NTU Journal of Pure Sciences (2023) 2 (3) : 9-18
DOI: https://doi.org/10.56286/ntujps.v2i3

IRAQI Academic Scientific Journals

ISSN: 2789-1089 EISSN: 2789-1097
NTU Journal of Pure Sciences
Available online at: https://journals.ntu.edu.iq/index.php/NTU-JPS/index

Electrochemical Sensors of Thyroid Hormones: A review of recent advances

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Article Informations

Received: 21-06- 2023,
Accepted: 08-08-2023,
Published online: 30-09-2023

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Key Words:
Thyroid Hormones,
Electrochemical Sensors,
Voltammetry,
Amperometry.

A B S T R A C T

The current review focuses on voltammetric and amperometric sensors measurement methods applied for the determination of thyroid hormones in the past seven years (2016-2023) and It provides an overview of their properties for medical applications. The analytical performance of voltammetric or amperometric chemical and biochemical sensors (linear range and detection limit) is highlighted.

Introduction

3,5,3-triiodothyronine (T3) and thyroxine (T4), which are required for tissue creation, regeneration, and metabolism control, are among the inactive precursors of thyroid hormones that go by this common term [1-4]. The feedback mechanisms that regulate the synthesis of thyroid hormone are mediated by the hypothalamic-pituitary-thyroid (HPT) axis. Thyrotropin-releasing hormone (TRH) production in the hypothalamus rises when thyroid hormone levels fall, which in turn causes the anterior pituitary gland to secrete more thyroid-stimulating hormone (TSH).

TSH promotes thyroid cells to produce thyroid hormones [5,6]. Thyroglobulin (Tg) synthesis, thyroid peroxidase (TPO), and the sodium isotope/iodide (NIS) is an active iodide adsorbent are all required for thyroid hormone synthesis. The breakdown of the Tg protein results in the release of the thyroid hormones thyroxine (T4) and triiodothyronine (T3). The thyroid gland releases T4 in much greater amounts (in a ratio of about 14:1) [7]. However, the type 1 and type 2 iodothyronine deiodinases (Dio1 and Dio2) convert the majority of T4 to T3 in target tissues [8,9].

Knowledge of any intrinsic, environmental, or inherited factors that may have an impact on thyroid hormone levels is crucial, as is a comprehension of the mechanisms underlying changes in the levels of thyroid hormones (TSH) and thyroxine [10].

However, iodine, BMI, and smoking revealed more pronounced associations with TSH and thyroid hormones (micronutrients taken in from the diet) but BMI levels were positively associated with free T3 and TSH levels, and smoking mostly caused In the decrease in TSH levels increase thyroxine and increase levels of triiodothyronine (T3) and thyroxine (T4) and decrease levels of thyroxine as a result of increased iodine. Most studies found that levels of thyroid hormone among the pollutants evaluated were reduced by exposure to perchlorate.

Although many other factors can potentially affect thyroid function, the account of genetic factors equals sixty-five percent of an individual's variability in TSH and thyroid hormone levels [11,12]. These variables include demographic ones like age and sex [13,14], intrinsic ones like microbiome [15], stress [16], medication use [17], and a variety of environmental ones [18–21]. Particularly crucial is the awareness of any circumstances that may impact TSH and thyroxine levels in susceptible populations, for example, those with expectant women and infection of thyroids [22].

Methods for Determination of Thyroid Hormones:

Abnormal blood levels of thyroid-stimulating hormone (TSH) is a major indicator of thyroid dysfunction because it can cause hypothermia and a lower basal metabolic rate when it falls below the lower limit of the reference range [23,24]. On the other hand, Decreased bone mineral density, mental retardation, significant atrial fibrillation, and decreased growth rate are among the diseases resulting from primary hypothyroidism, which is first detected in the laboratory when the TSH level is high in the blood [25-27], Therefore, the creation of quick and accurate TSH detection methods is essential for the early detection and treatment of thyroid illness. In this review, we investigated the latest progress in the modification of electrodes and their improvement in the detection of Thyroid hormones.

1. Non-Electrochemical Techniques:

Numerous methods for TSH detection have been published, including spectrometry of tandem mass, immunoassay of bioluminescent, and spectroscopy of infrared [28-30].

2. Electrochemical Sensor Techniques:

An electrochemical sensor is an instrument that translates chemical data, such as the concentration of a specific sample component or an analysis of the entire composition, into a signal that can be analyzed. Normally chemical (molecular) recognition systems (receivers) and physicochemical transducers are the two basic parts of chemical sensors [31,32].

