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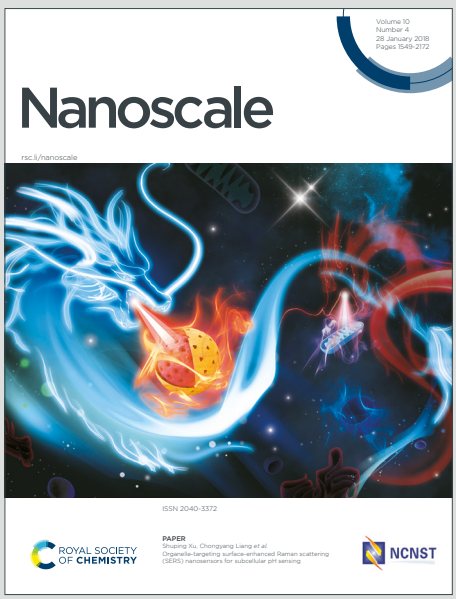

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Progress in Nanoparticle-based Electrochemical Biosensors for Hormone detection

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Hormones are chemical messenger that regulate a wide range of physiological processes including metabolism, development, growth, reproduction and mood. The concentration of hormones that orchestrate the numerous bodily functions is very low (1nM or less). Efforts have been made to develop highly sensitive tools to detect them.

This review represents a critical comparison between different types of nanoparticles-based electrochemical biosensors for the detection of various hormones, such as cortisol, sex hormones (estradiol, progesterone, testosterone), insulin, thyroid-stimulating hormone (TSH) and growth hormone (GH). The electrochemical biosensors investigated for each hormone were firstly divided on the basis of the biological fluid tested for their detection and successively on the basis of the electrochemical transducer utilized in the device (voltammetric or impedimetric). Focus is given on the nanoparticles employed and the successive electrode modification developed in order to improve detection sensitivity and specificity and biosensor stability. Limit of detection (LOD), linear range, reproducibility and possibility of regeneration for continuous reuse are also investigated and compared. The review addresses also the recent trends in the development of wearable biosensors and point-of-care testing for hormone detection in clinical diagnostics useful for endocrinology research, and the future perspectives regarding the integration of nanomaterials, microfluidics, Near Field Communication (NFC) technology and portable devices.

1. Introduction

1.1 The role of hormones in pathology and diagnosis

Hormones are signalling molecules transported to distal organs to regulate various physiological and behavioural functions.[1] Hormones are secreted by glands or specific cells, circulate in the bloodstream, and specialize in targeting certain cells. The knowledge of hormone precise amount, which is fluctuating in the human body, is of extreme importance in better understanding of their role and the potential impact of their imbalances on health and well-being. Levels of hormones are therefore significant diagnostic indicators for possible disease status.

They can be categorized into three systems, namely, steroids, peptide, and amino acid-derived, depending on their building units, as shown in **Figure 1**. Steroid hormones possess lipid solubility which enable them to traverse the plasma membranes of target cells and exert their effects within the nuclei. Peptide hormones are composed of amino acid chains, are therefore water-soluble and exert their primary physiological effects on the endocrine system by binding to cell surface receptors.[2-6] Some of them are glycoproteins (TSH, luteinizing hormone, LH, and follicle-stimulating hormone, FSH). Amino-acid derived hormones are small molecules with similar chemical structure to particular amino acids, such as tyrosine (catecholamines) and tryptophan (thyroid hormones).

In this review, we describe the most relevant works published in recent years about electrochemical nanoparticles-based biosensors for the detection of the above-described hormones. For each of them, a first division has been made according to the biological body fluid of detection and a sub-classification is further carried out depending on the electrochemical technique employed in each sensing device.

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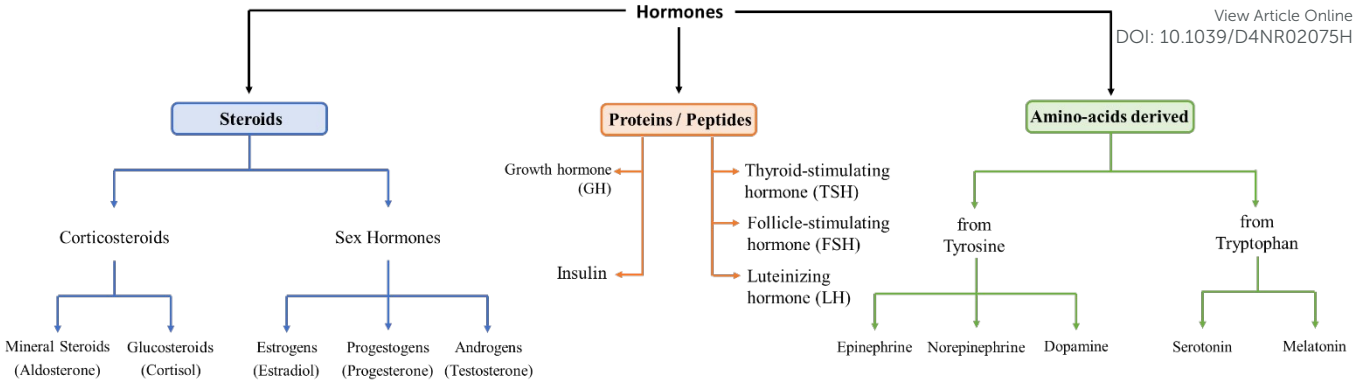

Fig. 1 Classification of hormones based on their building units.

Different voltametric techniques such as cyclic voltammetry (CV), differential pulse voltammetry (DPV), square wave voltammetry (SWV) and amperometry have been indicated with the general term "voltammetric" biosensors in order to distinguish them from those based on Electrochemical Impedance Spectroscopy (EIS) technique and referred as "impedimetric" biosensors.[7-10] Moreover, wearable non-invasive biosensors for hormone detection in biofluids, such as sweat and interstitial fluid (ISF) have been reported and discussed. Finally, the review has been concluded with challenges and future perspectives for nanoparticles-based biosensors to detect hormones in various human biofluids.

1.2 Relevant hormones for what? and current detection methods?

Scientists have identified over 50 hormones in the human body so far. In this review we focus on 7 key hormones which mostly affect the health in our body. In particular, 4 lipid hormones derived from cholesterol, i.e. cortisol, estradiol, progesterone and testosterone, and 3 peptide hormones, i.e. insulin, GH and TSH.

Table 1 displays the different concentrations of primary sexual hormones found in biological fluids, including serum, urine, and saliva.[11-22]

1.2.1 Cortisol

Cortisol (11β,17α,21-trihydroxypregn-4-ene-3,20-dione) is a steroid hormone synthesized by the zona fasciculata of the adrenal cortex. The release of cortisol is regulated by the hypothalamic-pituitary-adrenal (HPA) axis response, which is the main stress response system of the human body.

The process which leads to the release of cortisol from the adrenal cortex, began in the hypothalamus with the production of corticotropin-releasing factor (CRF). CRF interact specific receptors in the pituitary gland and causes the release of the adrenocorticotropic hormone (ACTH) which signal to the adrenal gland cortex to release cortisol in the bloodstream from which it can diffuse to target tissues **(Figure 2)**.[23]

Cortisol is a small molecule (362.46 g/mol) of neutral electrostatic charge and soluble in fat. It can pass across cytoplasmic membranes and act as messenger in several metabolic pathways. Because cortisol is released by the organism in response to stress, it regulates several pathways aimed to prepare the body to overcome the crisis. Cortisol is involved in the formation of glucose and its metabolism. At the same time, it is involved in the regulation of appetite and the concentration of cortisol determines carbohydrate, proteins or fat to be formed.[24] Furthermore, after a bleeding wound, cortisol regulates the activation of anti-inflammatory and anti-stress pathways.[25]

Persisting high levels of cortisol can lead to serious health conditions as insulin resistance, dyslipidaemia, hypertension and obesity. Longer exposure to high cortisol concentrations can lead to bone demineralization, difficulties in the intestinal absorption of calcium, water retention and muscles breakdown.[23, 24] The involvement of cortisol in numerous stress related diseases and conditions make it a prime diagnostic target for the detection of persisting stress level and to prevent the related conditions.[26] In absence of any pathology, cortisol level changes during the day in a reproducible cycle, peaking in the morning, approximatively one hour after waking up and progressively decreases to reach its minimum around midnight.[27]

Because cortisol is able to pass cytoplasmic membranes it can be found in detectable concentrations in many parts of the human body, the most relevant for its detection are hairs, saliva, sweat, urine, blood, and interstitial fluid.[28-34]

In an analytical laboratory setting, cortisol quantification is obtained using three main techniques: liquid chromatography paired with tandem mass spectroscopy/mass spectroscopy (LC-MS/MS), radioimmunoassay (RIA) and enzyme-linked immunoassay (ELISA).[27] LC-MS/MS is mostly used for quantitative analysis, because it has a low detection limit (LOD) of 0.1 ng/mL and it can test samples in a wide range of concentrations, but it requires sophisticated equipment and trained personnel.[35]

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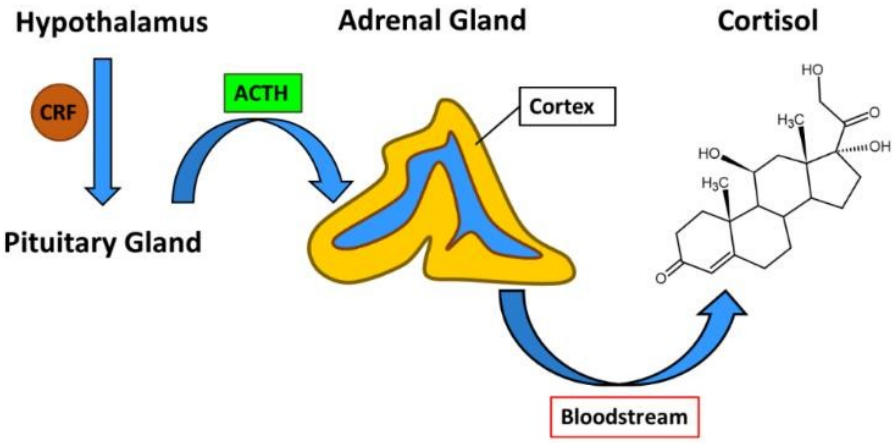

Fig. 2 Hypothalamic-pituitary-adrenal axis response chain and molecular structure of cortisol.

Commercial RIA kits have a LOD of 8.6 nM and they are easier to perform. But a great limitation to their use is the need of radioisotopes to use as markers which produce dangerous difficult waste to dispose.[36] ELISA immunoassays are the most recent and commonly used, they have good selectivity for cortisol compared to other steroids but their accuracy and reproducibility is still lacking compared to LC-MS/MS.[37, 38] All three of these techniques are expensive to run (LC-MS/MS, RIA), require specialized personnel and waste management (LC-MS/MS, RIA) and time consuming (ELISA 1.5 – 2 h).

Electrochemical measurements have several advantages compared to the above techniques: they are usually simpler to use than LC-MS/MS, they do not require toxic or radioactive material as RIA, they do not need to destroy the sample as the ELISA and they are usually faster with no direct intervention of an operator. It is interesting to note that these advantages are valid for the detection of all hormones and not only for cortisol detection. On the other hand, they offer some unique advantages, for example electrochemical cortisol detector can be deployed to the point of care (PoC), have limit of detection and selectivity similar to ELISA and can track the cortisol level during the day.[23, 26, 39]

1.2.2 Sex hormones

Sex hormones are steroid hormones derived from cholesterol, which play a crucial role in regulating both reproductive and non-reproductive systems, influencing sexual function and behavior. They serve as chemical messengers in the body, and their actions are mediated by receptors within various central nervous system structures, including the hypothalamus, midbrain, amygdala, cortex, and pituitary gland. Binding to specific receptors, sex hormones elicit cellular responses through both genomic and non-genomic mechanisms, involving signal transduction processes.[40, 41]

Sex hormones are vital for sexual development and function in both males and females, each eliciting unique effect. While androgens are commonly referred to as male sex hormones due to their masculinizing effects, estrogens, and progestogens are considered female hormones. It's crucial to note that all sex hormones are present in each gender, contributing to various functions across organs and systems.[42-47]

Estrogen is present in both blood and interstitial fluid. Upon binding, estrogen permeates the cell membrane, entering the cell nucleus and forming a hormone-receptor structure. This structure, known as a dimer, binds to specific sequences in the genome called estrogen-response elements situated in regions that regulate gene transcription. Estrogen acts through various nuclear receptors, influencing tissues such as the endometrium, vagina, and breast. Its non-genomic activities include the removal of granular calcium cells and modulate of uterine blood flow, occurring independently of cellular receptors. Additionally, estrogens counteract the effects of parathyroid hormone, minimizing calcium loss from bones and promoting bone strength. Estrogens find applications in hormone replacement therapy for menopause, oncology, and contraceptives. In summary, estrogens play a crucial role in developing and maintaining both internal and external genitalia, enhancing skin appearance and functionality, improving bone density through increased osteoblastic activity, regulating kidney retention of sodium, chloride, and water, and reducing overall cholesterol levels in the body.[48-54]

Progesterone, secreted by the corpus luteum and placenta, holds pivotal roles in both the menstrual cycle and pregnancy. Its applications extend to hormonal contraception, long-term ovarian suppression in conditions like dysmenorrhea, endometriosis, hirsutism, and bleeding disorders. Often combined with estrogen to mitigate uterine or cervical cancer risk, progesterone is integral to hormone replacement therapy and feminizing hormone therapy. In addition to its essential functions, progesterone serves as a crucial metabolic intermediate along the production pathways for various endogenous steroids, encompassing sex hormones, corticosteroids, and neurosteroids. Its effects are amplified in the presence of estrogen, with estrogen receptors upregulating or inducing the extraction of progesterone receptors. An imbalance in progesterone levels impacts aldosterone function, influencing sodium retention and extracellular fluid volume. In the reproductive system, progesterone engages in non-genomic

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signaling affecting sperm, modulating intracellular calcium signaling and sperm motility. During pregnancy, progesterone contributes to endometrial preparation for implantation, thickening vaginal epithelium, making cervical mucus impenetrable to sperm, and inhibiting lactation. In the absence of pregnancy, declining progesterone facilitates menstruation, considered a consequence of progesterone withdrawal. Beyond reproduction, progesterone plays a role in breast development and is implicated in breast cancer pathophysiology. Similar to estrogen, it influences skin health, with decreased levels during menopause contributing to skin atrophy and increased wrinkling. Hormone replacement therapy, including progesterone, enhances skin characteristics such as collagen content, thickness, elasticity, hydration, and surface lipids. In the brain, progesterone's impact extends to serotonin receptors, potentially influencing addiction. Insufficient progesterone levels may lead to behaviors aimed at enhancing serotonin activity, such as alcohol, cannabis, and nicotine consumption. Overall, like estrogen, progesterone exhibits protective effects against skin aging and has intriguing implications in neurochemical processes.[55-60]

Androgens serve as the primary steroid determining sex in males; nevertheless, both males and females produce these hormones. In men, insufficient androgen levels can lead to a diminished sex drive and the development of gynecomastia. Conversely, females with excessive androgen expression may experience symptoms like hirsutism, acne, and other related issues. Testosterone, the most prevalent androgen, is produced by both the ovaries and testicles. Across all genders, androgens play crucial roles in various physiological processes, including the regulation of bone density, muscular development, onset and maturation during puberty, red blood cell production, libido, and sexual function. In men, androgens induce specific effects such as voice deepening, hair growth on the face, scalp, underarms, chest, and genitals, and the development of sperm. In women, androgens regulate menstruation, help prevent osteoporosis by minimizing bone loss, assist in conception and pregnancy, and stimulate the growth of pubic and underarm hair.[61-66]

