine traits. There are also anabolic effects such as puberty growth spurts and skeletal muscle growth, as well as male hair patterns and vocal changes. ales have a higher hematocrit because testosterone increases erythropoiesis. Figure 1 shows the scheme of Testosterone hormone functions. Men typically experience a decline in testicular size, a loss of libido, decrease of muscle, fat mass increased, and a decrease in erythropoiesis that may result in anemia as their testosterone levels decline with age [4].

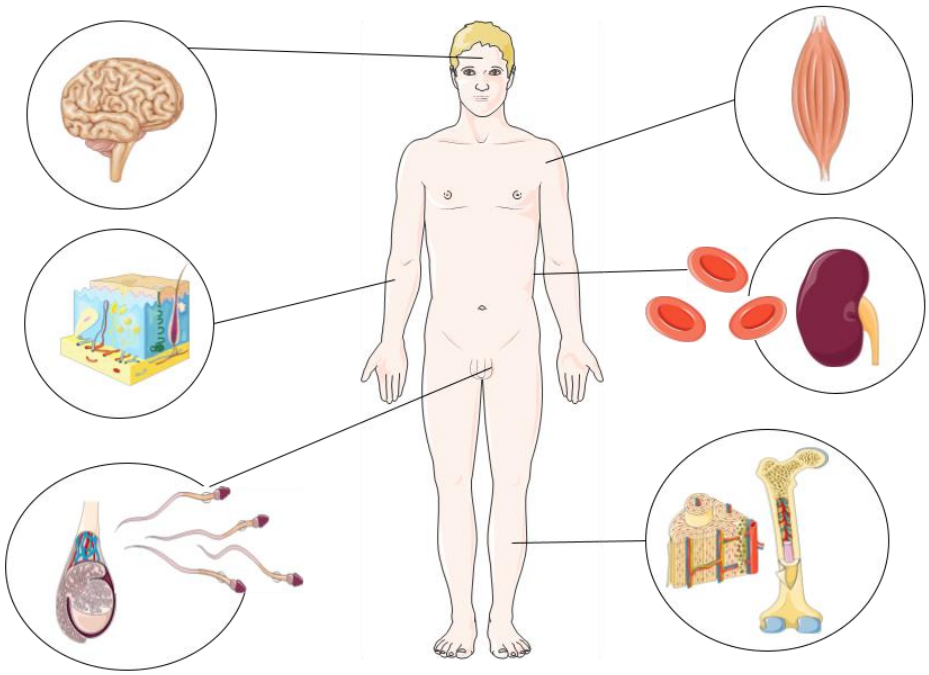

Figure 1. A diagram showing how Testosterone hormone works

The testes have cells called Leydig cells that change cholesterol into testosterone. The initial stage of this process is regulated by LH. Dehydroepiandrosterone and androstenedione are two intermediates in this process. There is an enzyme called 17-beta-hydroxysteroid dehydrogenase that changes androstenedione into testosterone. The majority of testosterone is bound to albumin and sex hormone binding globulin. Thanks to this supply of testosterone, which is mostly associated with proteins, the body receives an additional amount of testosterone. There is a direct impact of free testosterone in the blood on the seminal vesicles, the bones, the muscles, as well as the prostate gland. At the level of the cell, the enzyme 5-alpha-reductase changes testosterone into dihydrotestosterone. Both testosterone and dihydrotestosterone have the ability to bind to cellular receptors, regulating protein expression [5]. Therefore, both sexes should have levels of testosterone within normal ranges as a result of this. There is no doubt that testosterone and its analogues play an important role in global doping in sports. Therefore, hormone quantification is becoming increasingly important in health monitoring.

Usually, hormones circulate at low concentrations and ultra-high sensitivity is needed to detect hormones in order to diagnose diseases. Various methods for detecting hormones have been developed in recent years. Many of them involve lengthy procedures and need qualified personnel to carry out adequate analysis [6-8].

Techniques such as HPLC (High Performance Liquid Chromatography), HPLC-MS (HPLC/Mass Spectrometry), MS-GC (Mass Spectrometry/Gas Chromatography) are often used for hormone analysis with high sensitivity and high selectivity [9]. Hormone detection with electrochemical techniques is much faster, more sensitive, and less expensive than these techniques. These techniques don't require expensive equipment or specialists [10-12].