Typically, a typical biosensor consists of :

a) bioreceptors that bind to the glycolytic selectively; b) an interface architecture where a specific biological occurrence produces a signal that is detected by c) a transducer; the signal of a transducer, the laser beam coupling angle to the electrode generated current that is converted by the detector into electronic signal via the right reference and after that delivered into processing, d)a meaningful physical property representing the process under study from computer software; The resulting quantity must then be transmitted through an e) interface to the human operator.

Different methods can be used to identify an electrical signal shift brought on by an analyte's redox reaction. Electrochemical sensors use a reference electrode to detect changes in analyte characteristics brought on by the gain or loss of electrons. Instead of using direct redox reactions, certain electrochemical devices rely on the indirect or mediated electron transfer process. The intermediates facilitate electron flow between the electrode surface and the reaction site [33], and the sensitivity of electrochemical transducers and the high specificity of biological recognition processes are combined by electrochemical biosensors, as demonstrated by low detection limits [34]. Because of the biological recognition elements' instability and limited sensitivity, biosensors have not been used as widely in analytical applications as may have been expected. Electrochemical biosensors have more features than other types of biosensors due to their mobility, high sensitivity, wide linear response, stability, self-contained design, reproducibility, low cost, low detection limit, resilience, and capacity to work in turbid fluids [35-39].

A wide variety of samples, including food samples, body fluids, and cell cultures, in addition to using them to analyze environmental samples, can apply biosensors to them [40-46].

Electrochemical biosensors often use enzymes or antibodies as biorecognition components to detect hormones. Electrochemical biosensors work on the fundamental premise that target substances react chemically with immobilized molecules to produce electrons that change the characteristics of the solution [47-51]. Electrochemical techniques have high sensitivity, rapid reaction, and low cost in comparison to conventional methods, opening up new opportunities for the creation of straightforward and automated analytical processes [52].

2.1. Voltammetric and Amperometric Sensors:

Thyroxine (T4) and triiodothyronine (T3) have been evaluated with various electrochemical techniques [53], such as sensors [54,55] and immunosensors [56]. For the assay of TSH, immunosensors [57-60], biosensors [61], and optical sensors [62] have been reported. Recently, reliable methods of analysis for the detection of biomarkers unique to various disorders were proposed [63-64].

Different advanced nanomaterials have been used as markers for signal amplification, including gold nanoparticles, magnetic beads, and carbon nanotubes of carbon [64–69]. MBs are among the best broadly used resources to enhance immunosensitization efficacy due to their easy separation from reaction systems and their large surface areas for interactions. [69-74]. Modern analytical chemistry is renowned for its miniaturization, integration, and intelligence. Microchips that have been inkjet printed and include an electromechanical system are useful for creating a variety of miniature analytical equipment. Additionally, due to their quick assay times and minimal sample volume requirements, to enhance the effectiveness of immune reactions, inkjet-printed microchips can be used [75–78]. For example, Liu's team created a sensitive microchip-based electrochemical immunosensor to measure S100B, with a detection limit of 0.1 pg/mL and 0.1 pg/mL to 100 pg/mL range of linear concentration[79]. To detect atrazine, Marco's team developed a unique impedimetric immunosensor based on an array of interdigitated electrodes, and they were able to achieve of 0.04 g/L detection limit [80]. 8 channel electrochemical cell platform for the diagnosis of infectious diseases in humans and animals, including Chagas disease, was developed by the Comerci team. [81].

2.1.1. Voltammetric and Amperometric Sensors at Modified Electrodes:

A brand-new electrochemical sensor is built on Nanocomposite for accurate measurement of thyroid hormone (T4). With a nanocomposite electrode, hydrodynamic amperometry is carried out by dispersing. T4 oxidation occurs on the electrode surface at 0.85 volts versus silver /silver chloride, where the amperometric device operates. The sensor measures between 1.00 nM and a concentration range of 14 nM T4 in 0.1 molar HCl solution, the detection limit was 1.00 nanomolar [82].

Thyroxine T4 was evaluated using an antibody-based biosensor made of a self-assembled polythiol monolayer to which particular it was bound to the antibody. Impedance readings on a ferricyanide redox probe were used for electroanalytical analysis. Limits of quantification (LOQ) is 1.5- 4.0 nanogram per milliliter, was gained. As evidence of the ideal improvement of an electrochemical method for T4 detection at the point of care employed this biosensor [83].