The most common techniques for the detection of sex hormones in clinical settings are direct chemiluminescent immunoassays, which did not require any pre-analysis purification, but they have demonstrated some limitations on accuracy and reproducibility.[67] For this reason, from 2007 the Endocrine Society has suggested the use of mass spectrometry (MS) analysis for sex hormones characterization and quantification. Recently in 2013 a standard assay has been developed by the National Institute of Standard and Technology (NIST) to be able to analyze samples for sex hormones with liquid chromatography – mass spectrometry (LC-MS/MS).[67]

Test, as the one offered by nanoparticles based electrochemical biosensors, will greatly improve our capability to determine the concentration and role of sex hormones in clinical settings.[67]

1.2.3 Insulin

Insulin is a small peptide hormone composed of 51 amino acids in total with a molecular weight of *ca.* 5.7-5.8 kDa.[68, 69] The structure of human insulin consists of two peptide chains, namely the A-chain (21 amino acids) and the B-chain (30 amino acids), which are connected via two disulfide bridges. Insulin is a pancreatic hormone produced by B-cells and is responsible for regulating blood glucose levels in human.[68] Our bodies respond to the presence of high blood glucose by releasing insulin, which promotes carbohydrate metabolism and glucose uptake by adipose tissue and muscle.[68-70] This process attempts to convert a relative amount of glucose into a storage form of glycogen for cellular uptake and thus reduce the circulation of glucose molecules in the bloodstream. An impairment of glucose regulation activities leads to the development of diabetes mellitus (DM), which is clinically classified into type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM).[69-72] Both types of DM are characterized by an irregular increase in blood glucose, but they differ in their underlying mechanisms. T1DM is an autoimmune disease that contributes to the decline of pancreatic beta-cells and consequently hyperglycemia.[72] In the pathogenesis of T1DM, T-cell mediated autoimmunity destroys beta-cells leading to a declination of beta-cell mass. As a result, patients lose the ability to produce insulin, fail to adjust blood glucose levels to normal, and eventually develop dysglycemia and symptomatic T1DM. In T1DM patients, insulin secretion is negligible or disappeared, and treatment relies on lifetime insulin injection. Therefore, T1DM is diagnosed by the presence of T1DM-associated autoantibodies.[72]

On the other hands, T2DM is a significant change in blood glucose level primarily caused by insulin deficiency, insulin resistance, or both.[71] Insufficient insulin secretion is led by beta-cell dysfunction, resulting in the pancreas being unable to produce enough insulin to effectively control blood glucose levels. In some cases, insulin is physiologically secreted in responses to rising blood glucose levels. However, the body is poorly sensitive to insulin and the glucose regulation mechanism is rarely activated, thus a loss of the ability to reduce blood glucose, known as insulin resistance. Since insulin resistance is a feedback regulation mechanism, insulin is continuously released in an attempt to maintain glucose at normal level. As a result, a relatively high amount of insulin is circulated in T2DM patients. In this case, elevated blood glucose levels can be detected, and patients are diagnosed with T2DM. Individuals with metabolic insulin resistance are at risk of having subsequent complications, including cardiovascular disease, blindness, high blood pressure, kidney failure, obesity, and even fatality.[68, 70] To distinguish between these two types of DM, T2DM exhibits insulin resistance, while T1DM is characterize by the presence of autoantibodies.[72]

Insulin is indeed a vital biomarker, predominantly found in the bloodstream. Insulin level can vary depending on an individual's health condition. For effective diagnosis, the association between insulin concentration and diabetes should be analysed while considering the condition of the patient. In T1DM, insulin is virtually undetectable, especially in the later stages, due to insulin deficiency. In contrast, individuals with T2DM develop insulin resistant, leading to elevated blood insulin levels.

While blood is the primary source for insulin detection, insulin hormones can also be detected in other body fluids. Excess

blood insulin increases toxicity due to glucose overconsumption. In response, the body activates physiological process to reduce blood insulin levels. The kidney and liver play a role in regulating the level of blood insulin. The liver is primarily responsible for insulin removal and degradation, while the kidney is involved in the reduction of insulin levels, but with a lesser extent.[73] In general, the kidney diminishes toxic substances and restores vital compounds to bloodstream. In this case, blood insulin is filtered in the kidney through the glomeruli and reabsorbed by renal tubules, a process termed as insulin clearance.[74] The relative amount of insulin clearance is returned to the bloodstream and the remaining is excreted in urine.[75] Urinary insulin is found at low levels because most of the insulin (>98% of the glomeruli filtered insulin) is subjected to reabsorption by proximal renal tubes.

In non-diabetic people, the insulin clearance rate is stable and independent to serum insulin. On the other hand, insulin clearance is high in T2DM, primarily due to elevated blood insulin levels, which can exceed the transport capacity of renal reabsorption. Therefore, urinary insulin can be a potential biomarker for diabetes. However, to consider urinary insulin as a biomarker, other health conditions need to be accounted for. Insulin clearance varies with creatinine clearance.[74, 75] Furthermore, liver or kidney diseases can increase insulin clearance.[74] Renal disease significantly elevates urine insulin levels due to diminished tubular reabsorption of the hormone.[76] With derangement of kidney function, the filter mechanism is not as effective as normal, thus decreasing insulin reabsorption and increasing blood sugar level. Using urinary insulin for clinical diagnosis may be challenging with the derangement of kidney and liver functions and careful interpretation of results is necessary.

Insulin is indeed present in blood and urine due to insulin removal mechanism. Furthermore, it can be detected in other body fluids. A recent review has summarized the levels of insulin found in various types of body fluids.[77] **Table 1** outlines the types of fluids and their corresponding insulin concentration ranges. As evident from **Table 1**, insulin is most abundant in milk and interstitial fluids. However, these fluids are not suitable for home testing, especially interstitial fluids, which require skilled medical staff and equipment for sample extraction. Among the listed fluids, serum is the preferred choice of sampling due to decent insulin content. However, other fluids such as urine and saliva hold potential as insulin biomarkers, offering the advantages of convenience and detection, compared to traditional blood-based tests.[77]

Insulin in clinical setting is mostly detected using chromatographic techniques as high-pressure liquid chromatography (HPLC), capillary electrophoresis chromatography (EC) and more recently LC-MS/MS, but they require complicated procedure to prepare the samples and costly machinery. Immunosensors have also being used to determine the concentration of insulin but as seen for other hormones, they have a slow rate of analysis that limits their clinical use.[77]

1.2.4 Thyroid-stimulating hormone

Thyroid-stimulating hormone (TSH) is a glycoprotein hormone originating from the anterior pituitary, which serves as the primary stimulus for thyroid hormone synthesis by the thyroid gland. Additionally, it induces the growth of thyroid follicular cells, leading to the enlargement of the thyroid. The release of TSH is regulated by the hypothalamic-pituitary axis. Neurons in the hypothalamus release thyroid-releasing hormone (TRH), which, in turn, stimulates thyrotrophs in the anterior pituitary to secrete TSH. TSH then prompts thyroid follicular cells to release thyroid hormones, primarily in the form of triiodothyronine (T3) or thyroxine (T4). T3, the active form of thyroid hormone, constitutes only 20% of the released hormone, with the majority of T3 originating from the peripheral conversion of T4 to T3. Thyroxine (T4), also known as tetraiodothyronine, makes up more than 80% of the secreted hormone. Upon entering circulation, T4 undergoes de-iodination to form T3. Both T4 and T3 exert negative feedback on the anterior pituitary, with elevated levels suppressing TSH secretion, and low levels stimulating TSH release. Moreover, TSH stimulates the thyroid gland to secrete prolactin.[78]

TSH levels are typically monitored in serum and urine samples. **Table 1** presents the concentrations of TSH in these biological fluids among healthy individuals.[79, 80]

Clinical analysis of TSH requires a great sensitivity for the different forms of the hormone. In clinical settings this is achieved by using immunoassay with sensitivity < 0.01 mIU/L or by MS/LC-MS tandem.[81] Electrochemical biosensors have better sensitivity, faster results and selectivity compared to immunoassay and MS/LC-MS tandem.

1.2.5 Growth hormone

Growth hormone (GH) is a peptide hormone that stimulates growth, reproduction, and cell regeneration in humans. Moreover, it stimulates the production of insulin-like growth factor 1 (IGF-1) and increases the concentration of glucose and free fatty acids.

GH is secreted episodically with approximatively two third of the daily secretion of GH happening during the night at the onset of slow-wave sleep. With the first episode of slow-wave sleep of the night triggering secretory pulses releasing the 70% of the total daily secretion of GH. In normal healthy individual GH has a minimal basal secretion constant during the 24 h that is too low to be detected with immunoassay, which account for the 50% of the day, interrupted by peaks of secretion which accounts for the majority of the whole secretion. The ultradian rhythm of GH secretion is the result of the interaction of many different factors, for example jet-lag increases temporarily the GH peaks amplitudes, physical stresses increase the levels of GH

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Table 1 Normal concentrations of cortisol, estradiol, progesterone, testosterone, insulin, TSH and GH for healthy subjects in different human body fluids.

Hormone	Body fluid	Normal concentration
Cortisol	Saliva	Morning: 10.2 – 27.3 ng mL^{-1}; Midnight: 2.2 – 4.1 ng mL^{-1}
	Hairs	55 pg mL^{-1}
	Urine	14 – 78 mg / day
	Serum	Morning: 250 ng mL^{-1}; Midnight: 50 ng mL^{-1}
	Interstitial fluids	≈30 ng mL^{-1} averaged during the day
	Sweat	8.2 – 141.7 ng mL^{-1}
Estradiol	Saliva	Female: 9 – 15 pg mL^{-1}; Male: 2.6 – 10.6 pg mL^{-1}
	Urine	Female: 30 – 400 pg mL^{-1}; Male: 10 – 50 pg mL^{-1}
	Serum	Female: 30 – 400 pg mL^{-1}; Male: 10 – 50 pg mL^{-1}
Progesterone	Saliva	Female: 27.1 – 103.6 pg mL^{-1}; Male: 15.5 – 49.5 pg mL^{-1}
	Urine	Female: 0.1 – 0.3 ng mL^{-1} (prepubescent), 0.1 – 0.7 ng mL^{-1} (follicular stage), 2 – 25 ng mL^{-1} (luteal stage); Male: 0.3 – 0.9 ng mL^{-1}
	Serum	Female: 0.1 – 15.9 ng mL^{-1}; Male: 0.1 – 0.9 ng mL^{-1}
Testosterone	Saliva	Female: 35 – 300 pg mL^{-1}; Male: 70 – 274 pg mL^{-1}
	Urine	Female: 2 – 10 mg / day; Male: 40 - 120 mg / day
	Serum	Female: 15 – 46 ng dL^{-1}; Male: 265 – 923 ng dL^{-1}
Insulin	Plasma	< 1 ng mL^{-1}
	Serum	0.2 – 0.4 ng mL^{-1}
	Urine	0.1 – 0.8 ng mL^{-1}
	Saliva	0.02 – 0.04 ng mL^{-1}
	Tears	0.3 – 0.5 ng mL^{-1}
	Milk	4.6 – 12.2 ng mL^{-1}
	Interstitials fluids	1.9 – 3.4 ng mL^{-1}
	Cerebrospinal fluid	0.2 – 0.3 ng mL^{-1}
TSH	Serum	0.5 – 5.0 mU L^{-1}
	Urine	0.4 – 4.0 mU L^{-1}
GH	Serum	1.8 ± 1.2 ng mL^{-1}
	Saliva	0.6 ± 0.5 pg mL^{-1}
	Urine	5.1 ± 1.9 pg mL^{-1}

while psychological conditions as depression reduces the GH levels.[82] GH secretion is also related to nutrition, with malnutrition and fasting causing an increase of GH while obesity and the ingestion of glucose suppress the secretion of GH.[83]

GH concentration changes in pulses and it peaks during puberty.[84]

In a healthy subject the concentration of GH regulates the metabolism of carbohydrates, proteins and lipids while at the same time stimulates the expression of the insulin growth factor (IGF-1) which regulates the growth of cartilage and bones.[85, 86]

The hyperexpression of GH is connected with gigantism or acromegaly while the ability of detecting the increase of GH peaks and concentration would give important information on the development of aggressive form of tumours.[87, 88]

For its action on the metabolism and the response of the body to physical exertions GH is also consider a doping substance from the world anti-doping association.[89]

Concentrations of GH in biofluids other than blood is less studied, in 2016 Gough *et al.* measured the levels of GH in serum, urine and saliva in 11 male candidates before and after physical activity (**Table 1**).[90]

The immunoassays are the most common clinical analysis for the serum concentration of GH, but recently there have been reports on the poor comparability between the different immunoassays and on their analytical value.[91]

1.3 Biosensors: advantages and drawbacks compared to traditional techniques

Biosensors are appealing tools with the extraordinary capacity to detect a biological event on a transducing device utilizing a

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signal proportionate to the analyte concentration.[92-99] Both microfluidic innovations and nanotechnology advances have made easier for the design and development of miniaturized devices that can operate as PoC devices.[100, 101]

The identification of molecules in the human body specifically for the purpose of keeping track of one's health is referred to as molecular diagnostics, which covers both early and emergency diagnostics.[101] Early disease detection can improve patients' quality of life while they are receiving treatment and enhance the likelihood that they will survive.

Biosensors can be classified according to their recognition elements (bioreceptors) or type of transducer signal that was employed for target detection (**Figure 3A**). Bioreceptors are antibodies, enzymes, DNA or aptamers, molecular imprinted polymers (MIPs), and cells. Their corresponding biosensors can be: immune-, enzymatic-[102], apta-, DNA/nucleic acid (geno-), cell-based sensors (**Figure 3B**).

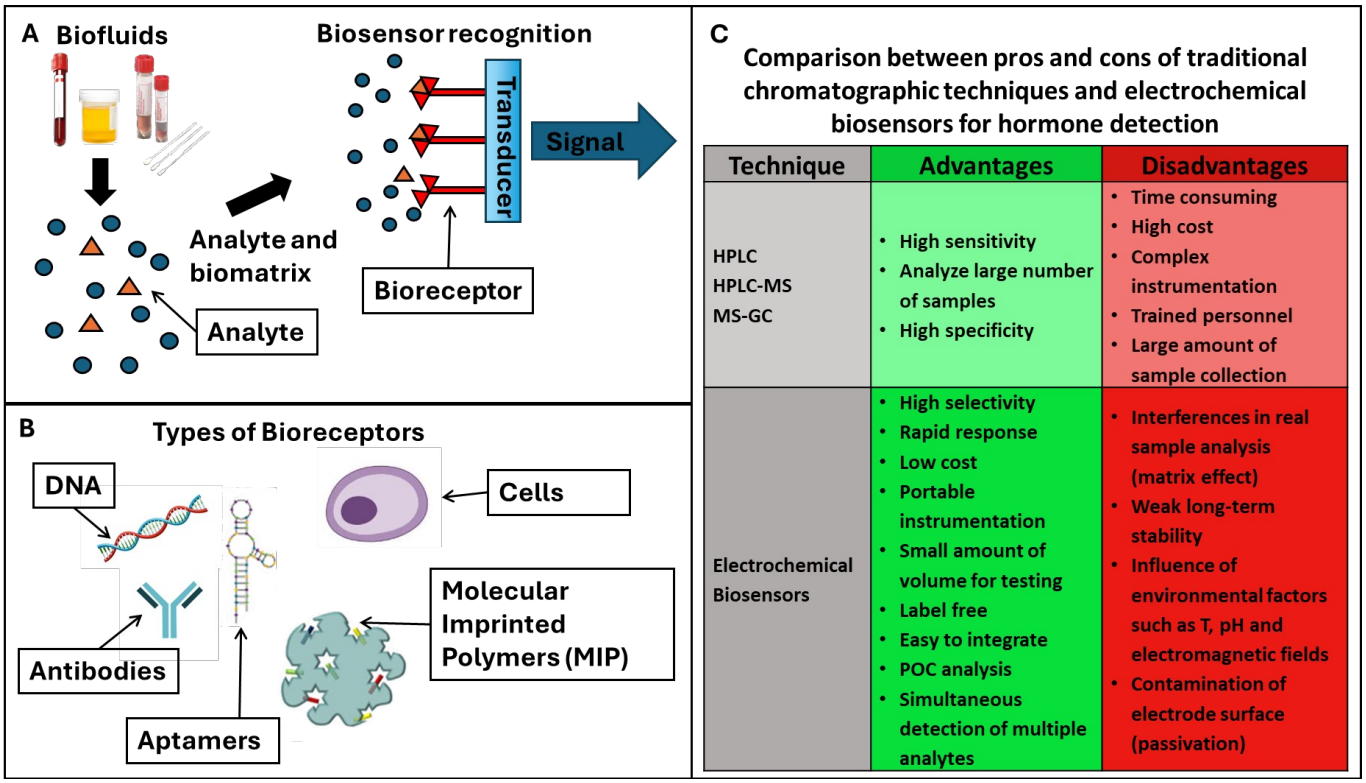
Aptasensors are a particular class of nucleic acid biosensors, based on aptamers (single-stranded DNA/RNA) that "fit" specifically to a target. They are synthetic antibodies, DNA receptors that can replace antibodies, or complete DNA strands. MIPs are best described as synthetic analogues to the natural, biological antibody - antigen systems. As such, they operate by a “lock and key” mechanism to selectively bind the molecule with which they were templated during production. MIPs potentially offer the specificity and selectivity of the biological receptors with the explicit advantages of durability with respect to environmental conditions and low cost.