Using electrochemical sensors and biosensors for medical or pharmaceutical research is a quick and cheap option [12,13-17]. The use of biosensors as an alternative to complex bioanalytical systems is becoming increasingly popular due to their ease of use and portability in treating relatively complex samples. A biosensor consists of the bio-receptor and the transducer. The first component, known as the bio-receptor, is a biomolecule that is capable of recognizing the target analytes, and the second component, the so-called converter or transducer is responsible for converting the detection event into a measured signal. Bio-recognition components can be anything from an enzyme to an antibody to nucleic acid to a lectin to a hormone to a cell structure or even a tissue [18].

Enzymes or antibodies are frequently used as bio-recognition components in electrochemical biosensors that detect hormones. The idea behind electrochemical biosensors is that target compounds react chemically with chemically immobilized molecules to produce electrons that alter the solution properties [6]. Since the reaction modifies the solution's electrical properties, four distinct types of electrochemical transducers have emerged to account for these changes: amperometric transducers, which measure changes in current,

potentiometric transducers, which measure changes in potential, conductometric transducers, which measure changes in the medium's conductivity, and impedimetric transducers, which measure changes in the medium's impedance between electrodes [19]. Figure 2 shows the scheme of biosensor. Some issues with biosensing processes include the fact that biomolecules do not last forever, biosensors must sometimes be treated in a specific way before they can be used, and some sensors do not remain stable over time. We now have to differentiate between biosensors that are good for only one measurement and those that can be reused and recalibrated a large number of times. The important characteristics of biosensors include selectivity, sensitivity, response time linear, regeneration, concentration range and lifetime, etc. [20].

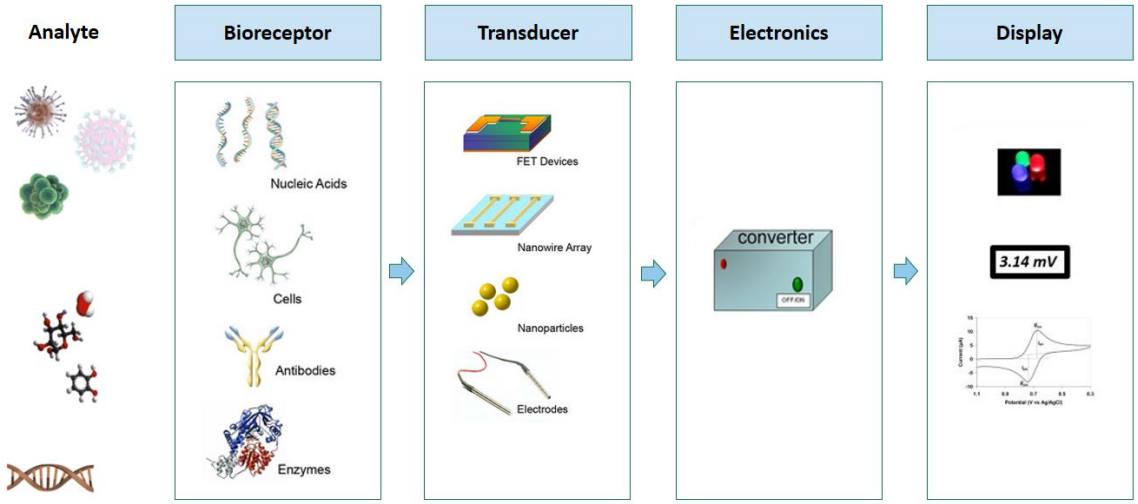

Figure 2. shows the scheme of biosensor

Biosensors and nano biosensors are useful tools for medical diagnosis because of their small size, speed of response, and low cost [21-23]. A nano biosensor is a piece of analytical equipment that looks for the presence of chemicals molecules in the samples [24].