Using an immunoelectrochemical sensor, thyroid stimulating hormone was detected based on the compound azo(E)-5-[(4-dodecyloxyphenyl)diazenyl]isophtalic acid which is considered to be electroactive. The principle of action of the immunosensors is a decrease in the electrochemical response due to the presence of the TSHantigen, which reacts exactly with the abTSH antibodies fixed on the electrode surface, as a result of the azo-complex film placed on a glassy carbon electrode surface. Cyclic and square-wave voltammetry, as well as electrochemical impedance spectroscopy, was used to examine the electrochemical performance. A linear range of 0.2 to 20.0 $\mu IUmL^{-1}$ was achieved, with an estimated detection limit of 0.04 $\mu IUmL^{-1}$[84].

A technique for the sensitive detection of thyroid-stimulating hormone was established by using a chip of printed inkjet and a technique of double signal amplification using alkaline phosphatase, The reaction of aminophenyl phosphate reaction, and magnetic beads. The immunosensor was described using differential voltammetry, a cyclic voltammogram, and an amperometric (i-t) curve. Good selectivity and high sensitivity are noted. Peak currents increased in proportion to the concentration over the linear range of detection from 0.01 IU/mL to 10 IU/mL. At S/N = 3, the detection limit was 0.005 IU/mL. The range of relative standard deviations 1.3 - 3.1% and recoveries range 98.0- 101.8%, the immunosensor was also used to detect TSH in human serum, indicating its potential use in clinical diagnostics[(85].

Using plasma-enhanced chemical vapor deposition PE-CVD, a flexible molybdenum disulfide MoS2-based biosensor was successfully created. For the direct synthesis process on a polyimide substrate, the temperature was set at 150 Celsius. to avoid a transfer step. To detect three different hormones triiodothyronine (T3), thyroxine (T4), and parathyroid hormone (PTH) with their corresponding AAP substrates. In synthetic sera, T4, PTH, and T3, and concentrations were found by values for the calibration ranges of 10-500, 10-500, 1-10 ng/mL respectively. This newly created biosensor was able to identify the hormones with excellent accuracy and detect them with extreme sensitivity [86].

Based on the versatile DNA 3-way junction (3WJ) structure and porous rhodium nanoplates pRhNPs, for the detection of T4 a unique electrochemical biosensor was created at different concentration ranges, T4 was detected in samples established through sequential dilution of synthetic and medical T4 samples was accomplished by electrochemical impedance spectroscopy EIS to achieve the sensing performance 10.33 and 11.41 pM, respectively, were the lower limits of detection. The ability of the biosensor system described in this study to find T4 in medical and synthetic samples was confirmed [87].

2.1.2. Voltammetric and Amperometric Sensors at Modified Electrodes with Gold Nanoparticles:

An electrochemical immunosensor that is very sensitive for the recognition and evaluation of thyroid stimulating hormon. The covalent amide bond formation between amino-coated AuNPs and carboxyl groups of thyroid stimulating hormone-specific antibodies is catalyzed by the electrode surface modified with gold nanoparticles. The rising immunocomplex capacitive layer is detected by the direct immunoassay format as an increase in electron transfer resistance. Using cyclic and linear sweep voltammetry, the electrochemical performance of the manufactured immunosensor was assessed. The best-known electrochemiluminescent biosensor has a detection limit of 0.005 IU/mL and a dynamic range of 0.005-150 IU/mL, whereas the immunosensor has a lower detection limit of 0.001 IU/mL and a wider dynamic range of 0.001-50 IU/mL and 0.001-150 IU/mL, respectively, using linear sweep voltammetry and cyclic voltammetry [88].

Based on a modified carbon paste electrode CPE, a new immunosensor was used for the electrochemical measurement of human TSH. The antigen (TSH) was sandwiched between a thyroid-stimulating hormone antibody on the surface of a CPE modified with gold nanoparticles and a secondary antibody, a horseradish peroxidase-tagged anti-human polyclonal antibody, to create an immunoassay structure (HRP-labelled anti TSH). As the TSH concentration grew from 0.2 - 90.0 ng/mL the peak current increased, and limit of detection was set at 0.1 ± 0.02 ng/mL[89].

For thyroid disease detection, a sensitive, one-step nanosensor designed to yield a covalently linked antibody through peptide bond formation between amino coated with nanoparticles of gold and antibody of anti TSH, is catalyzed by nanoparticle screen-printed carbon electrode. The nanoscale biosensor senses the effective resistance provided by the electrode to identify and quantify thyroid hormone in the sample.

The covalent connection between the surface amino group and the carboxylic Fc made possible by the functionalization of gold nanoparticles with cystamine dihydrochloride ensures that the amount of active antibody is maximized. Due to the greater loading capacity and improved detection range provided by the bigger effective surface area presented, this technique guarantees a substantially lower limit of detection, due to these two immunosensor construction techniques, the limit of detection was reduced to 0.001 μIU/mL and the detection range was increased to 0.001-150 μIU/mL. As a result, the constructed immunosensor can be used for investigational purposes covering a clinically relevant range and is a potential applicant for a point of care device for the diagnosis of hypothyroidism with its direct detection method[90].