Most biosensors are based on enzymes; they can recognize the target employing electroactive species[102] as by-products of their enzymatic reaction. The target recognition of the bioreceptor can be quantified by measuring the transductor signal at the interface of the biosensing device.[103] There are several transduction signals, based on mass variations, dielectric constant, heat, redox signal, and, consequently, biosensors can be also classified in piezoelectric, optical, calorimetric and electrochemical, respectively.[104] The superior characteristics of the electrochemical biosensors in terms of sensitivity and selectivity, operational simplicity and possibility of miniaturization made these devices suitable candidates for the development of marketable PoC devices.

1.4 Electrochemical biosensors

Additionally, depending on the type of transducer, the electrochemical biosensors can be subdivided in into the following categories: amperometric, potentiometric, conductometric and impedimetric biosensors, depending on the interaction of the biomolecules at the electron surface which may generate. These sensors based on the interaction of the biomolecules to the electrode surface, which may generate a measurable current at a particular potential (amperometric), a measurable potential or charge accumulation (potentiometric), or may alter the conductive properties of the medium (conductometric) or the charge transfer resistance (impedimetric).[105]

In recent years, the advance in the design and synthesis of novel nanomaterials with different dimensions have enabled their



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Comparison between pros and cons of traditional chromatographic techniques and electrochemical biosensors for hormone detection

Technique	Advantages	Disadvantages
HPLC HPLC-MS MS-GC	• High sensitivity • Analyze large number of samples • High specificity	• Time consuming • High cost • Complex instrumentation • Trained personnel • Large amount of sample collection
Electrochemical Biosensors	• High selectivity • Rapid response • Low cost • Portable instrumentation • Small amount of volume for testing • Label free • Easy to integrate • POC analysis • Simultaneous detection of multiple analytes	• Interferences in real sample analysis (matrix effect) • Weak long-term stability • Influence of environmental factors such as T, pH and electromagnetic fields • Contamination of electrode surface (passivation)

Fig. 3 Schematic representation of (A) biosensor components, (B) types of bioreceptors and (C) comparison between the pros and cons of the traditional techniques for hormone detection and electrochemical biosensors.

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incorporation in the development of biosensing electrochemical platforms, including 1D (e.g. nanotubes, nanowires, nanorods), 2D (e.g. graphene, nanosheets) and 3D (e.g. nanoparticles, quantum dots) nanomaterials. In particular, nanoparticles (NPs) have been largely used in biosensing technology electrode surface modification due to their excellent amplification of the detection signal.

Owing to their very small size (1-100 nm), they exhibit unique and very attractive chemical, physical and electronic features different from those of bulk materials, and they can be employed successfully in the development of promising electrochemical sensing devices. Usually, metal NPs, present good conductivity and catalytic characteristics, which enable them to act as "electrical wires" to improve the electron transfer (ET) between the redox center of the enzymes and the electrode surface, and moreover to act as catalysts to increase the electrochemical reactions.

Many kinds of nanoparticles (metal, oxide and semiconductor ones) are widely used in electrochemical analytical devices. Their principal functions can be summarized as follows in: (i) biomolecules immobilization (thanks to their large specific surface area they can adsorb strongly the bioreceptor on the electrode surface and preserve their bioactivity); (ii) catalysis of electrochemical reactions (due to their excellent catalytic properties); (iii) electron transfer enhancement, acting themselves as redox mediators (NPs act as mediators); (iv) molecules labelling molecules (due to their small size and modifiability) and (v) acting as reactants (thanks to their chemical activity).[106-108]

As far as hormones detection, biosensors offer several advantages compared to the most widely used conventional techniques, such as HPLC, HPLC-M and MS-GC. Unfortunately, it is well known that these methods involve lengthy procedures and need qualified personnel to carry out the analysis. Hormone detection with electrochemical biosensors is much faster, more sensitive, and less expensive compared to these, as they do not require expensive equipment or specialists.

Moreover, they allow rapid response and ease of miniaturization for PoC diagnostics.[109] In addition to the advantage of electronic miniaturization of electrochemical biosensors for PoC diagnostics[109] there are many mobile applications, and the possible integration of this technology into the internet-of-things (IoT) would help with both the real-time monitoring of patients and by the rapid transmission of clinical data on infectious diseases. Moreover, the users of this diagnostic technology are able to perform an assay using their own devices, thus opening the door to easy, affordable techniques based on PoC technology. With these benefits, electrochemical biosensors are able to meet the technological needs and public health appeals of PoC, which involving widespread accessibility of people for early diagnosis and treatment of diseases.

When compared with electrochemical biosensors, the most common techniques used for the detection of hormones are expensive to perform and maintain, while requiring long sample preparations and time of analysis or lack of the necessary sensitivity to be used for clinical evaluations. Electrochemical biosensors have similar sensitivity to MS techniques without the cost and the training requirement for techniques such as MS (lengthy sample preparation) or ELISA (30 min – 4 h of testing time).[67] The convenience and the speed of electrochemical analysis allows the quick determination of multiple hormones for the same duration and resources required for a single sample.

On the other hand, the biosensors have also some pitfalls such as interferences in real sample analysis, weak long-term stability and contamination of the biosensor surface which, in some cases, may limit their reusability.

Figure 3C summarizes the advantages and disadvantages of the electrochemical biosensors compared to conventional chromatographic methods for hormone detection.

2. Nanoparticles-based electrochemical biosensors for hormone detection in biological fluids

Biofluids produced by the human body for biosensing hormone detection are blood, urine, saliva, sweat and tears. Blood is collected from the patients with a blood draw by inserting a needle, and it is therefore an invasive procedure with consequent patient discomfort which may limit its application. Moreover, it has to be repeated at regular time intervals when it is necessary to measure the hormone levels during this time. Serum and plasma are both fluids derived by the liquid part of the blood: serum is obtained by letting the blood cloth and collecting the supernatant, while plasma is obtained by stabilizing the blood sample with anti-clotting agent (ethylenediaminetetraacetic acid, citrate etc.) and then separating the liquid part of the blood from the solids by centrifugation.[110]

The use of blood, serum and plasma has been limited due to the invasiveness of the procedure to collect the samples.

Urine, saliva, sweat and tears are externally secreted biofluids which have the advantage of their non-invasive detection. In particular, urine and saliva are considered vital biofluids due to their easy accessibility and large availability. Moreover, they show good correlations with most blood analytes, which make them a valid alternative to blood analysis. The collection of urine and salivary samples is usually quick and not particularly invasive, which allow to repeat the sampling several times during the day.

Sweat and tears represent other interesting biofluids for their good correlation with blood, although their collection may be not always easy. For example, in many applications outside athletics, it is often necessary to use wearable bands to locally stimulate sweating.

Saliva, sweat and tears show also some limitation and lead to contamination. The concentration of their bioanalytes is more variable compared to the composition of blood, and can be influenced by different factors such as the presence of microbes on the skin, in the case of sweat and tears, or by food ingestion, in the case of saliva.[111]

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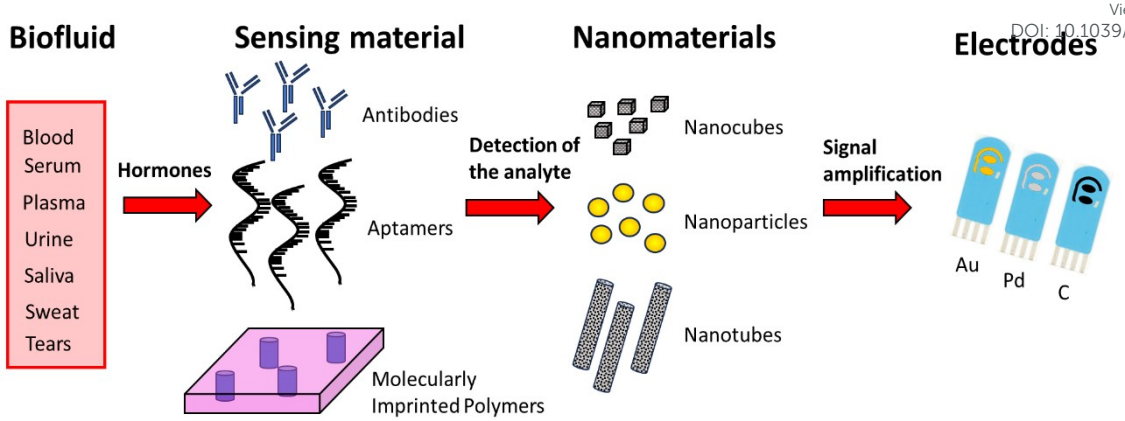

Fig. 4 General principle of nanoparticle-based electrochemical biosensors for detecting hormones from biological fluids.

All these disadvantages may be overcome with the interstitial fluid. The interstitial fluid (ISF) is definitely the most minimally invasive accessible fluid of the body. It basically acts as a mediator between blood vessels and cells in constant supply of nutrients and waste product and therefore it represents the most reproducible matrix showing excellent correlation with blood.[112] However, ISF extraction requires the realization of a complex integrated wearable device which allows extraction, collection and detection of the desired bioanalytes in a continuous manner. The hormone detection with wearable biosensors will be discussed in section 3.

The simplest strategy for electrochemical-based monitoring of hormones is their direct electrochemical signal at the electrode surface at a specific potential. In this approach, the hormone of interest is directly oxidized or reduced at the electrode surface producing an electroactive product, which can be electrochemically measured, whose concentration is proportional to the target hormone. However, direct oxidation usually occurs at a high overpotential, which can lead to electroactive interference from other species present in the biological matrix. To this aim, nanomaterials have been successfully utilized for electrode surface modification with the result of a higher selectivity. However, to better address this issue, indirect detection methods have been realized based on the use of specific biorecognition elements coupled to the electrode surface, allowing for sensitive and selective detection of the target hormone.

The electrochemical nanoparticles-based biosensors described in this review, namely immunosensors, aptasensors, and MIP-based biosensors, depending on which biorecognition element is used, i.e. antibodies, aptamers, or MIPs, respectively, may involve direct, competitive, or sandwich configurations and

Table 2 Electrochemical nanoparticles-based biosensors for cortisol detection.

Nanoparticles	Electrode	Receptor	Transducer	Bodily Fluid	LOD	Linear Range	Ref.
AuNPs	Au	Aptamers	DPV	Saliva	0.8 pM	0.28 pM - 0.28 µM	114
Ni nanoclusters-N-CNT	GCE	MIP	DPV	Saliva	2.37 fM	10 fM - 1 nM	115
AuNPs/MoS_2/AuNPs	C	AntiC	DPV	Saliva	0.18 nM	0.5 nM - 200 nM	116
$SmMoO_4$ nanoflowers	GCE	Aptamers	DPV	Saliva	45 fM	100 fM- 10 pM	113
AuNPs	Au	MIP	SWV	Saliva	200 fM	1 pM - 0.5 µM	119
NiCo-MOF/SnS_s-Thioacetamide/AuNPs	GCE	AntiC	SWV	Saliva	80 fM	0.28 pM - 0.28 µM	120
$CuWO_4$@MoS_2 NPs/Chitosan-AuNPs	GCE	AntiC	CV	Saliva	38.6 aM	2.8 fM - 2.8 µM	122
ZnO nanoflakes	Au	AntiC	CV	Saliva	1 pM	100 pM - 100 nM	123
ZnO nanorods	Au	AntiC	CV	Saliva	1 pM	10 pM - 100 nM	123
DNA-AuNPs superlattice	Au	Aptamers	EIS	Saliva	0.13 nM	0.5 nM - 10 nM	124
MWCNTs/AuNPs	PDMS	AntiC	DPV	Sweat	0.83 fM	2.8 fM - 2.8 µM	125
AgNPs and AuNPs	GCE	Aptamers	DPV	Sweat	0.25 pM	0.28 pM - 28.6 nM	126
Fe_2O_3 nanosized ellipsoid	Carbon Yarn	AntiC	CV	Sweat	8.3 aM	2.8 fM - 2.8 µM	127
MnO_2 nanocacti	GCE	AntiC	EIS	Sweat	23 fM	0.1 pM - 1.5 nM	128
ZnO nanocrystals	Pd	AntiC	EIS	Sweat	2.8 nM	27.6 nM - 0.5 µM	129
Ag@AgO core/shell	Au	AntiC	CV	Serum	1.8 pM	2.8 pM - 2.8 µM	130
Fe_3O_4 NPs and AuNPs	GCE	AntiC	DPV	Serum	0.14 nM	0.28 nM -2.8 µM	131
AuNPs	Au	AntiC	SWV	Blood	44.1 pM	137.9 pM - 6.9 nM	132

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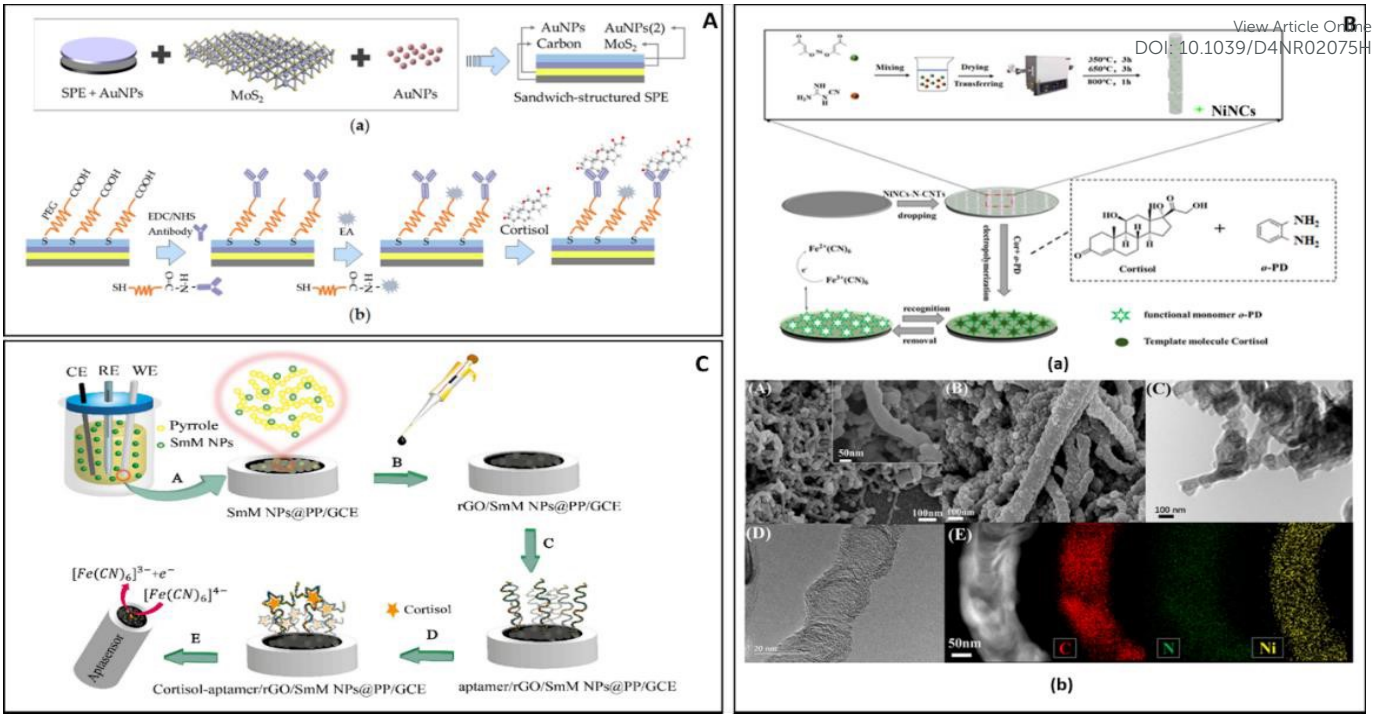