The use of electrochemical sensors based on nanomaterials is an emerging class of sensor technology that finds applications in environmental monitoring, food analysis, and disease diagnosis [28-31]. Different nanomaterials such as inorganic nanoparticles, quantum dot and carbons can be used in electrochemical and colorimetric sensors [32]. Nanoparticles with high surface to volume ratio show unique and extraordinary properties and in many cases are different from bulk materials [33]. These unique and extraordinary properties have caused the application of nanoparticles to expand in different fields such as energy, photocatalysts, biotechnology and almost all other practical and technological fields [34]. Due to the creative evolution of nanostructures and their wide applications in various sciences, during recent years, various methods, including sol-gel [35-37], co-precipitation [38-40] solid-state [41], and sonochemistry [42-44] have been developed to fabricated nanostructures. Since the

morphology and size of nanostructures are crucial factors in how well they perform in various applications, scientists have tried to synthesize different nanostructures with different morphologies and sizes by changing the conditions affecting the properties of nanostructures in these methods [45-50]. For example, by varying the concentration of reactants, temperature, and surfactants used in the co-precipitation method, the size and morphology of the synthesized nanostructures can be controlled [51-55]. Also, in the hydrothermal method, it is possible to control the size and morphology of nanostructures by management the temperature, concentration of reactants, reaction time and some other parameters [56,57].

Biosensors can be categorized according to the bio-recognition elements used for target detection (Nucleic acids, antigen, enzyme and etc.). Furthermore, biosensors are frequently classified based on the transduction methods they use: electrochemical, piezoelectric and optical [58]. In medical diagnostics, optical biosensors and electrochemical biosensors are most useful [59].

When chemical information is transformed into an electrical signal and analyzed, the sensor is referred to as an electrochemical sensor (amperometric, potentiometric, conductometric/ impedimetric biosensors). Electrochemical biosensors have a high affinity for detecting medical diagnostic analytes (Cancer antigens, markers for heart attacks, medications, hormones, allergies and antibodies) [60].

Today accurate and novel biosensors have been developed for testosterone testing. The review compares various types of electrochemical biosensors used for testosterone detection. Amperometric, impedimetric, potentiometric, and conductometric biosensors were inspected. Following a concise explanation of the testosterone hormone, a summary of these biosensors' structures as well as their analytical capabilities is presented. We compared LOD (limit of detection) and linear range. An amplifier's linear range is the input range for which it produces linear output. Depending on the resolution and range of analyte concentrations tested, the linearity of the test is affected. A substance's LOD is the lowest detectable amount. The LOD depends on the instrument's sensitivity and resolution [61].

2. DETECTION OF TESTOSTERONE USING AMPEROMETRIC BIOSENSORS

Biosensors are mostly amperometric, where oxidation or reduction of an active species causes a current [30,31,62-65]. At a fixed potential, a constant amplitude voltage is maintained at the working electrode (usually carbon, platinum, gold), and current is passed through the sample. Usually, current is measured with a constant potential, which is called the amperometric method. Voltammetry measures current during a controlled change of potential. There are many advantages to using the amperometric sensor, including its availability, simplicity, and cost [66].

A disposable electrochemical immunosensor that detects testosterone in serum was developed by Egulaz and colleagues. Testosterone measurements were performed by

amperometry, and calibration curves for testosterone were obtained under optimized conditions with a linear range between 5.0×10^{-3} and 50 ng/ml. There was a detection limit of 1.8 pg/mL and an EC_{50} of 0.25±0. Analyzing human blood serum comprising between 1 and 10 ng/mL of testosterone. demonstrated the application of the immunosensor, as well as good selectivity for other steroid hormones. Furthermore, this immunosensor was found to be stable for at least 25 days [67].

Another study used immunochromatographic assays to measure testosterone. Utilizing an electrode chip, nitrocellulose membrane electrochemical detection was combined. Three electrodes—a gold working electrode, a counter electrode, and a pseudo-reference electrode were constructed into the chip's bottom. Over the membrane, testosterone was incubated with HP-labeled testosterone (a competitor) to initiate competitive immunoreactions. An electrode chip was well filled with ferrocenemethanol (FcOH) and H_2O_2 solution, and amperometry was used to detect enzyme reaction. Using HRP-labeled membranes, FcOH was oxidized and then reduced electrochemically by electrode chips by labeling HRP on them. Increasing testosterone concentration decreased the electrochemical response of the reduction current [68].

In other study, gold nanoparticle-carbon nanotube-Teflon composite electrodes were used as a testosterone electrochemical immunosensor. By coupling carbon nanotubes with gold nanoparticles, this material was able to immobilize biomolecules while retaining their biological activity. In this experiment, testosterone and HRP-testedosterone were compared for binding site affinity. In the presence of H_2O_2 and catechol as redox mediators, an affinity reaction was monitored by amperometry at 0.05 volts vs Ag/AgCl. Within a range of point 0.1 and 10 ng/mL testosterone, the calibration plot was linear.Approximately the LOD was 85 pg/mL in spiked human serum [69].