A nanoparticle-based electrochemical sensor can identify and measure the hormone triiodothyronine (T3). The anti-T3 antibody and gold nanoparticle immunosensor were used to measure the amount of T3 antigen using

differential pulse and cyclic voltammetry techniques. The electrochemical response of the built-in immunosensor is highly associated with the amount of antigen present in the sample. Redox current falls as antigen concentration rises because more immunocomplexes develop on electrode surfaces. The immunosensor has a lower limit of detection of 1 $pg.ml^{-1}$ and a dynamic range of 1 to 500 $pg.ml^{-1}$. The sensitivity of the immunosensor was found to be 29.81 $pg/mL/cm^2$ [91].

To detect parathyroid hormone(PTH), based on a carbon electrode with screen printing, an electrochemical platform was created. A combination of multi-walled carbon nanotubes and gold nanoparticles was applied to the SPCE to facilitate the binding of antibodies and horseradish peroxidase (HRP). Due to its effective capacity to transport electrons and cylindrical structure, MWCNT increased the stability and conductance of the immunosensor. The AuNP increased the electrochemical signals for enzyme-linked immunosensing in addition to offering a significant surface area for antibody immobilization. Cyclic voltammetry showed that the enhanced electrode had increased electron transport and efficient surface area. The interference of cellular proteins in human serum did not affect the electrochemical performance of this PTH immunosensor, which had a linear detection range of 1-300 pg.ml^{-1}. For differential pulse voltammetry and square wave voltammetry, the detection limits for this PTH immunosensor were 0.886 and 0.065 pg.ml^{-1}, respectively [51].

2.1.3. Voltammetric and Amperometric at Modified Electrodes with Polymer:

It may be possible to create a screen-printed carbon electrodes SPCE-MIP sensor for the electrochemical analysis of thyroxine T_0AM. Because the analyte (T_0AM) and one of the probable interference investigated 3-iodothyronamine (T_1AM) are not commercially accessible, they had to be synthesized. Electropolymerization of the monomer (4-aminobenzoic acid) yielded a poly 4-ABA film with sufficient gaps for the T_0AM to be physicochemically suitable. The EIS of the SPCE-MIP sensor stands out because it exhibits suitable MIP production and strong analyte binding. Limits of detection (LOD) and quantification (LOQ) of 0.081 and 0.27 molar (1.9 and 6.2 μg. dl^{-1}) and a linear range of up to 10 molar (0.23 x 10^3 μg. dl^{-1}) with a R^2 of 0.998 [92].

The production of electrochemical immunosensors for the sensitive detection of thyroid hormone utilizing copolymers of poly aniline - 4 - aminophenol and anti-triiodothyronine has been made possible thanks to a new platform (T3). To interact with the probe and biocompatible milieu, the copolymer demonstrated strong electrochemical properties, making it effective for immobilizing biomolecules.

On copolymer poly(aniline-4-aminophenol), a particular antibody for T3 was immobilized. When target T3 is present, an immune response with anti-T3 happens, and it is noticed that the current decreases proportionally to the T3 concentration. Following the fall required the use of differential pulse voltammetry, which could detect down to 0.60 pg. ml^{-1} and had good stability by maintaining 90 percent of its response even after sixty days of storage. The immunosensor exhibits potential for use in the detection of additional disease indicators as well as suitability for the detection of thyroid hormones in actual serum samples[93].

A brand-new, simple immunosensor based on polyamidoamine dendrimers has been created. The gold electrode was immobilized with the assistance of a cysteamine self-assembled monolayer. The immunosensor was created using a polyamide crosslinker to expand the surface area where Ant-TSH was immobilized. Cross-linking has also been accomplished using glutaraldehyde. After each immobilization, the electrode surface was observed using energy-dispersive X-ray spectroscopy, cyclic voltammetry techniques, electrochemical impedance spectroscopy, scanning electron microscopy, and optimization studies were carried out. The suggested immunosensor had linear detection limits of 0.026 and 0.086 mIUL^{-1} respectively, and a linear detection range of 0.1 to 0.6 mIUL^{-1} [94].