Fig. 5 A) The construction and detection procedure of cortisol immunosensor: (a) Fabrication of layer-by-layer self-assembled gold nanoparticles/molybdenum disulfide/gold nanoparticles (AuNPs/MoS2/AuNPs) sandwich-structured screen-printed electrode (SPE), (b) covalent immobilization of cortisol antibody through self-assembled monolayer of polyethylene glycol (PEG).[116] **B**) Preparation of the NiNCTs/MIP electrode and SEM characterization: (a) Multisteps fabrication of the NiNCs-N-CNTs/GCE electrode and MIP layer, (b) SEM images of NiNCs-N-CNTs (A) and MIP/NiNCs-N-CNTs (B), TEM images of MIP/NiNCs-N-CNTs, (C) and NiNCs-N-CNTs (D), STEM-EDS mapping (C, N and Ni elements) of NiNCs-N-CNTs (E).[115] **C**) Aptasensor design: (A) electro-polymerization to form polymeric nanocomposites, (B) rGO dropping, (C) aptamer loading, (D) aptasensor immersion in the cortisol solution, (E) electro-chemical investigation of ferricyanide as a probe on the surface of the aptasensor.[113]

label-free or label-based approaches. Less frequently enzymes are employed, corresponding to the so-called enzymatic biosensors.

In the following, recent various electrochemical detection strategies used for measuring the selected hormones in several sample matrices are presented and schematized in **Figure 4.**

2.1 Detection of Cortisol

Cortisol is not an electroactive molecule and lacks a specific enzyme catalysing its redox reaction. Consequently, most cortisol biosensors are affinity biosensors based on bioreceptors, such as antibodies (immunosensors), aptamers (aptasensors) and MIPs.

Regular sampling and analysis of the cortisol concentrations during the day it is fundamental to study the metabolic behaviour of a hormone that naturally has a peak of concentration in the morning and slowly decrease with time to reach a minimum around midnight.

Table summarized the characteristics and performances of nanoparticles-based electrochemical biosensors for cortisol in literature.

2.1.1 Saliva samples.

Cortisol concentrations in saliva are always fairly low, less than ≈30 ng/mL in the morning and as low as 2.2 ng/mL in the night, which requires the development of very sensitive sensors.[113]

2.1.1.1. *Voltammetric biosensors*.

Examples of differential pulse voltammetry (DPV) electrochemical biosensors for the detection of cortisol in saliva can be found in the work of Sharma V. *et al.*, Duan D. *et al.*, Liu J. *et al.* and Rezapoor-Fashtali Z. *et al.*[113-116]

Sharma V. *et al.* in 2023 developed an electrochemical sensor based on a printed Au electrode on which AuNPs are deposited and then functionalized with aptamers to detect cortisol. *et al.*[114]

Liu J. *et al.* (**Figure5A**) described an electrode based on a AuNP modified carbon electrode, on which a layer of MoS_2 was deposited. On it another layer of AuNPs was deposited and modified with a PEG-COOH attached to antibodies. In this work the AuNPs are used both to anchor the different layers of the sensor and to improve the electrical contact between the electrode and the antibody. One of the advantages of this sensor was to be easily controlled using a smartphone app.[116]

Duan D. *et al.* in 2022 (**Figure 5B**) offered a different approach in the construction of a biosensors, their DPV electrochemical sensor was based on a glass carbon electrode on which were dropped nitrogen doped carbon nanotubes modified with nickel nanoclusters (NiNCs-N-NCTs). The presence of the NiNCs-N-NCTs on the surface of the electrode greatly expanded the surface and the sensitivity of the sensor thus allowing it to register the minute change in current caused by the binding of cortisol on the matrix formed by the polymerization of o-phenylenediamine in presence of cortisol (0.5 mM). After the reticulation the polymer formed was able to retain cavities shaped as the molecules of cortisol present during the process

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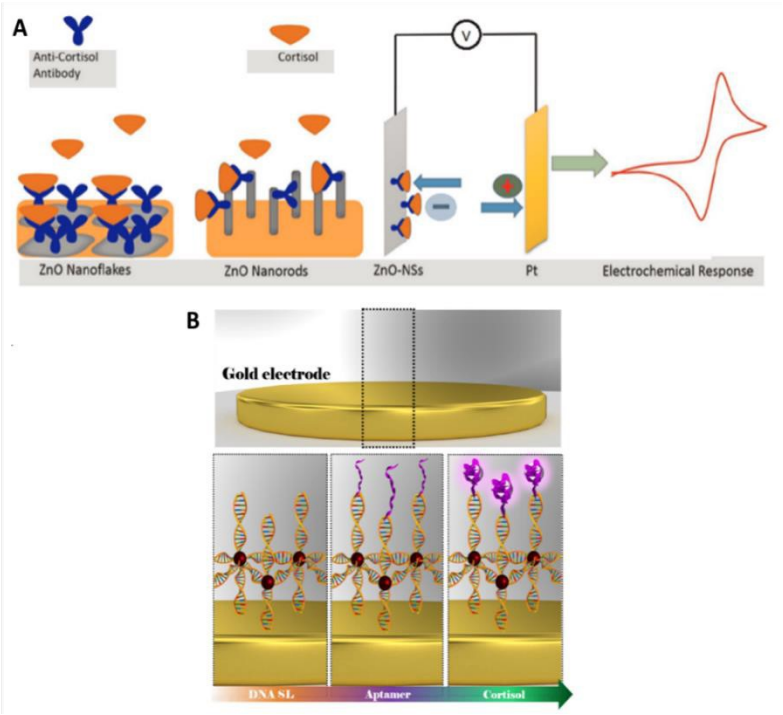

Fig. 6 A) Illustration of ZnO-nanorods and ZnO-nanoflakes immobilized on gold electrodes and functionalized with monoclonal anti-cortisol antibody (left); electrochemical response at the fabricated electrodes (right).[123] B) Structure of the superlattice/AuNPs/Aptamer biosensor.[124]

and created sites where cortisol in the sample could interact. According to Duan D. *et al.* the bamboo-like structure of the electrode improved the transmission of the signal from the MIP to the electrode thus providing an enhancement of the signal detected.[115]

Rezapoor-Fashtali Z. *et al.* (**Fig. 5C**) for their sensor utilized samarium molybdate ($SmMoO_4$) nanoflowers deposited on a glassy carbon electrode (GCE) and trapped in a layer of polypyrrole (PP). The resulting mesh was then covered with reduced graphene oxide (r-GO). On this base, aptamers were attached by exploiting their terminal -NH moiety and used to interact with cortisol. In this set up the layer of r-GO maintains a weak negative charge that slightly repel DNA sequences (negatively charged), thus preventing entanglement and improving the interaction with cortisol. The sensor requires 15 min of incubation in a solution containing the analyte in order to maximize its response to the presence of cortisol. *et al.*[113]

Another interesting voltammetric technique is SWV, which is faster than DPV and can sometimes offer better sensitivity and being less effected by non-Faradic currents although it is less suitable for very diluted samples.[117, 118]

Both Sanjida Y. *et al.* and Yang B. *et al.* apply SWV to the detection of cortisol in salivary fluid, while utilizing different electrodes and methods of detection.[119, 120] In the work of Sanjida Y. *et al.*, AuNPs and a MIP are formed on the surface of a gold-plated electrode. The reactions which lead to the simultaneous formation of Au NPs and MIP is done by applying between 0 V to 1 V to a solution of o-phenylendiamine and $HAuCl_4$ in presence of cortisol. The resulting polymer will have Au NPs trapped in its matrix and, after washing, cavities shaped as molecules of cortisol. During the analysis of a sample some of the cavities will be occupied by cortisol and the amount of current passing throughout the electrode will be reduced.[119]

Yang B. *et al.* used a complex metal-organic frameworks (MOF) electrode based on a) of $Ni(NO_3)_2$, $K_3[Co(CN)_6]$ and SnS_2. The NiCo-MOF was shaped as nanocubes and embedded in a matrix of SnS_2 and thioacetamide. In the same matrix AuNPs were formed. On this complex structure, monoclonal antibodies are attached and used to detect cortisol.[120]

CV is a fundamental technique for the study of redox reactions, based on the fast application of a potential range to a sample while measuring the current produced.[121]

Nong C. *et al.* developed an electrochemical biosensor for cortisol in saliva based on a modified glassy carbon electrode (GCE) on which core shell particles of $CuWO_4$ covered in MoS_2 were deposited. On the functionalized surface of the electrode a network of chitosan ornated with AuNPs was layered and functionalized with anti-cortisol monoclonal antibodies foetal bovine serum (FBS) was then used to reduce the possibility of non-specific binding*l.*[122]

Vabbina P.K. *et al.* have developed two electrochemical biosensors based on ZnO nanoflakes or ZnO nanorods immobilized on a gold electrode (**Figure 6A**). On these nanostructures antibodies were attached and used to detect the presence of cortisol.[123]

The width of the linear range for this system doesn't completely represent the differences between the two particles morphology tested by Vabbina *et al.* ZnO nanorods were able to give a linear response for quantities of cortisol 10 times smaller than the flakes, but the larger exposed surface of the flakes and their shape improved the contact with the electrode and the binding with the antibody thus generating sharp and defined

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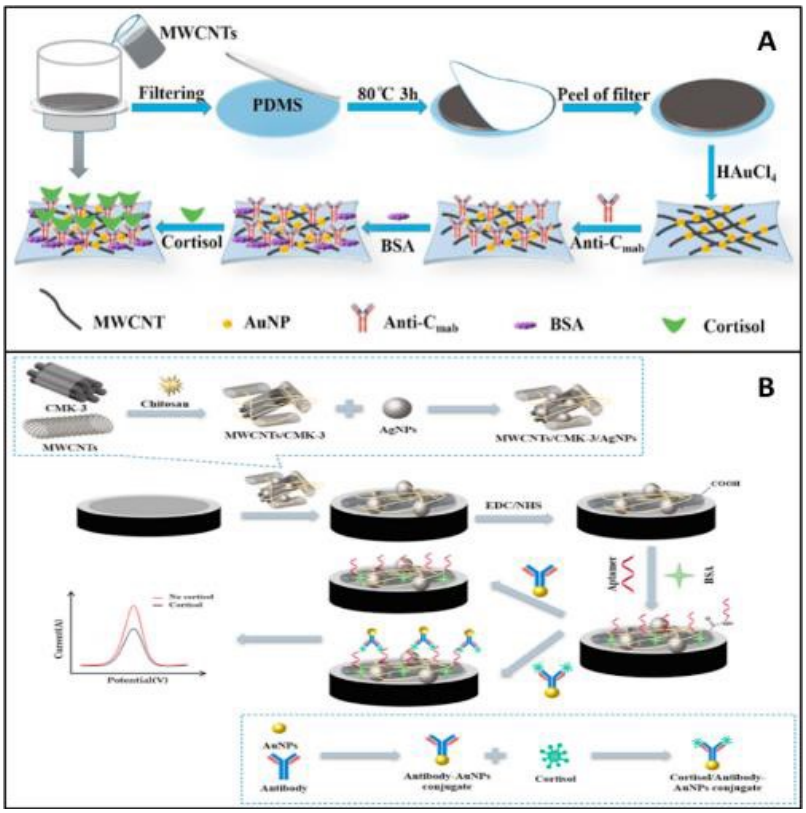

Fig. 7 A) Stepwise fabrication of the immunosensor (Anti- Cmab /AuNPs/MWCNTs/PDMS)[125]; B) Preparation and functioning of the cortisol sensor described by Huang et al. [126]

peaks when used for CV. This gain in resolution and the relative ease in manufacture made the ZnO nanoflakes the most likely candidate for further development.

2.1.1.2. *Impedimetric biosensors*.

Cantelli *et al.* used EIS for cortisol detection, a technique able to collect information about the processes happening on the electrode surface by measuring the fluctuations of impedance caused by the biorecognition process at the electrode surface in presence of a redox probe in solution (**Figure 6B**). In this work the authors have realized a superlattice of DNA sequences attached to an Au electrode and attached to AuNPs and to specific aptamers.[124]

2.1.2 Sweat samples.

According to the most diffused studies sweat can contain between 8.16 ng/mL and 141.7 ng/mL of cortisol, a concentration comparable to blood and plasma.[34] Recently, there have been new evidences that these values may have been overestimated, with the actual concentration of cortisol in the sweat attesting around 0.5 ng/mL (1.4 nM) to 1.7 ng mL^{-1} (4.7 nM), with large variations caused by the stress level and the diet.[111]

2.1.2.1 *Voltammetric biosensors*.

DPV results the most commonly used techniques for analysing the concentration of cortisol in sweat samples. Obviously, several different electrochemical platforms and different NPs have been employed for electrode modification. Liu *et al.* used multi-walled carbon nanotubes (MWCNTs)[125] to increase the sensitivity of thin microelectrodes whereas Huang *et al.* in 2021 used MWCNTs in combination with mesoporous carbon material (CMK-3) bound with chitosan and ornated with Ag NPs as a way to detect the presence of cortisol in sweat.