Another study described an electrochemical immunoassay that contains anti-testosterone antibodies in sol-gel polyvinyl butyral films doped with gold nanowires. In this study, an antibody for testosterone was conjugated covalently to a surface of gold nanowires formed using nanopore polycarbonate membranes made from nanoporous polycarbonate. In addition to increasing the amounts of immobilized biomolecules and improving the immunosensor's sensitivity, gold nanowires can also create a biocompatible microenvironment for biomolecules. Biomolecule leakage from the composite film was prevented by gold nanowire-functionalized probes as opposed to gold nanoparticle-conjugating probes. When optimal conditions are met, the developed immunosensor showed good linearity with testosterone, ranging from 1.2 to 83.5 ng ml^{-1}, with a detection limit of 0.1 ng ml^{-1} (at 3δ). Furthermore, it showed high sensitivity, good reproducibility, and long-term stability. Testosterone levels in human serum samples were measured using immunosensors as prepared. Using the immunoassay developed for testosterone detection in clinical diagnosis is a promising alternative method. Unlike conventional ELISAs, the proposed immunoassay method had no multiple labeling or separation steps [70].

To detect 19-nortestosterone and methyltestosterone in urine, Conneely and colleagues developed disposable immunosensors that use screen-printed electrodes. In the early stages of the development of electrochemical immunosensors, an ELISA (enzyme-linked immunosorbent assay) was developed first. A chronoamperometric detection of horseradish peroxidase (HRP) was carried out using tetramethylbenzidine/hydrogen peroxide (TMB/H_2O_2) substrate system at +100 mV with HRP as the enzyme label. The assays were indirect, and used immobilised antigen-protein conjugates. Nortestosterone immunosensor has an EC50 value of 936 pg/ml, with a LOD value of 10.5 pg/ml and EC50 of methyltestosterone in urine was 274 pg/ml with LOD of 14.8 pg/ml. There were cross-reactive responses between nortestosterone and testosterone, as well as α-testosterone. Methylboldenone showed the most significant cross-reactivity with anti-methyltestosterone. Both immunosensors were found to be accurate, precise, and stable [71].

The Conneely and colleague study also developed a screen-printed electrode immunosensor for detecting testosterone in bovine urine. Regulations in the EU prohibit the use of steroid hormones to stimulate animal growth that are used for food production. For the purpose of identifying the use of these compounds illegally, all member states have put in place strict screening procedures. It has been successfully used an ELISA to develop competitive immunoassays, which have then been adapted into an electrochemical immunosensor format by printing disposable carbon electrodes on a screen in order to develop competitive immunoassays. Chronoamperometric detection was performed with a TMB/H_2O_2 substrate system at +100 mV using HRP as the enzyme label. In both buffer and urine, the EC50 values were relatively similar, 710 pg mL^{-1} and 960 pg mL^{-1} respectively. In urine, it ranged from 0.03 ng mL^{-1} to 1.6 ng mL^{-1}; In buffer, the linear range was 0.03 ng mL^{-1} to 40 ng mL^{-1}. In urine and buffer, LODs were 1.8 pg mL^{-1} and 26 pg mL^{-1} respectively. The antibody showed notable cross reactivities with 19-nortestosterone and boldenone 11.6% and 9.86% respectively. Reproducibility and repeatability of the sensor were adequate. The recovery information obtained from spiking studies and the known analyte concentrations were found to be in good agreement. In a temperature range of +4 degrees Celsius, sensors remained stable for 4 days. In order to detect testosterone in bovine urine, a sensitive, highly specific, inexpensive, disposable immunosensor was developed. The device displayed excellent overall performance in detecting testosterone [72].

Any study used an electrochemical sensor based on recombinant Fab fragments to detect testosterone in bovine urine. This technique consists of a screen-printed electrode with a testosterone conjugate on the surface, followed by a recognition of the testosterone conjugate and then by an anti-testosterone Fab fragment. Competition was determined by using a conjugate of IgG to horseradish peroxidase. A 100 mV potential was used for chronoamperometry to measure the product of 3,3',5,5'-tetramethylbenzidine catalysis. Assays were examined using ELISA before being transferred to SPE. In the final sensor, the linear

range was 300-40,000 ppm, while the detection limit was 90±13 ppm. It's also possible to determine testosterone directly after dilution without extraction or hydrolysis thanks to the developed Fab sensor. Several quantitative and semi-quantitative results were obtained by comparing administered bovine urine samples with GC-MS data, allowing the identification of suspect samples for additional examination. The suggested method offers additional benefits for meat quality control in addition to its simplicity, low detection limit, and repeatability [73].