Recently, a unique, highly specific electrochemical bioassay that makes use of antibodies and laccase to measure free thyroid hormone was disclosed (free triiodothyronine, f T3). The glassy carbon electrode that served as the biosensor's foundation was modified using a Fe_3O_4 graphene nanocomposite, and the electrode modification procedure was assessed using cyclic voltammetry. All of the biosensor's normal operational parameters were looked into using differential pulse voltammetry. The biosensor responded sensitively to fT3 in a concentration range of 10-200 M, with a limit of quantification of 45.9 nanomolar and a detection limit of 27 nanomolar. The created biosensor offers potential for usage in upcoming medical diagnostics and has demonstrated good stability [95].

Table 1. Some Analytical Performances Obtained in the Electrochemical Determination of Thyroid Hormones**:**

Thyroid Hormones	Type of Detection	Electrode Type	Linear Response	Detection Limit	Ref.
TSH	Linear Sweep Voltammetry LSV	An interdigitated electrode on a glassy Electrode	0.02-100 mIU. L-1	0.012 mIU. L-1	57
T4	Differential Pulse Voltammetry and Chronoamperometry	screen-printed carbon electrode (SPCE)		3 nM	54
Thyroxine T4	Cyclic Voltammetry CV	Graphene-based nanocomposite Electrode	1-14 nM	1.00-0.02 nM	82
Thyroxine T4	Cyclic Voltammetry CV	screen-printed gold -	1.5-4 ng		83

		microelectrode (SPAuE)	mL-1		
TSH	Cyclic Voltammetry, Square Wave Voltammetry	Glassy Carbon Electrode GCE	0.2 to 20.0 µIUmL-1	0.04 µIUmL-1	84
TSH	Differential pulse Voltammetry DPP, Cyclic Voltammetry CV	inkjet printed microchips	0.01-10 IUmL-1	0.005 IUmL-1	85
T3 T4 PTH	Cyclic Voltammetry CV	modified MoS2−Au−PI flexible sensor	1-10 10-500 10-500 ng mL-1		86
T4	Cyclic Voltammetry CV	pRhNPs-modified Au electrode		10.33 pM and 11.41 pM	87
TSH	Cyclic Voltammetry, Linear Sweep Voltammetry (LSV)	Modified electrode with gold nanoparticles	0.005-150 IUmL-1 0.001-150 IUmL-1	0.005 IUmL-1 0.001 IUmL-1	88
TSH	Differential Pulse Voltammetry (DPV)	Modified CPE with gold nanoparticles	0.2-90.0 ng. mL-1	0.1±0.02 ng. mL-1	89
TSH	Electrochemical impedance spectroscopy EIS	Gold nanoparticles modified screen-printed carbon electrode catalyze	0.001–150 µIU.mL-1	0.001 µ IU.mL-1	90
T3	Cyclic Voltammetry, Differential Pulse Voltammetry	screen printed electrode with gold nanoparticles	1 - 500 pg.mL-1	1 pg.mL-1	91
PTH	Differential Pulse Voltammetry, Square Wave Voltammetry	screen-printed carbon electrode (SPCE) with gold nanoparticles and multi-walled carbon nanotubes	1-300 pg.mL-1	0.886 0.065 pg.ml-1	51
T0AM (thyronamine)	Cyclic Voltammetry (CV)	screen printed carbon electrodes with molecularly imprinted polymer (MIPs)	(0.23x 103 µg dL-1)	1.9 µg dL-1	92
T3	Differential Pulse Voltammetry (DPV)	Copolymer poly(aniline-4-aminophenol) and anti-triiodothyronine were used to modify graphite electrodes	0.2 - 8.8 ρgmL−1	0.60 pg .mL−1	93
TSH	Cyclic Voltammetry (CV)	Gold electrode with poly-amidoamine dendrimer	0.1–0.6 mIUL-1	0.026 mIUL-1	94
T3	Differential Pulse Voltammetry	a nanocomposite made of Fe3O4 and grapheme with a glassy carbon electrode	10-200M	27nM	95

Conclusions

This study describes current advances in electrochemical sensors that are used to quickly and precisely detect thyroid hormones for therapeutic purposes. It also includes information on the type of detection, linear response, and detection limit. Because they are simple to use, unassuming, and affordable, electrochemical sensors provide excellent analytical tools for clinical analysis.

Because they are straightforward, dependable, quick, and affordable, biosensors are an alternative to conventional detection technologies and a pioneer in the fields of accuracy and modern healthcare, medicine, drug development, and pharmacogenomics.

These can be transformed into compact, portable gadgets that offer quick point-of-care (POC) detection. These gadgets have successfully detected critical indicators in bodily fluids such as blood, saliva, perspiration, and urine. According to the reviewed literature, voltammetric and amperometric biosensors are the most often employed electrochemical biosensors.

Acknowledgments. We are thankful to the Department of Chemistry, College of Science, University of Mosul for providing all the necessary facilities.

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