The voltametric biosensor described by Liu *et al.* is formed by a thin layer of MWCNTs supported on a film of polydimethylsiloxane (PDMS), which was functionalized with AuNPs and cortisol specific antibody (**Figure 7A**). Later the electrode was incubated with BSA to saturate all the locations for nonspecific binding. The prepared electrochemical biosensor was thin and flexible enough to be worn as a patch and detect the level cortisol in real-time.[125]

Z. Huang *et al.* described an electrochemical biosystem based on a GCE electrode on which MWCNTs and CMK-3 were deposited and blocked with chitosan (**Figure 7B**). On this ornated surface, Ag NPs were absorbed and used as support for aptamers, which are able to bond cortisol with antibodies and Au NPs which are used to produce the analytical signal.[126]

CV is relatively less popular as a detection technique for cortisol in sweat samples, however Sekar *et al.* have successfully created a electrochemical biosensors based on this technique.[127] The proposed biosensor was fabricated by deposition of ellipsoidal Fe_2O_3 nanosized ellipsoid, 300 – 350 nm of diameter and 850 nm length on a carbon yarn. Followed by the immobilization of cortisol specific antibodies. The excess of non-specific binding sites was blocked by BSA. The proposed

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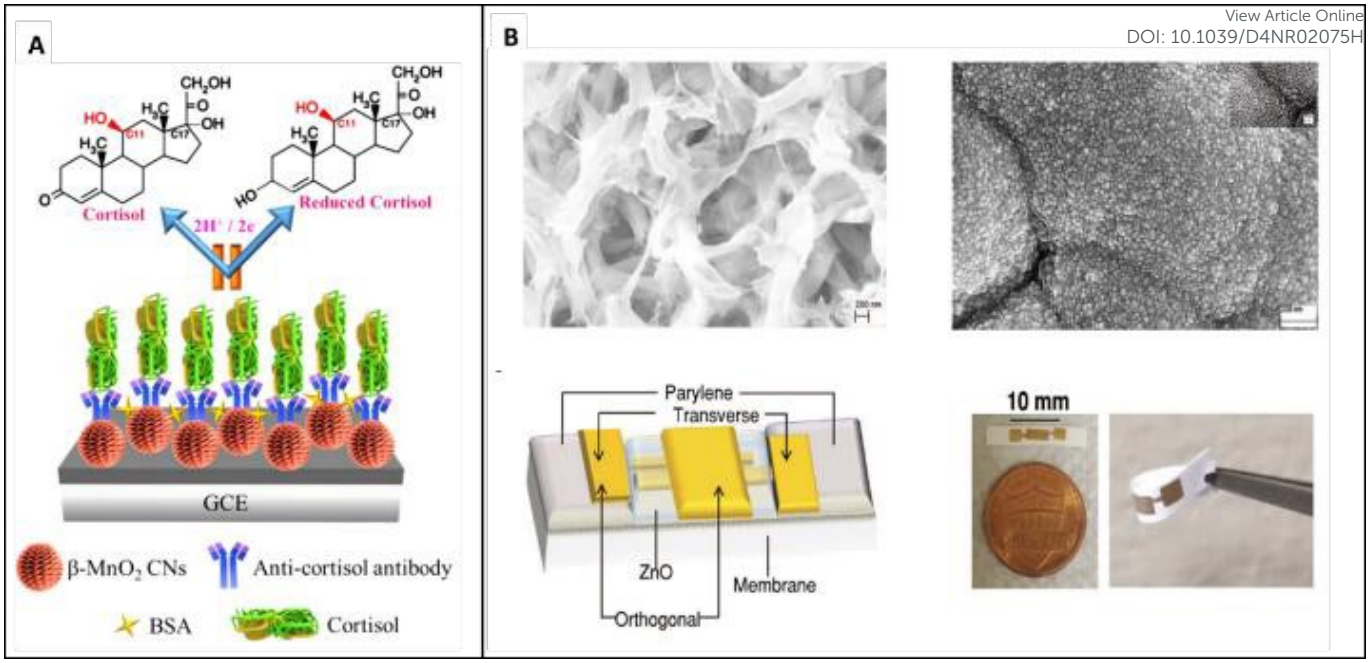

Fig. 8 A) Schematic representation of MnO_2 nanocacti electrochemical biosensor; [128] B) TEM pictures of the polyamide matrix before and after the deposition of ZnO, scheme of the electrochemical biosensor and real-life image of the biosensor.[129]

immunosensor was able to fully analyse sweat samples in only 120 s.[127]

2.1.2.2. *Impedimetric biosensors*.

Zhou *et al.* developed a biosensor based on MnO_2 nanostructures, while Munje *et al.* developed a biosensor based on the deposition of NPs of ZnO on the surface of polyamide substrate.[128, 129]

Zhou *et al.* studied the possibility of fabricating an electrochemical biosensor based on MnO_2 nanostructures as nanorods, MnO_2 nanoparticles/carbon and MnO_2 nanocati (**Figure 8A**). Nanocacti are nanoparticles of approximatively spherical structures with spikes and crumpled thin surfaces similar to the leaves of a succulent plant. Of the three nanostructures only MnO_2 nanocacti showed some resistivity which is necessary for EIS analysis. These structures were used to form the biosensors, modified with monoclonal antibodies for cortisol and the non-specific binding sites blocked with BSA.[128]

The last NP-based electrochemical biosensor for the detection of the concentration of cortisol in sweat based on EIS listed in this review has been developed by Munje *et al.* (**Figure 8B**). In their work, Munje R.D. *et al.* deposited a thin layer (≈ 90 – 100 nm) of ZnO on a polyamide matrix by pulsed laser deposition, on this layer antibodies specific for cortisol are attached by using dithiobis(succinimidyl propionate) (DSP) as crosslinker.*et al*.[129]

This system is on the threshold between nanostructured material and proper NPs, the pores in the polyamide matrix are nanosized as well as the size of the ZnO nanocrystals laser deposed on it, but in its complex the structure is macroscopic.

2.1.3 Other body fluids.

Although the initial stage of cortisol biosensing used only blood serum as a sample, only a few papers are reported in literature in recent years for the detection of cortisol in blood and serum, which have been now overtaken by sweat and saliva for their non-invasive nature of detection.

Both Kaushik *et al.* and Sun *et al.* developed two immunosensors to detect cortisol in human serum.[130, 131] Kaushik *et al.* developed an electrochemical biosensor based on alternate electrodeposition of layers of polyalanine and core-shell Ag@AgO NPs on a gold electrode. On the modified electrode monoclonal antibody for cortisol is attached and the non-specific absorption was prevented by the deposition of BSA on the surface of the electrode. The presence of cortisol and its concentration was established by CV.[130]

Sun and collaborators developed an electrochemical biosensor based on a glass carbon electrode GCE cover in Nafion on which a layer of polyethylenimine graphene oxide (rGO-PEI) modified with Fe_3O_4 NPs is attached. On the electrode a layer of AuNPs is also deposited. Cortisol will attach to the surface of the electrode and be used as an anchor point for monoclonal cortisol antibodies modified with an enzyme able to induce the dimerization of phenylethylenediamine in presence of H_2O_2 which could be detected by DPV.[131]

Liu et al. have developed an electrochemical biosensor for detecting the presence of cortisol in blood samples. On a Au electrode, AuNPs were deposited on this substrate thiolated protein G and monoclonal antibodies for cortisol are attached and tested using SWV.[132]

2.2 Sex Hormones

Most electrochemical biosensors for the three main sex hormones, estradiol, testosterone and progesterone, in all biological samples reported in literature are based on

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Table 3 Electrochemical nanoparticles-based biosensors for sex hormones detection.

Sex Hormone	Nanoparticle	Electrode	Receptor	Transducer	Bodily Fluid	LOD	Linear Range	Ref.
Testosterone	MNPs	SPE	Antibody	CV	Urine	23.7 ng mL^{-1}	50 - 1000 ng mL^{-1}	133
Estradiol	AuNPs	GCE	Enzyme	DPV	Urine	6.0 x 10^{-14} M	5.0x10^{-13} - 5.0x10^{-9} M	134
Estradiol	RhNPs	GCE	Enzyme	DPV	Urine	0.54 pM	0.9 - 11 pM	135
Estradiol	AuNPs	GCE	Aptamer	DPV	Urine	0.7 pM	0.001 - 1 nM	136
Estradiol	AgNPs	GCE	Antibody	Amperometry	Urine	65 pg mL^{-1}	0.1 - 50 ng mL^{-1}	137
Progesterone	N-HCSs	GCE	Aptamer	DPV	Urine	3.3x10^{-6} nM	1x10^{-5} - 4.2x10^{-3} nM	138
Estradiol	AuNPs	GCE	Aptamer	DPV	Serum	1.5 pM	12 pM - 60 nM	140
Estradiol	AuNPs	SPCE	Antibody	CV, DPV	Serum	10 pg mL^{-1}	0.01 - 100 ng mL^{-1}	141
Estradiol	AuNPs	Au electrode	Antibody	SWV	Serum	0.84 pg mL^{-1}	0.54 - 1.36x10^{4} pg mL^{-1}	142
Estradiol	AuNPs	GCE	Aptamer	DPV	Serum	5 pg mL^{-1}	0.01 - 500 ng mL^{-1}	143
Estradiol	Au@Pt	GCE	Aptamer	DPV	Serum	8.0x10^{-14} M	1.0x10^{-13}-1.0x10^{-9} M	144
Progesterone	NiO-AuNFs	SPCE	Aptamer	DPV	Serum	0.58 pg mL^{-1}	0.003 - 314.46 ng mL^{-1}	145
Progesterone	AuNPs	GCE	MIP	SWV	Serum	0.17 nM	0.2 - 125 nM	146
Testosterone	AuNPs	ITO glass	Antibody	EIS	Saliva	3.9 ng mL^{-1}	10 ng mL^{-1} - 0.5 μg mL^{-1}	149

antibodies and enzymes. **Table** summarized the characteristics and performances of NP-based electrochemical biosensors for estradiol, progesterone and testosterone in literature.

2.2.1 Urine Samples.

Analyzing urine for the detection of sex hormones has been a potential avenue, yet its application has been constrained by the nature of sample collection procedures.

2.2.1.1 ***Voltammetric biosensors.***

CV was largely used for the detection of the three sex hormones. Sanli *et al.* developed a portable testosterone biosensor based on the use of a screen-printed working electrode (SPWE) functionalized with magnetic NPs (MNPs), in particular iron oxide NPs.[133] The designed biosensor was analyzed in spiked synthetic urine sample by CV and demonstrating a high selectivity and sensitivity towards testosterone, with a linear range between 50 to 1000 ng mL^{-1} and LOD value of 23.68 ng mL^{-1}.[133]

DPV was also largely used because of its superior sensitivity. Huang *et al.* used AuNPs for the design of a selective and sensitive enzyme biosensor for 17-β-estradiol.[134] A GCE was modified with copper sulfide nanosheets (CuS), that act as electrical conductor, and the dual modification of glucose oxidase and gold nanoparticles (AuNPs) on the electrode generate the electrochemical signal amplification. The combination of AuNPs and CuS nanosheets favored the electron transfer and amplified the DPV electrochemical signal, this made it possible to obtain a high sensitivity with a LOD of 60 fM.[134]

Povedano and colleagues developed and electrochemical enzymatic biosensor based on rhodium nanoparticles (RhNPs) for 17-β-estradiol detection.[135] The innovative platform was obtained by a one-pot reaction that generate a nanomaterial based on graphene oxide/Rhodium nanoparticles (rGO/RhNPs). In particular, the simultaneous reduction of Rhodium(III) chloride (RhCl3) and graphene oxide with sodium borohydride ($NaBH_4$) generate a novel platform subsequently filed on GCE. The rGO/RhNPs/GCE was used as a support for bind the laccase enzyme thus obtaining a biosensor used for 17-β-estradiol monitoring. The DPV had shown a high sensitivity, high selectivity and a low LOD (0.54 pM) for the designed biosensor.[135]

Huang *et al.* developed a sensitive aptasensor based on a thiol group tagged 17-β-estradiol aptamer on a GCE previously modified with AuNPs and cobalt sulfide nanosheets (CoS) for monitoring the level of 17-β-estradiol in urine sample [136]. Through differential pulse DPV it was possible evaluate the LOD value and the linear concentration range, 0.7 pM and 0.001-1 nM respectively. This electrochemical aptamer biosensor based on a hybrid nanomaterial composed by AuNPs and CoS demonstrated a high sensitivity, high selectivity and a good stability.[136]

AgNPs were used by Cincotto *et al.* to build an immunosensor for estradiol monitoring.[137] After the synthesis of mesoporous silica-coated reduced graphene oxide (SiO2/GO), that allows anchoring metal NPs safely onto graphene support and favouring the catalytic performance, the AgNPs were used to obtain the AgNPs/SiO2/GO hybrid. This hybrid structure was absorbed onto GCE and then grafting with 4-aminobenzoic acid to create a desirable surface for covalent bond of the capture antibody. This strategy allowed the development of a competitive immunosensor for the determination of estradiol hormone that exhibited a good reproducibility and stability, a linear range between 0.1 to 50 ng mL^{-1} and a LOD of 65 pg mL^{-1}.[137]

Ghanbarzadeh *et al.* have developed a novel non-invasive sensor aimed at monitoring progesterone levels.[138] In their study, they designed an ultrasensitive electrochemical aptasensor capable of detecting progesterone in human urine. The sensor utilizes nitrogen-doped hollow carbon spheres (N-HCSs) to covalently immobilize high-density aptamer (Apt) sequences, which serve as the bioreceptor for progesterone. To fabricate the sensor, N-HCSs are drop-cast onto the surface of a GCE, followed by the addition of Apt specific to progesterone onto the N-HCSs modified electrode. Subsequently, BSA is employed as a blocking agent to prevent nonspecific

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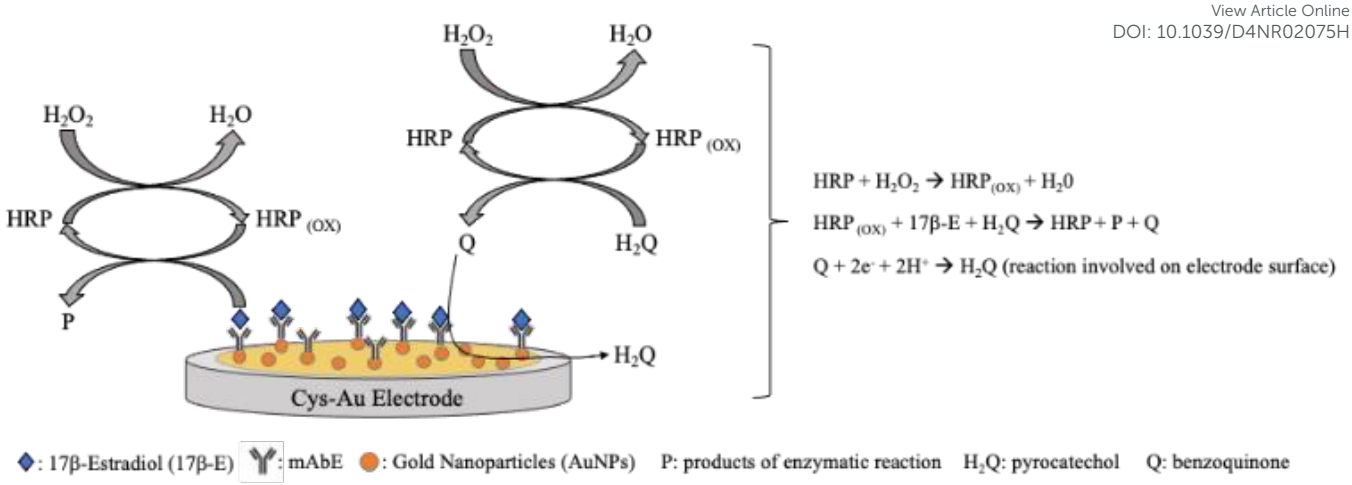

Fig. 9 Schematic presentation of the 17-β-Estradiol electrochemical immunosensor based on Cys-Au electrode modified with gold nanoparticles and anti-17-β-estradiol monoclonal antibody.[142]

interactions. By employing DPV, the sensor demonstrates a limit of detection (LOD) value of 3.33 fM and a linear concentration range spanning from 10 fM to 5.6 µM. This aptasensor exhibits a high selectivity even in the presence of various off-target species, showcasing its effectiveness in detecting progesterone in human urine samples.[138]

2.2.2 Serum Samples.

The biosensors used for sex hormones detection in serum sample are obtained by the modification of SPWE, GCE or other type of electrode with different type of NPs to obtain sensitive and selective platforms.

2.2.2.1 *Voltammetric biosensors*.