For the detection of testosterone, the researchers used a new benzenediamine-benzodithiophene polymer. Drop coating of poly(benzenediamine-Bis[(2-ethylhexyl)oxy]benzodithiophene) (pBDBT) onto a screen-printed carbon electrode produces the sensory platform. Glutaraldehyde attaches to the electrode backbone via amino functional groups and immobilizes testosterone antibodies. Various electrochemical techniques are used to investigate testosterone binding, including electrochemical impedance spectrometry, cyclic voltammetry, differential pulse voltammetry and contact angle measurements. Using atomic force microscopy, modified electrode surfaces are characterized. In this step, the linear range of the sensor will be calculated, as well as its detection limit. The sensory platform is also used to analyze testosterone in synthetic biological fluids [74].

The use of amperometric microsensors and modified carbon nanoparticles was used in another study in order to measure estradiol, dihydrotestosterone, and testosterone levels in children's saliva. Differential pulse voltammetry (DPV) was used to analyze the response characteristics of microsensors. Carbon nanotube-based microsensors could only measure testosterone and dihydrotestosterone, whereas carbon nanoparticle microsensors modified with maltodextrin measured estradiol, dihydrotestosterone, and testosterone. A carbon nanoparticle microsensor modified with maltodextrin was used to detect testosterone at the lowest detection limit of 0.427 pmol L^{-1}, and a microsensor based on carbon nanotubes was used to detect dihydrotestosterone at the lowest detection limit of 0.442 pmol L^{-1}. In order to construct a highly sensitive estradiol microsensor (0.555 p mol L^{-1}), carbon nanoparticles and maltodextrin were combined. In children's saliva, estradiol, dihydrotestosterone, and testosterone were reliably measured using the two microsensors. Due to ELISA's high detection limits, it is inappropriate to use it to measure these hormones in children's saliva [75].

Graphene oxide/glassy carbon electrodes (rGO/GCE) were used in another study for testosterone electro-reduction. There was an increase in testosterone reduction peaks with the use of CTAB, a cationic surfactant. In contrast to Ag/AgCl, CTAB-testosterone displayed a reduction peak at -1.1 V in a borate buffer (pH 5.4). With testosterone concentrations of 2.0 to 210.0 nM, A rectilinear increment of peak current was obtained by subtracting CTAB-testosterone's reduction peak current, with a detection limit of 0.1 nM. The sensor was employed to measure the amount of testosterone present in drugs and biological fluids [76].

A new technique to measure testosterone levels in aqueous and aqueous/surfactant solutions using bismuth-film electrodes (BiFEs) was developed as part of a new study.

Bismuth-film electrodes (BiFEs) were created by ex-situ electrodeposition onto glassy carbon electrodes. In cyclic voltammetry, the substance displayed a single irreversible and adsorption-controlled reduction peak. Based on the findings of BiFE, testosterone concentrations ranging from 1 to 45 nmol L^{-1} in Britton-Robinson buffer, pH 5.0, containing 3 mmol L^{-1} cetyltrimethylammonium bromide, demonstrated good linear response. Limits of detection were 0.3 nmol L^{-1} (0.09 ng mL^{-1}). To conclude, the BiFE was successfully applied to quantify testosterone in pharmaceutical samples (oil-based ampoules) as well as biological samples (human urine) [77].

A glassy carbon (GC) electrode was used in a different study to look into the electrochemistry of testosterone in aqueous and aqueous/surfactant solutions. An irreversible, adsorption-controlled reduction peak was observed by cyclic voltammetry. As a result of an interaction between a cationic surfactant CTAB (cetyltrimethylammonium bromide) and an anionic surfactant SDS (sodium dodecylsulfate), and a non-ionic surfactant (Tween 80), the reduction current signal of testosterone was enhanced. In Britton-Robinson buffer with pH 5.0 containing 3 mM CTAB, the current increased linearly with concentration when measured using sq.are-wave adsorptive stripping voltammetry. For a concentration level of 35 nM (n = 11), it was determined that the detection limit would be 1.18 nM (0.34 ng mL^{-1}) and the relative standard deviation would be 4.12 percent. This method worked for testosterone analysis in oil-based pharmaceutical preparations and urine samples without separation [78].

Voltammetry was used to determine the levels of testosterone and epitestosterone using EPPGE electrodes that had been SWNT-modified. The simultaneous determination of isomeric steroids has been performed using square wave voltammetry (OSWV). It was noted that well-defined voltammetric peaks were seen as the two isomers were reduced in a pH-dependent, 2e, 2H process. Under ideal experimental conditions, both steroid calibration curves are linear for concentrations between 5 and 1000 nM, and the detection limits for testosterone and epitestosterone are 2×10^{-9} and 4×10^{-9} M, respectively. Both normal and testosterone-treated urine samples are successfully analyzed using the developed protocol. Comparing the voltammetric results with HPLC results, similar results were obtained from the proposed method [79].

The determination of nandrolone using Osteryoung square wave voltammetry (OSWV) in phosphate buffer media was investigated using edge plane pyrolytic graphite substrates, indium tin oxide, glassy carbon, and gold. Basal plane pyrolytic graphite substrates were also compared. To ascertain the impact of embedded metallic fullerene impurities on the determination of nandrolone, the voltammetric response of nandrolone on untreated, purified, and super-purified edge plane pyrolytic graphite electrodes was examined. Among the substrates studied, EPPGE has been found to serve as the best one for casting fullerene. Nandrolone's peak potential shifts to a more positive potential without metals, and peak current decreases. This calibration curve exhibits linearity over the concentration range of 0–50 nM,

with detection limits and sensitivity of 1–5 10^{-11} M and 1–838 A nM^{-1}, respectively. A number of commercially available medicinal samples were successfully tested for the presence of nandrolone using the developed method [80].

3. BIOSENSORS FOR THE DETECTION OF TESTOSTERONE BY POTENTIOMETRY

When conducting potentiometric measurements, a voltammeter is used to determine the potential difference between the working electrode and the reference electrode when no current is flowing. As species in sample solution oxidize and reduce, the potential difference is measured. Potentiometry is also used to determine ion activity in electrochemical reactions. There are several types of transducers, including anion selective electrodes (ISEs) and selective membranes as recognition elements. A potential signal is generated when the ISE converts bio-recognition into analytical information [81].

In a study, it was shown how to detect the anabolic steroids stanozolol (Stz) and methylboldenone (MB) by using arrays of carbon nanotube field-effect transistors (CNTFETs), two specific antibodies, and specific antibodies. Antibodies specific for Stz and MB were immobilized on carbon nanotubes (CNTs) either noncovalently or covalently. A covalent or noncovalent bond between CNTFETs and specific antibodies allowed steroid detection. Transistors recognized steroids based on changes in threshold voltage and drain current that were statistically significant. The specific antibodies could only detect Stz and MB in the steroid samples because there were no statistically significant changes in the transistors. As a result of the polymer, antibodies on the electrodes do not aggregate and transistor hysteresis is decreased. However, this method cannot prevent non-specific adsorption of streptavidin, so it is only useful for purified samples due to non-specific adsorption on CNTs. According to other authors, hysteresis modification can be attributed to electron/hole trapping, Schottky barriers, SB modifications, and scattering potentials. In the presence of polymer, the electrode-CNT contact appears to be hindered by modulation [82].

Molecularly imprinted polymers (MIPs) can also be used to bind target molecules in a way that is highly selective and specific. Conductive polymers are often characterized by aromatic rings and functional groups, which, when combined with a similarly shaped target (or template) molecule, are capable of exhibiting hydrogen bonding interactions. To imprint electrodes with testosterone, this study used an electrochemical method to optimize poly (aniline-co-metanilic acid) self-assembly. The detection limit for testosterone was as low as a few pM, with a linear sensing range of 0.1 to 100 pg/mL. TIECP sensors were used to dilute random urine samples by 1000 times in order to measure the testosterone concentration. A commercial system, the ARCHITECT CI 8200, was compared. In the tested samples, testosterone concentrations ranged from 0.33±0.09 to 9.13±1.33 ng/mL. The average accuracy of the TIECP-coated sensors was 90.3±7.0 % [83].