Electroanalytical techniques such as linear sweep voltammetry (LSV), CV, DPV, SWV, and chronoamperometry offer comprehensive qualitative and quantitative insights into electroactive species. Consequently, they are increasingly recognized as potential alternatives to more traditional spectrometric or chromatographic techniques. These methods are extensively employed for monitoring various hormones, including sexual hormones, owing to their ability to provide detailed information on their concentrations and behavior.[139]

A novel nanocomposite based on the interaction between AuNPs and thionine (Thi) was studied by Liu and colleagues to develop an electrochemical aptasensor to quantify the concentration of17-β-estradiol in serum samples.[140] The cationic dye Thi has been used as electrochemical indicator and was mixed with carbon nanotubes (CNTs) to form Thi/CNTs composite. After that Thi/CNTs solution was added into AuNPs solution forming AuNP/Thi/CNTs, and this support was casted onto a GCE. The designed platform modified with a specific aptasensor have been studied by DPV in presence of different concentration of 17b-estradiol and exhibit a very low limit of detection of 1.5 pM and a linear range from 12 pM to 60 nM.[140]

In another work AuNPs were combined with carbon nanotubes (CNTs) to obtain an electrochemical immunosensor for 17-β-estradiol detection.[141] Wang *et al.* fabricated a microfluidic device based on AuNPs, MWCNTs and Thi used to modify a SPWE. In this designed composite, Thi molecules act as electrochemical mediator while MWCNTs and AuNPs, like any nanomaterials, favouring electron transfer for the signal amplification. The synergistic effect of electrochemical mediator and catalytic material produced a sensitive electrochemical immunosensor that revealed a low limit of detection of 10 pg mL^{-1}.[141]

SWV was also utilized, Monneris *et al.* designed a sensitive immunosensor based on AuNPs for 17-β-estradiol (17b-E) monitoring in real bovine serum sample.[142] The immunosensor was constructed onto a gold disk electrode modified with a simple layer of cysteamine (Cys-Au electrode) grafted with AuNPs. Over this structure was immobilized the anti-17b-E monoclonal antibody (mAbE) thus obtaining a highly sensitive and selective immunosensor towards the target molecule (**Figure 9**).[142]

Ming *et al.* have introduced a novel folding aptasensor platform featuring microfluidic channels specifically designed for the label-free electrochemical detection of 17-β-Estradiol.[143] They utilized an innovative nano-assembly comprising amine-functionalized SWCNTs, methylene blue, and AuNPs to modify a GCE working electrode, thereby enhancing the detection sensitivity of estradiol. The calibration curve derived from experimental data demonstrated a linear range spanning from 10 pg mL^{-1} to 500 ng mL^{-1}, with an achieved detection limit of 5 pg mL^{-1}. Moreover, the platform underwent experiments for E2 detection in clinical serum, yielding results highly comparable to those obtained using a large electrochemical luminescence apparatus. The proposed device is a portable, inexpensive, and highly sensitive aptasensor platform that is capable of detecting with high resolution for estradiol in a real human sample.[143]

Zhao *et al.* have presented another electrochemical aptasensor tailored for monitoring 17-β-estradiol in serum samples.[144] They electrodeposited poly(3,4-ethylenedioxythiophene) (PEDOT)-graphene oxide (GO) coupled with Au@Pt nanocrystals (Au@Pt) onto a GCE. Notably, the Au@Pt nanocrystals, synthesized through a one-step reaction, were utilized for immobilizing the

aptamer specific to 17-β-estradiol, thereby enhancing detection sensitivity. Upon the addition of 17-β-estradiol the signal obtained through DPV gradually decreased. Under optimal conditions, the calibration curve for 17-β-estradiol displayed a linear range spanning from 0.1 pM to 1 nM, with an impressively low LOD of 0.08 pM. The developed aptasensor exhibited high selectivity, stability, and reproducibility.[144]

An electrochemical progesterone aptasensor was successfully developed by Samie *et al.*[145] They utilized a NiO-Au hybrid nanofibers (NiO-AuNF) synthesized via the electrospinning technique, combined with graphene quantum dots (GQDs) along with MWCNTs to modify a screen-printed carbon electrode (SPCE). This modification aimed to create an effective immobilization matrix rich in carboxylic functional groups essential for binding the progesterone-specific aptamer. Transmission electron microscopy (TEM) images of the NiO-Au nanocomposites revealed a highly porous structure composed of numerous nanoparticles, providing a large surface area and minimizing transport hindrance for subsequent catalytic reactions. The aptasensor demonstrated a dynamic concentration range spanning from 0.01 to 1000 nM, with an impressive detection limit of 1.86 pM. Furthermore, the proposed aptasensor was successfully employed for determining progesterone levels in human serum samples.[145]

A simple biosensor based on MIP was built by Laza *et al.* for the determination of progesterone in calf serum samples.[146] Initially, AuNPs were electrochemically generated on GCE to enhance the surface activity. Subsequently, the MIP was deposited through the electropolymerization of aminophenol in the presence of the target analyte. The linear range and LOD values achieved with this platform were determined via SWV and found to be 0.2 to 125 nM and 0.17 nM, respectively. The biosensor's selectivity was evaluated against compounds with similar structures to progesterone, demonstrating no interference effects.[146]

2.2.3 Saliva samples.

Saliva is gaining increasing recognition as an appealing diagnostic fluid due to several reasons. Firstly, it contains various salivary biomarkers that accurately reflect both normal physiological states and disease conditions in humans. These biomarkers provide valuable insights into the health status of an individual. Secondly, saliva offers significant advantages over traditional blood sampling methods. Collecting saliva is non-invasive, painless, and relatively easy compared to venous blood collection. Additionally, saliva collection does not require highly trained medical personnel, specialized equipment, or strict storage conditions, reducing logistical challenges and costs associated with sample collection and transportation. Overall, the presence of disease-related biomarkers in saliva coupled with the ease of sample collection makes it a promising diagnostic fluid for a wide range of applications in healthcare and biomedical research.[147]

2.2.3.1 Impedimetric biosensors.

The identification of testosterone in saliva has been accomplished, prompting studies into the reliability of salivary testosterone analysis. These studies aim to establish a strong correlation between salivary testosterone levels and serum testosterone concentration.[148]

Sun *et al.* developed an impedimetric immunosensor for testosterone monitoring in saliva sample.[149] In this work the self-assembled monolayer (SAM) of (3-aminopropyl)triethoxysilane (APTES) formed onto indium tin oxide (ITO) glass electrode by immersion into APTES solution and then AuNPs were drop-cast on the modified electrode. Finally, the plates were washed and left to dry under N_2 gas flow. The final step consisting of drop casted a solution of Ab-testosterone for the immobilization of the antibody on the electrode thus obtaining the desired immunosensor (Ab-testosterone/AuNPs/APTES/ITO glass electrode). This developed sensor provided a linear concentration of testosterone from 10 ng mL^{-1} to 0.5 µg mL^{-1}, with a limit of detection of 3.9 ng mL^{-1}.[149]

2.3 Insulin

The traditional electrochemical biosensors for insulin detection rely on the electroactivities of insulins due to the presence of electroactive amino acid residues, such as tyrosine, histidine, and cysteine, which are electro-active.[150] In the redox process, insulin contains three disulfide bonds the redox behaviour of which play a crucial role in electrochemical detection.[151, 152] Under electrochemical activation, these disulfide bonds can undergo reduction and oxidation processes, influencing the electrochemical properties. In electrochemical studies, insulin molecules absorb onto the electrode surface, forming a monolayer. This adsorption allows for the reduction of disulfide linkages, resulting in the formation of thiol moieties. These thiol groups can undergo re-oxidation, leading to the reformation of the original disulfide bonds and release of electrons. Therefore, insulin absorbed onto the electrode surface to which insulins transfer their electrons due to oxidation process, resulting in a measurable electrochemical signal. However, conventional bare electrodes are limited by slow response (ca. 100 s) due to slow electron transfer kinetics and high potential required to electrocatalytically oxidize insulins.[151] Bare electrodes generally require high energy to induce electro-oxidation reaction of insulins, causing overpotential, a common problem found in electrochemical electrodes. Electrodes are rapidly deactivated resulting in surface fouling issue. This is contributed by radical products of the disulfide oxidation process generated by anodic electrochemical reaction of insulins, which absorb and accumulate on the electrode surface.[153, 154] It therefore reduces stability and lifetime of the electrodes. Due to surface fouling issue, NPs have been incorporated into the electrodes to improve the efficiency and performance of electrochemical sensors. It is known that NPs with inherent catalytical activities can be used as electron transfer mediators or catalyzers to facilitate electron transfer at the interface between an electrode and insulins, leading to the reduction of voltage required for insulin oxidation, which could significantly prolong

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Table 4 Electrochemical nanoparticles-based biosensors for insulin detection.

Nanoparticles	Electrode	Receptor	Transducer	Body fluid	LOD	Linear range	Ref.
AuNPs	Gold electrode	aptamer	SWV	serum	0.1 pM (0.58 pg/mL)	10 pM - 10 nM	[157]
IONPs and rGO	MGCE	MIP	DPV	serum	3 pM	0.01 nM - 1 nM	[158]
AuNP@MoS_s nanocomposites	GCE	antibody	DPV	serum	0.05 pM	0.1 pM - 1 nM	[159]
Au@Cu_5Zn_8/HPCNC and AuNPs/NHG	GCE	antibody	amperometry DPV chronoamperometry	serum	0.453 fg/mL 0.341 fg/mL 0.0124 pg/mL	0.022 pg/mL - 222 ng/mL 0.022 pg/mL - 11 ng/mL 0.1 pg/mL - 50 ng/mL	[160]
PdNP@MoS_x	GCE	antibody	amperometry	serum	0.52 pM	1.72 pM - 17.2 nM	[161]
Zn_2SiO_4-PdNPs and AuINCs	GCE	antibody	chronoamperometry SWV	serum	0.25 fg mL^{-1} 80 fg mL^{-1}	0.1 pg mL^{-1} - 1 to 50 ng mL^{-1} 0.1 pg mL^{-1} - 50 ng mL^{-1}	[162]
CQDs	GCE	aptamer	EIS	serum	106.8 pM	0.5 nM - 10 nM	[163]
AgNFs-decorated rGO	ITO micro-disk electrode	antibody	EIS	serum	8.62 pM in buffer and 12.07 pM in serum	172.4 pM - 172.4 nM	[165]
AuNPs	PGE	aptamer	EIS	Plasma and urine	0.27 nM (1.57 ng/mL)	1.0 - 1000.0 nM	[168]
Nano MIPs	screen printed platinum electrodes	MIP	DPV	plasma	26 fM	50 - 2000 pM	[169]
AuNPs	SPCE	aptamer	SWV	Saliva	45 pM (0.26 ng/mL)	0.05 - 15 nM	[170]

the lifetime of electrodes and increase electrochemical activities.

However, other interfering substances, such as uric acid, glucose, and ascorbic acid, in sample matrices are also electroactive and potentially influence electrochemical detection causing false positive results.[155] Regarding specificity and selectivity of non-immune electrodes, biological insulin recognition molecules, such as antibodies, aptamers, and MIPs are combined with NP-modified electrodes to allow insulin binding and prevent undesired compounds approaching the electrodes. However, the use of biological molecules requires proper storage conditions to prevent protein denaturation, with short lifetime of the modified electrodes.[156]

Table 4 summarized the characteristics and performances of NP-based electrochemical biosensors for insulin in literature.

2.3.1 Serum samples.

Serum is the most used as the sample in the diagnosis as it contains a high level of insulin. For this reason, most studies selected insulin serum as models for their tests in human samples.

2.3.1.1 *Voltammetric biosensors*.

An electrochemical aptasensor based on a dual-signal strategy for detecting serum insulin was developed and demonstrated.[157] Instead of directly measuring insulin oxidation, the assay utilized a combination of methylene blue (MB) and a redox reporter (Fc) to generate simultaneously detectable electrochemical signals. Additionally, AuNPs were employed as a catalyst. The preparation of the dual-probe aptasensor and its SWV response are shown in **Figure 10**.

The gold electrode was modified with a "signal-off" probe (DNA1@MB-IBA) and a "signal-on" probe (DNA2Fc@GNPs/DNA1@MB-IBA) (**Figure 10A**).

Redox activities were generated by Fc and MB for the "on" and "off" signals,

respectively. In the absence of insulins, the MB probe is initially closer to the electrode than the Fc complex, allowing MB to effectively transfers electrons, resulting in a dominant off-signal observed at ~0.28 V (**Figure 10B**). Conversely, the on-signal of the Fc probe becomes more influential in the presence of insulin as the Fc complex approaches the electrode, resulting in an

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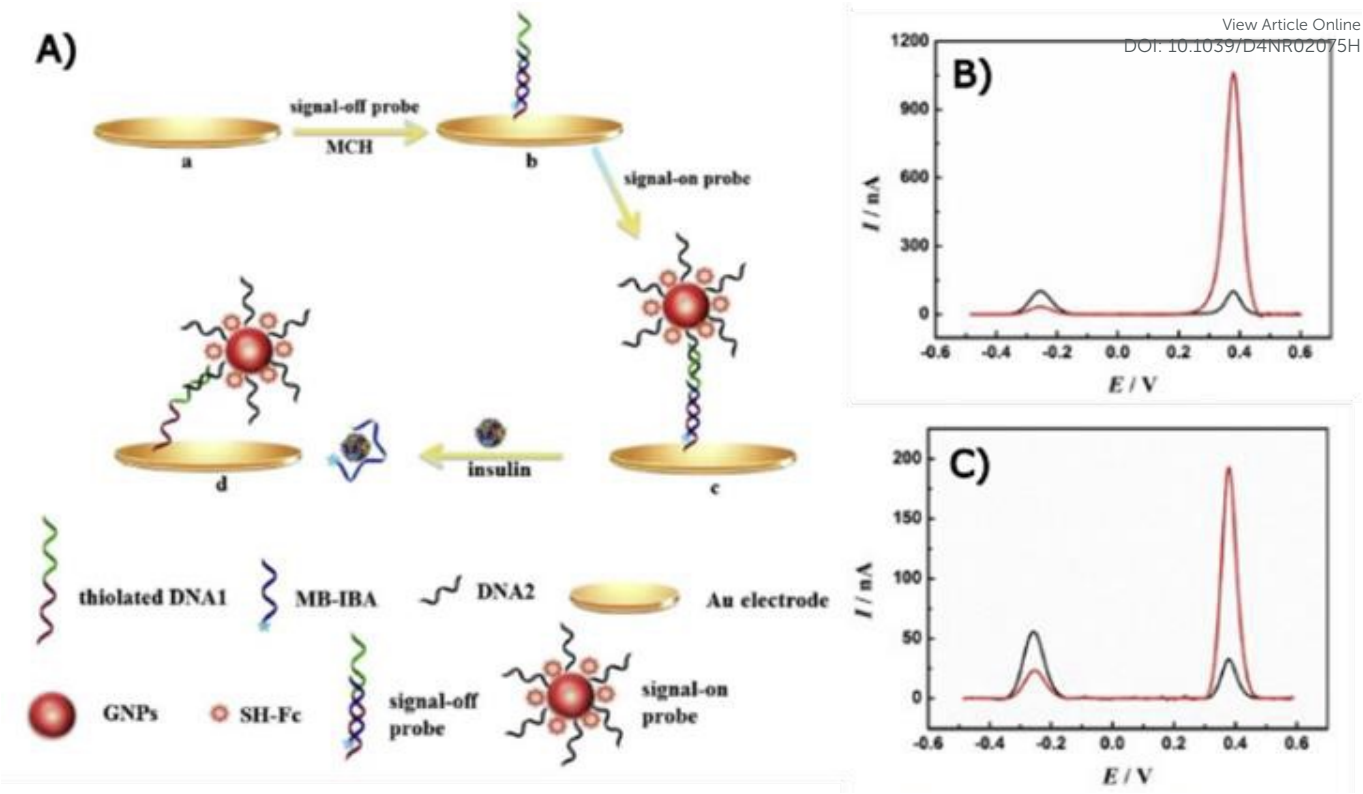

Fig. 10 Schematics of a dual-probe electrochemical aptasensor. A) Preparation of the Fc and MB probes on a gold electrode and the displacement of the MB probe upon the presence of insulin. The SWV response of the aptasensor B) with and C) without AuNP modification. The SWV response of the MB probe ("signal-off") and the Fc probe ("signal-on") at approximately 0.28 and 0.38 V, respectively. The black lines present the SWV response before insulin addition and the red lines present the SWV response after 100 pM insulin addition.[157]

enhanced oxidation peak at 0.38 V while decreasing that of the MB probe due to the displacement of the MB probe. AuNPs play a crucial role in mediating electron transfer to amplify electrochemical signal. In **Figure 10C**, the SWV signal of the electrode without AuNP-modification is displayed and it points out an enhancement in the electrochemical response compared with the AuNP-modified electrode in **Figure 10B**.