Another study used a disc-ring microelectrode array device to detect testosterone on a 3D sensing platform. Each component of a microelectrode array contains a large number of distinct microdiscs. Each microelectrode has a microring that acts as an electrode for electrochemical measurements. Biocomponent layers can be incorporated between these two electrodes due to their physical separation. The suggested sensor displayed a linear range between 0 and 10 ng/mL and a maximum detectable level of 12 and 5 pg/mL testosterone with a 45-minute detection time [84].

Saliva is now frequently used as a non-invasive sample to identify a variety of biomolecules. Stefan-van Staden et al. have described an electrochemical sensor for the detection of testosterone in young children's saliva that is based on diamond paste and modified with electroactive materials. The main benefit of the suggested method is the ability to measure testosterone in just a few minutes using small sample volumes. The limits of quantification obtained for testosterone 1 pmol/L using the proposed sensing method. It's better than ELISA, the standard method used in clinical laboratories to measure testosterone [85].

4. IMPEDIMETRIC BIOSENSORS FOR TESTOSTERONE DETECTION

Monitoring biorecognition activities at the surfaces of modified electrodes is possible with impedimetric measurements. An efficient way to characterization and fabricate a biosensor is through electrochemical impedance spectroscopy (EIS). Quantification of target molecules and charge transfer resistance can be accomplished with EIS [86].

One study used electrochemical impedance spectroscopy (EIS) measurements to determine testosterone at ultrasensitive femtomolar to micromolar levels. Graphene-oxide sheets were electrochemically grafted with a molecularly imprinted polymer film. A change in interfacial impedance caused by the recognition of a target molecule is explained as the detection mechanism for this sensor. In addition to the nanosheet structure, graphene-oxide has a large surface area, which greatly enhances the sensitivity of the MIP sensor. Under an optimized condition, a wide linear range from 1 fM to 1 μm ($1\times10^{-15-1}\times10^{-6}$ mol L^{-1}) and a detection limit of 0.4 fM (4.0×10^{-16} mol L^{-1}) was obtained. It displayed strong selectivity against structurally related steroid hormones, and it remained stable at room temperature for a considerable amount of time. These benefits make it possible for the MIP/GO electrochemical biosensor to take the place of testosterone immunosensors and detect other endogenous substances as well [87].

A high-quality, inexpensive, and reliable replacement for expensive and fragile natural receptors is now possible with the aid of molecularly imprinted polymers (MIPs). The lack of straightforward and affordable methods that enable reliable fixation and uniform receptor distribution makes it difficult to develop sensors using MIPs. An easy method is presented using microfluidics and in situ photopolymerization on functionalized diamond substrates. It is efficient, low-cost, simple and time-saving. It also ensures that MIP material amount and

distribution are tunable and consistent across different sensor substrates, enabling active sensing surface control. The electrochemical impedance spectroscopy-based detection of physiological testosterone levels in buffer, urine, and saliva was successfully tested using a patterned MIP structure as a selective sensor platform. The highest added testosterone concentration (500 nM) in buffer resulted in an impedance signal of $10.03 \pm 0.19\%$ and the lowest concentration (0.5 nM) led to a measurable signal of $1.8 \pm 0.15\%$ for the MIPs. The MIP signals with a detection limit of 0.5 nM showed good linearity between a concentration range of 0.5 and 20 nM. These MIP structures provide excellent and selective recognition as well as stability during and after dynamic sensor measurements. A simple washing procedure can regenerate the MIPs, and their reusability has been demonstrated [88].

Another study constructed a phage display library of heavy chain antibodies or nanobodies (Nb) after immunizing a Bactrian camel with testosterone. Nbs are a promising tool for the upcoming generation of diagnosis and medical applications because of their smaller molecular size (15 kDa), improved solubility, high affinity, specificity, and lower immunogenicity. For the purpose of measuring testosterone, an immunosensor based on electrochemical impedance spectroscopy (EIS) and NBS was created in this study. A library of immune phage display was successfully used to isolate antitestosterone Nbs. The in vivo BirA system was used to biotinylate one Nb, which showed the highest yield and stability. Moreover, the biotinylated antitestosterone Nb was applied to the EIS immunosensor for testosterone detection. The biosensor had a linear detection limit of 0.045 ng mL^{-1} and a linear range of 0.05 to 5 ng mL^{-1}. Testosterone levels in serum samples could also be accurately determined using a proposed immunosensor.Using the proposed immunosensor, testosterone can be detected sensitively and accurately, making it a valuable tool [89].