Due to the immobilization of aptamers, their aptasensor demonstrated high specificity for insulin, even in the presence of interfering substances. When tested in insulin-spiked serum, the aptasensor showed analytical performance comparable to ELISA kits. Despite its high accuracy, sensitivity, and specificity for insulin determination, this dual-probe aptasensor is single-use and cannot be utilized for continuous measurement due to the immediate displacement of insulin upon insulin binding.

Specificity of electrochemical sensors toward insulin is one of the utmost factors to be considered as several blood components are electroactive. Employment of biomolecules certainly, enable high specific binding property, however, it may decrease the stability of the sensor and need extraordinary conditions for a long-term storage. To overcome the limitations of using biorecognition molecules, researchers have recently combined MIP technique to electrochemical sensor due to its high specificity, available multiple binding sites, signal regeneration capability.

Despite the high targeting capability of immune-based electrochemical sensors for analytes, the stability of biorecognition molecules remains a major concern. To address this limitation, Zhu et al. demonstrated electromagnetic MIP (EMMIP) showing the use of magnetic NPs in electrochemical biosensors.[158] They prepared an insulin-MIP membrane using ternary Fe_3O_4@rGO/PANI NPs (MGP NPs). Fe_3O_4 NPs were prepared on rGO sheets followed by polymerization of polyaniline (PANI) in the presence of insulin templates (**Figure 11**).

This method exploited the unique characteristics of various materials to improve efficiency of electrochemical biosensors. PANI polymer and rGO are electrically conductive and influence electrocatalytical activity, promoting electron transfer at the interface. PANI was also used as an MIP membrane for insulin recognition. Unlike other studies, the role of metallic NPs did not involve an enhancement of electrochemical reaction, but the magnetic NPs were utilized to facilitate electrode regeneration.

Due to the embedding of Fe_3O_4 NPs, the EMMIP membrane is responsive to the external magnetic force of a magnetic glassy carbon electrode (MGCE). Consequently, the MIP membrane is magnetically controllable. The absorption of the EMMIP membrane can be controlled by applying or removing the external magnetic force of the MGCE.

Disadvantages of the conventional molecular imprinting technique is the degradation of protein during polymerization, such as exposing to high temperature, and it is difficult to imprint large and structural complex molecule, which restrict

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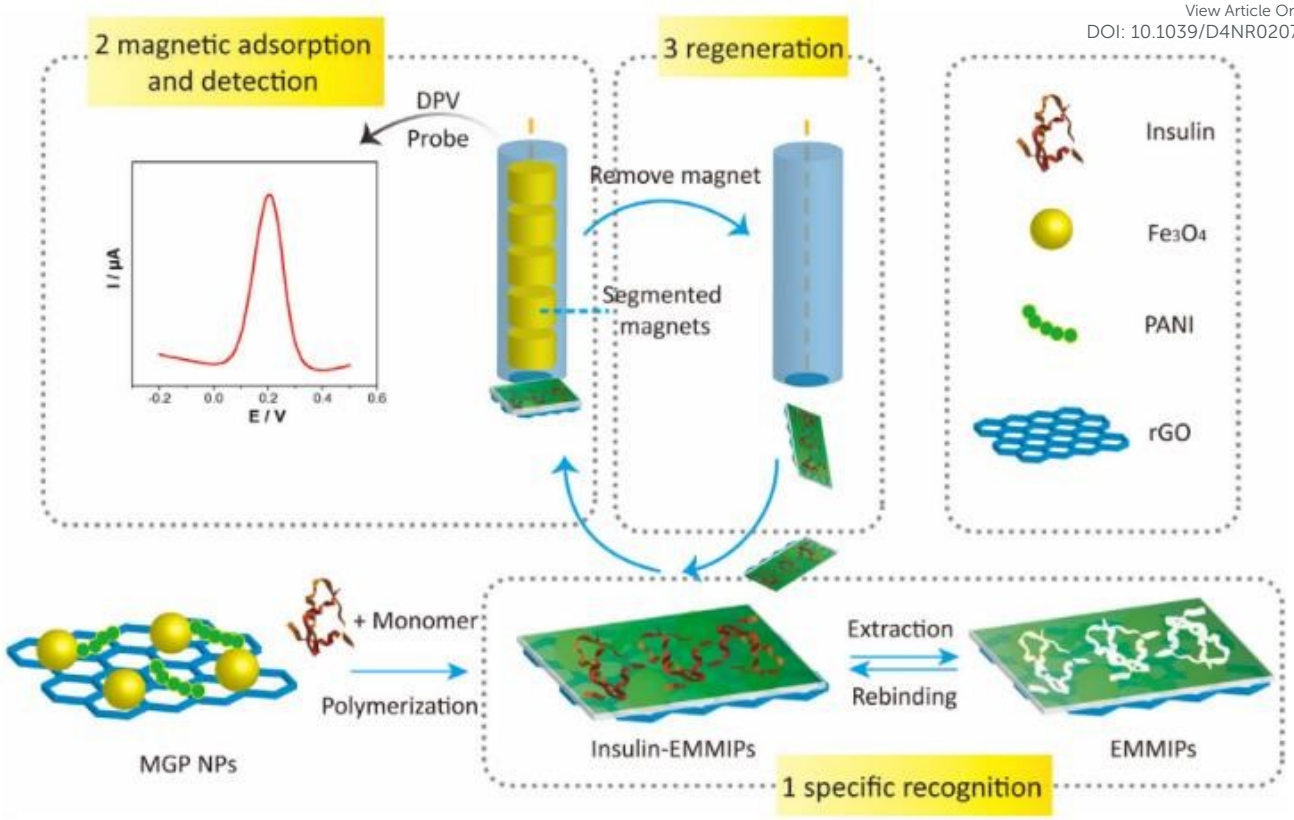

Fig. 11 Preparation and electrochemical detection operation of EMMIPs.[158]

their diffusivity. Surface imprinting is important as the imprinted sites are close to or at the surface of MIPs, avoiding the protein embedment in the polymer matrix, and thereby making the elution and rebinding of the target protein easier. It is superior to conventional imprinting process.

In conventional MIP electrodes, the electrode regeneration process is often complex and time-consuming. The EMMIP technique offers a convenient and rapid method for electrode regeneration by utilizing the magnetic properties of Fe_3O_4 NPs. The EMMIP membrane preparation and insulin elution can be performed independently without the electrode, allowing for the removal of the EMMIP membrane and reloading of a fresh EMMIP membrane within 15 - 20 min under magnetic activation. This facilitates high-throughput analysis.

The study by Zhu et al. demonstrated the crucial role of PANI in both insulin binding and electrochemical performance. Insufficient PANI loading resulted in reduced binding sites and a weaker electrochemical signal, while excessive PANI loading led to excessive crosslinking and hindered insulin template removal.

The EMMIP sensor exhibited good selectivity towards insulin in the presence of interfering substances and ions. The EMMIPs maintained their reusability for up to 10 times of insulin elution, absorption, and measurement. Moreover, they could be stored for up to 20 d while retaining 90% of initial performance. The EMMIP assays showed a strong correlation with the results obtained from radioimmunoassays, validating their accuracy.

Overall, the EMMIP technique is a facile, simple, and convenient method for the sensitive and specific determination of insulin in complex serum samples. The magnetic properties of Fe_3O_4 NPs enable rapid electrode regeneration and facilitate high-throughput analysis. The EMMIP sensor exhibits high sensitivity, selectivity, and reusability, making it a promising tool for insulin detection in clinical and research settings.

A sandwich-based electrochemical immunoassay for determining insulin was developed by Sun et al.[159] An enhancement of sensitivity was achieved using MoS_2 nanosheets decorated with AuNPs while its specificity was enabled by the immobilization of antibodies. The presence of MoS_2 nanosheets gives advantages of high specific surface area and excellent synergistical enhancement of electrochemical response with metal NPs. AuNPs were in situ grown on MoS_2 nanosheets in their study. As a result, the nanocomposites are conductive and accelerate charge transfer at electrode surface. Furthermore, hybridization chain reaction (HCR) was introduced to their immunoassay to further amplify electrochemical signal. The signalling molecules, RuHex, were electrostatically attached to DNA helices, resulting in 2.8-fold signal enhancement. This immunoassay showed good specificity, reproducibility, and stability. In the interfering investigation, their immunoassay was selective to insulin and other feasible molecules such as BSA, carcinoembryonic antigen (CEA), prostate-specific antigen (PSA), platelet derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) did not interfere with the detection. The immunosensor was

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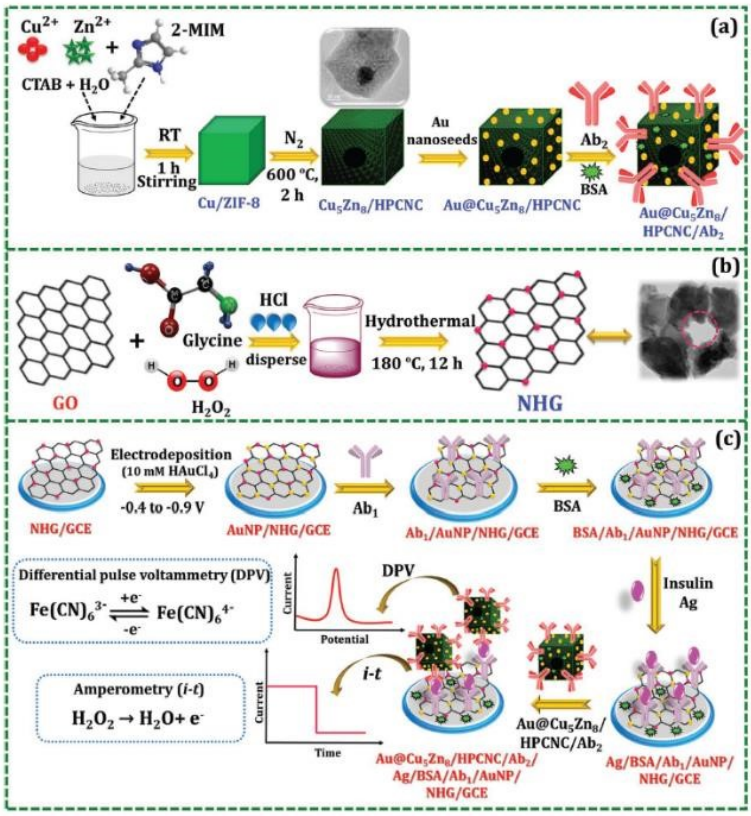

Fig. 12 Preparation of A) Au@Cu_5Zn_8/HPCNC/Ab_2 and B) NHG. C) Preparation of immune-based electrochemical biosensors.[160]

stable for 2 wk when being stored at 4 °C. Moreover, the immunosensor can be used in a complex matrix of serum showing recoveries between 96.0-106.4%, probably because the presence of impurities in the sample. However, this method requires multiple steps which raise complication of analysis.

Amperometry is also utilized among the voltametric techniques. Each material presents unique advantages for enhancing electrochemical performance. Recent research has combined the strengths of various nanomaterials to achieve improved electrochemical detection of serum insulin.[160] This study involved the fabrication of nanocomposites: Au-adhered bimetallic Cu_5Zn_8 hollow porous carbon nanocubes (Au@Cu_5Zn_8/HPCNC) and AuNPs deposited nitrogen-doped holey graphene (AuNPs/NHG), which were employed in a sandwiched immunoassay (**Figure 12**).

Excellent performance of insulin detection is attributed to the synergy between the great electrocatalytical active area of Au@Cu_5Zn_8/HPCNC and the electrical conductivity AuNPs/NHG. Bimetallic Cu_5Zn_8 nanomaterials possess inherent catalytic properties, making them excellent catalysts. When coupled with Au, they accelerate electron transfer at the GCE interface. Additionally, Cu_5Zn_8/HPCNC catalyzes the reduction reaction of H_2O_2 during amperometric measurements and enhances the sensitivity of DPV technique by quickening electron transfer. Therefore, AuNPs/NHG enhances electron transport while Au@Cu_5Zn_8/HPCNC acts as a signal amplifier enabling more sensitive insulin detection.

The high conductivity and surface area of AuNPs/NHG, which contribute to accelerate electron transfer, were confirmed by CV and EIS in their study. No interference from other hormones, including leptin (LEP), ghrelin (GHRL), or other molecules such as dopamine (DA), BSA, glucose (GLU), was observed.

Despite the incorporation of antibodies, the sensor exhibited good stability with 20 cycles of measurement and long-term storage of 11 d at 4 °C. The developed immunosensor was validated in human serum using electrochemical and ELISA techniques. The results obtained from these methods showed good correlation, indicating high reliability. Nonetheless, the total analysis time was 170 min, with 90 min for insulin binding and 80 min for AuNPs/NHG binding. Additionally, the efficiency of this immunosensor was temperature-dependent.

Another research group demonstrates the fabrication of H_2O_2 based electrochemical immunosensor.[161] To improve analytical performance, the electrode was modified with PdNPs functionalized with MoS_x followed by antibody immobilization. Amorphous MoS_x provides high surface area for the attachment of PdNPs and antibodies. The presence of PdNPs promote catalysis of H_2O_2 reduction via synergistic effect leading to great current response enhancement. In terms of specificity, the immunosensor exhibited high specificity toward insulin in the presence of prostate specific antigen, IgG, and CEA. The electrode can be performed for 60 cycles and the lifetime is 18 d. Moreover, the immunosensor was tested with insulin in serum matrix, showing recoveries of 95.3-102.8%, suggesting high accuracy.

Development on the electrochemical enhancement technique related with metallic NPs is also demonstrated by Li et al. in 2018.[162] Their sandwich immunosensor is based on the use of PdNPs decorated zinc silicate spheres (Zn_2SiO_4-PdNPs) in the detection of insulin in human serum. This is a dual-function technique which is suitable for both SWV and chronoamperometric measurements resulting in ultralow LOD. There are two NPs were included in their dual-function signal enhancement technique: Zn_2SiO_4-PdNPs and gold icosahedra nanocrystals (AuINCs). Zn_2SiO_4-PdNPs possess excellent electrocatalytical properties toward H_2O_2 reduction, and thus improving the sensitivity of the chronoamperometry. In the meantime, Zn_2SiO_4-PdNPs hinder electron exchange at the electrode interface which, on the other hand, increases the current change and therefore positively affects the sensitivity of SWV detection. In case of AuINCs, they were introduced into the immunosensor for the immobilization of antibodies as they can attach with biomolecules tighter than the spherical shape.
The electrochemical signal exhibits maximized analytical efficiency at physiological pH making the immunosensor in their study suitable for clinical applications. PSA, hepatitis B surface antigen (HBsAg), CEA and human serum albumin did not show significant electrochemical responses compared with the signals of insulin. After 2 wk of storage, the signal of this dual-function assay reduced by 4.8%. Their immunosensor was performed with insulin-spiked serum samples and exhibited the good relative standard deviation (RSD) and recovery rate. Hence, selectivity and stability are acceptable while the LOD is extremely low with wide linear working range.