Utilizing a molecularly imprinted polymer (MIP) sensor, testosterone can be impedimetrically detected. First, a self-assembled monolayer of thiolamine (11-amino-1-undecanethiol) was grafted onto the 4,4'-azobis(4-cyanovaleric acid) initiator. A photopolymerization was performed in the presence of testosterone to prepolymerize methacrylic acid and ethylene glycol dimethacrylate, followed by spincoating and polymerization on the functionalized electrode surface. The various stages of MIP sensor fabrication were managed by EIS, Fourier transform infrared spectroscopy, and secondary ion mass spectrometry. In the presence of testosterone concentrations up to 50 $\mu g.L^{-1}$ the biosensor response, expressed as the variation in charge transfer resistance at the electrode/electrolyte interface, increased linearly with testosterone concentration after template removal. The sensitivity was 0.28 per log C for testosterone, while it was only 0.020 and 0.0085 for 17b-estradiol and methyltestosterone, respectively. The limit of detection for testosterone was very good (103 $ng.L^{-1}$). In absence of the template, the sensitivity of a non-imprinted polymer sensor was 18 times lower than that of a MIP sensor [90].

There's always a need for new, high-performance portable devices. In the athletic field, doping is a constant problem, and mobile and trusted detection methods are imperative. The current work suggests a portable testosterone detection biosensor based on screen-printed electrodes (SPEs) and functionalized magnetic particles. After SEM analysis of the functionalized magnetic particles, pulse voltammetry (DPV), cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS) were used to measure amperometry. Results demonstrate linearity between 50–1000 ng/mL with a LOD of 23.68 ng/mL. Comparative analysis of serum and urine samples was performed using Q-TOF/MS. Various interferents were also tested to determine the biosensor's selectivity. Due to its versatility of applications, the designed biosensor has great potential for detecting doping and preventing illicit use [91].

5. CONDUCTOMETRIC BIOSENSORS FOR TESTOSTERONE DETECTION

Conductance can be measured using conductometric measurements, Biorecognition events and conductance are related in conductometric measurements. The amount of ions in a solution can alter how electrically conductive it is. The conductometric biosensor is composed of two electrodes made of metal (generally platinum or silver). Measurement of conductance between metal electrodes is done with an ohmmeter (or multimeter) [92].

Silicon nanowire field-effect transistors (SiNW-FETs) are the biosensors with the highest sensitivity and power. In addition to antibody, DNA, protein, and virus detection, it can also detect changes in conductivity caused by changes in charge on the surface of the SiNW-FET. By overcoming a SiNW-FET's inherent weakness, one study sought to build a platform for the detection of steroids. A protein known as 5-Ketosteroid Isomerase was created and engineered to function as a steroid acceptor. It was subsequently modified with a 1.5-EDANS moiety and immobilized on a silicon nanowire surface. If there is steroid present on the nanowire surface, the negatively charged 1,5-EDANS moiety is expelled. The electrical response generated by the 1.5-EDANS moiety is used to calculate a concentration. A level of femtomolar sensitivity can be reached with this new nano-bio-device [93].

6. CONCLUSION

Male development is greatly influenced by testosterone, which also helps to build bone and muscle, as well as the testis, penis, and prostate. Testosterone levels should be normal in both men and women. Developing accurate biosensors for the measurement of testosterone is crucial due to its importance. Detection of hormones and other phenomena is possible thanks to the development of biosensors. In order to detect hormones that regulate and control the human body's metabolism, highly sensitive tools are being developed. In this review, we summarize the various electrochemical sensors used for detecting testosterone and its analogs. These reviews enable the development of quick, easy, and non-invasive electrochemical biosensing platforms for the detection of testosterone. However, several issues, that needs to be resolved:

stability of sensing platform and reproducibility. Addressing these issues will allow this technique to become reliable method for fast and simple diagnosis of testosterone in clinical samples. Yet, the field of testosterone detection by electrochemical sensors is continually growing. It is believed that electrochemical sensors and biosensors will focus on single use sensing platform. One of the best candidates for single-use sensing platform is paper-based electrode. Such sensing platform often provide advantages over reusable sensors by reducing contamination and eliminating the need for calibration. How to simply and successfully implementation on disposable surfaces without affecting their performance, has become a critical step.

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