2.3.1.2 ***Impedimetric biosensors.***
A simple, cheap, and facile preparation to modify electrodes using carbon quantum dots (CQDs) was proposed by Abazar and Noorbakhsh.[163] CQDs were dispersed in chitosan matrix to homogenously distribute on the electrode surface via electrostatic attraction. CQDs-chitosan nanocomposite offers feasibility for further biofunctionalization to enable selectivity toward insulin. Moreover, it was reported that CQDs are effective in preventing electrode fouling.[164] Insulin exposure leads to blocking effect because of structural conformation of aptamer to bind insulin molecules. This results in extremely increase in resistance using EIS measurement. Feasible interfering species including BSA, ascorbic acid, uric acid, and L-cysteine, do not disturb insulin detection due to the presence of aptamers. The analytical performance of the impedimetric aptasensor developed in this study was investigated with serum samples and revealed the recoveries of 96.8-105.6%, showing great accuracy. Moreover, in comparison with commercial method, their developed CDQ-chitosan based aptasensor showed the recovery rates of 98.5-101.9%. The modified electrode can be kept at 4 °C for a week. However, as aptamer was used, the reaction was optimized at 60 min incubation.
Noble metallic silver nanoflowers (AgNFs) coupled with rGO nanocomposites are reported on the modification of electrode for insulin detection.[165] Insulin molecules are recognized by antibody-immobilized AgNF-rGO on the electrode. Both AgNFs and rGO possess excellent electrical conductivity. The presence of AgNFs increase the intensity and density of electric filed, making the nanocomposites more electrically conductive. Therefore, synergistic effect between AgNFs and rGO results in significant acceleration of electron transfer rate and therefore enhancement in impedance response. The binding of insulin causes the increment of impedance which is proportional to insulin concentration. Their impedimetric immunosensor is selective to insulin in the presence of C-reactive protein and BSA. The study points out the reusability, reproducibility, and stability (1 wk) of the immunosensor.

2.3.2 Plasma samples.
Beside human serum, relative amount of insulin can be found in plasma sample to be extracted for electrochemical quantification. Previous study suggests that there is no significant difference between the concentration of insulin in serum and plasma.[166] However, plasma insulin is more scattered than the serum insulin, leading to lower reproductive result of insulin diagnosis.[167] Certain number of research still focus on the quantification of plasma insulin and will be discussed in this section.

2.3.2.1 ***Impedimetric biosensors.***
An immune-based electrochemical sensor integrating with the AuNPs and a conductive polymer was developed by Ensafi et al. for plasma insulin determination.[168] The surface of a pencil graphite electrode (PGE) was electrodeposited with a conductive poly-orthophenylene diamine polymer to enhance charge transfer and provide a porous structure for the attachment of AuNPs and insulin-recognized aptamers. The successful fabrication resulted in a dramatic increase in insulin-electrode interaction and electrocatalytic activity. Attributing to the stability of aptamers, the modified electrode retained acceptable electrochemical activity up to 10 d of storage. Aptamers provided the modified electrode with the ability to recognize insulin. Nonetheless, the interaction between aptamers and insulin took 90 min to complete, possibly due to the slow binding kinetics of aptamers. Furthermore, poly-orthophenylene diamine polymer was introduced to the PGE to increase analytical performance. However, it was found that the polymer coating could reduce specificity. Polymeric pores induced non-specific absorption of plasma proteins as the electrooxidations of BSA, luteinizing hormone, and follicle stimulating hormone were observed. The study pointed out the importance of the optimizing polymer thickness. Despite these limitations, the proposed electrode exhibited impressive outcomes with insulin-spiked plasma samples. The evaluation of their aptasensor with 50 nM and 70 nM insulin in plasma showed recoveries of 103% and 88.9%, respectively.

2.3.2.2 ***Voltammetric biosensors*****.**
The integration of biomolecules into electrochemical biosensors leads to the increment of the logistic and storage costs. Although biomimetic MIP has been utilized in electrochemical biosensors, it has suffered with the complexity of biological samples, thus decrease in reliability, and may be not practical for clinical diagnosis for insulin. To address the

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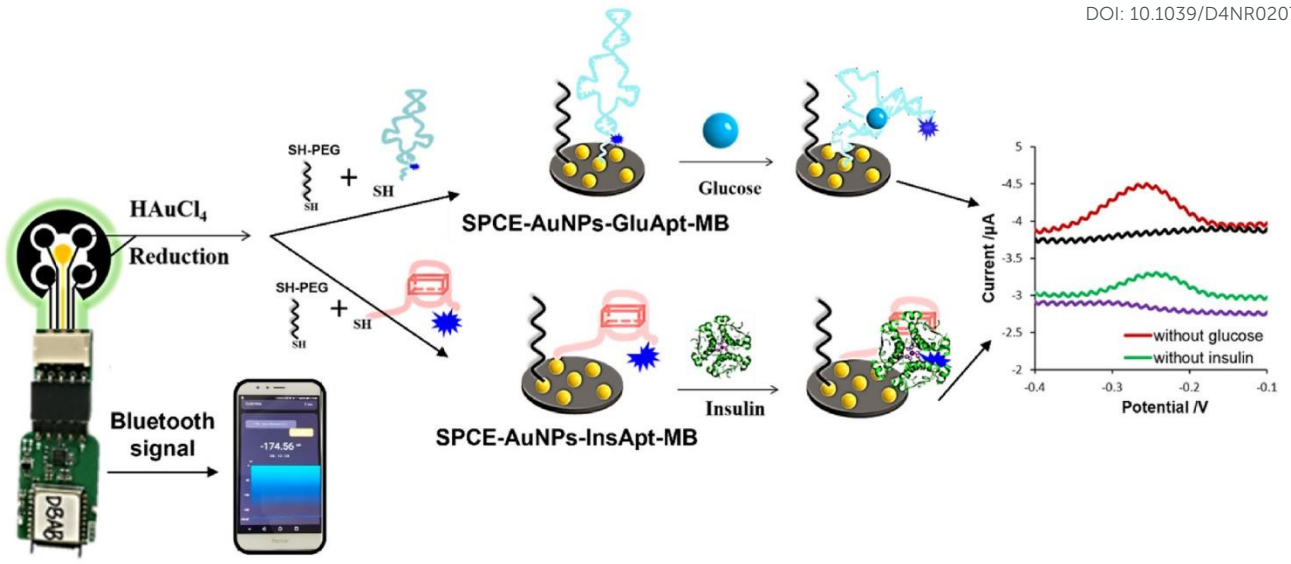

Fig. 13 The diagram of the simultaneous detection of insulin and glucose using the smartphone-assisted electrochemical aptasensor, measured by amperometric technique.[170]

challenge, Cruz et al. developed an electrochemical sensor using MIP nanoparticles (nanoMIPs) which is compatible with complex matrix of serum.[169] Polymeric monomer formulation was computationally designed and then optimized in their research to achieve maximum sensitivity and selectivity for insulin determination.

Electroactive ferrocenylmethyl methacrylate monomer was presented in nanoMIP during fabrication as a redox probe. Hence, nanoMIPs provide insulin-capturing and signal-reporting activities.

The recognition of insulin results in the change of electron transfer of ferrocence and subsequent increase in current response, which was found to be proportional to insulin concentration.

The nanoMIP-based electrochemical sensor developed in this study is highly robust as it can be kept for 168 d at 4°C showing extreme stability, compared with other reports mentioned earlier. In selectivity test, it showed negligible response of potential interferences in blood including haemoglobin, human serum albumin, and human proinsulin C-peptide. According to their report, the nanoMIP-based electrochemical detection is reproducible and accurate. Utilizing nanoMIPs offers several advantages such as high number of insulin-recognition sites, high stability, and high feasibility to be clinically used. Moreover, the cost of production is lower than other biomolecule-based sensors, making it more friendly for mass production in industrial scale.

2.3.3 Other biological fluids.

Blood is the predominant source of insulin, making it widely favourable for insulin detection in clinical settings. However, traditional blood-based insulin detection methods are invasive, requiring a small volume of blood to be drawn. Since insulin is not only available in the bloodstream, alternative body fluids, such as urine, sweat, and saliva, offer more non-invasive approaches for insulin detection. This section will discuss the use of alternative body fluids for insulin detection.

The use of urines as an alternative source for insulin detection offers several advantages over blood-based methods. Urine collection is non-invasive, painless, and can be performed multiple times throughout the day, allowing for continuous monitoring of insulin levels. Additionally, urine samples are less susceptible to interference from other proteins and compounds, potentially enhancing the accuracy of insulin detection.

The aptamer-based electrochemical sensor developed by Ensafi et al., as mentioned earlier in this review, also demonstrated the ability to identify insulin levels from urine sample.[168] Using the same PGE modification with AuNPs and poly-orthophenylene diamine polymer, EIS measurements revealed that the recovery rate for insulin determination in insulin-spiked urine (50-100 nM) was 93.0 – 94.0%. Other proteins did not interfere with the detection.

Nonetheless, urine-based insulin detection presents certain challenges. The concentration of insulin in urine is considerably lower than in blood, necessitating the development of more sensitive detection methods. Additionally, the presence of other metabolites and compounds in urine can potentially interfere with insulin detection.

The fabrication of a gold-modified aptasensor for insulin detection has been previously described. However, these methods typically rely on invasive blood collection. Recent efforts have demonstrated a promising non-invasive method to translate electrochemical insulin and glucose detection to a PoC device.[170] The authors demonstrated a miniaturized electrochemical assay for the prediction of prediabetes from saliva samples using a smartphone to facilitate home testing. An illustration of the configurative structure of the dual-marker aptasensor for insulin and glucose detection is shown in **Figure 13**. AuNPs were chemically formed on the SPCE, followed by immobilization of MB-modified aptamers to simultaneously

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Table 5 Electrochemical Au nanoparticles-based biosensors for TSH detection in the serum using antibody as receptor

Electrode	LOD	Linear Range	Ref.
SPCE	0.001μ IU mL^{-1}	0.001 – 150 μIU mL^{-1}	[174]
CILE	0.1 ng mL^{-1}	0.2 – 90 ng mL^{-1}	[175]
Gold-SPE	-	-	[176]

recognize insulin and glucose from the samples. This assemble was then integrated into a portable biochip for wireless analysis.

In the assay, the MB compound was responsible for generating redox activity, while AuNPs enhanced conductivity at the interface. Initially, the MB tags were positioned close to the sensing interface leading to substantial current responses for both redox probes. The insulin- and glucose-aptamers responded to the presence of insulin and glucose by undergoing conformational changes that allows them to capture these target compounds. The morphological alteration of the aptamers dislocated the MB tags farther away from the electrode surface, decreasing in the electrical currents of the corresponding channels.

The study not only presented the simultaneous detection capability and exceptional analytical efficiency of their aptasensor but also exploited the switchable structure feature of the aptamers to enable real-time monitoring of these analytes. The reversibility of the current signal could be easily controlled by disrupting the G-quadruplex formation of the aptamers.[171]

Owning to the high specificity of engineered aptamers, particularly towards insulin and glucose, no cross-reaction between insulin-aptamers and glucose, or between glucose-aptamers and insulin, was observed. Moreover, human immunoglobulin (IgG), BSA, interferon- γ (IFN- γ), and VEGF did not interfere the detection of insulin and glucose. The modified aptasensor provided long-term storage of 30 d at 4 °C.

The feasibility of the portable aptasensor was evaluated with spiked saliva samples, demonstrating high reliability with recovery rates of 92.0-98.8% and 95.1-104.1% for insulin and glucose, respectively. Under optimization, aptasensor required 30 min for antigen-aptamer interaction completion to maximize sensitivity. The operation period was shorter than that of the continuous monitoring MIP cryogel sensor (55 min)[172] and electrochemiluminescence (ECL) aptasensor (135 min).[173]

Overall, this aptasensor offered simple electrode preparation, continuous measurement, non-invasiveness, and feasibility of being a POC device for predicting pre-diabetic progression.

2.4 Thyroid stimulating hormone

Table 5 summarized the characteristics and performances of nanoparticles-based EIS biosensors for TSH in literature.

2.4.1. Serum samples.

TSH is monitored in serum samples by electrochemical biosensor principally based on AuNPs combine with other chemical compounds to obtain highly sensitive platforms.

2.4.1.1 *Impedimetric biosensors.*

Saxena *et al.* presented an immunosensor for the quantification of TSH by EIS technique that shown a high sensitivity and selectivity of the sensor, with LOD value of 0.001 μIU/mL and a linear concentration range between 0.001 to 150 μIU/mL.[174] A SPCE was functionalized by the addition of APTES that added free amino groups onto the surface of the electrode. After that the electrode was further modified with AuNPs and cystamine dihydrochloride which binds with NPs and provide free amine groups. At the end anti-TSH antibody was immobilized over the electrode with a direct immobilization. Thanks to EIS analysis it was possible to note a high affinity and selectivity for TSH molecule.[174]

In another work AuNPs were used to build a biosensor for TSH monitoring in serum samples by Beitollahi *et al.*[175] An ionic liquid carbon paste electrode (CILE) was modified with AuNPs and thioglycolic acid (TGA) that activated the surface of the electrode. After that, the thyroid stimulating hormone immunosensor was developed with the conjugation of TSH antibody by a covalent bond to TGA/AuNPs/CILE platform. Before the measurement by EIS, the electrode was incubated with various concentrations of TSH and with horse radish peroxidase (HRP) labeled TSH antibody. This TSH immunosensor shown LOD of 0.1 ng mL^{-1} and the peak current growth with the increase in the concentration of TSH from 0.2 to 90.0 ng mL^{-1}.[175]

Saxena and colleagues proposed an impedimetric immunosensor based on AuNPs for TSH monitoring in serum samples.[176] Through EIS the concentration of TSH was detected by a novel immunosensor obtained by the modification of a gold SPE. Firstly, cysteamine hydrochloride was added onto a SPE, this was followed by the drop-cast of colloidal AuNPs onto functionalized working electrode. Cysteamine hydrochloride was added again onto AuNPs modified electrode leading to AuNPs catalyzed amide bond formation with free amine groups on the platform. As a final step anti-TSH antibody was added to the built immunosensor. This work was based on the choice of the most suitable circuit used in EIS analysis for obtaining an EIS-based diagnostic devices for TSH detection.[176]

2.5 Growth hormone

Table 6 summarized the characteristics and performances of NP-based electrochemical biosensors for GH in literature.

2.5.1 Serum samples.

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Table 6 Electrochemical nanoparticles-based biosensors for GH detection.

Nanoparticles	Electrode	Receptor	Transducer	Bodily Fluid	LOD	Linear Range	Ref.
AuNPs	Au	Anti-hGH	EIS	Serum	93.0 fM	135.6 fM - 4.52 pM	[177]
Diphenylalanine nanoflowers	GCE	Anti-hGH	EIS	Serum	17.2 fM	4.52 fM - 4.52 nM	[178]
Fe_3O_4	GCE	MIP	EIS	Plasma	2.71 pM	4.52 pM - 4.52 nM	[179]
Magnetic beads	Au/CPE	Anti-hGH/hGH/Anti-hGH-labeled	SWV	Serum	226 fM	452 fM - 4.52 nM	[89]
CNT/PEDOT NPs	CPE	Anti-hGH	DPV	Serum/Saliva	199 aM	45.2 fM - 45.2 nM	[180]

Because the concentration of growth hormone is very variable, and very low in the periods between the impulses, analysis of the serum fraction of blood samples is a promising target for analysis for its relative concentration.

2.5.1.1. *Impedimetric biosensors*.

For its sensitivity EIS is the most common technique used to detect the presence of growth hormone in serum.

Two research groups, Rezaei B. *et al.* and Allafchain A. R. *et al.* have developed two different types of NP based electrochemical biosensors for the detection of growth hormone.[177, 178]

Rezaei *et al.* describe an electrode made of gold covered in AuNPs modified with 1,6-hexanedithiol (HDT) and functionalized with growth hormone selective antibodies.[177]

While Allafchian *et al.* utilized nanostructures of diphenylalanine deposited on the surface of GCE and modified with GH antibodies. When deposited and dried diphenylalanine self-assemble in a flower like structure of approximatively 20 µm of diameter.[178]

The technique of EIS is also used to measure the quantity of GH that can be found in plasma as reported by Bohlooli *et al.* in 2021. In their report Bohlooli *et al.* have created an electrochemical biosensor based on a glass carbon electrode on which were deposited Fe_3O_4 NPs, the electrode was then functionalized with a layer of MIP (PANI) to detect the presence of growth hormone.[179]

2.5.1.2 Voltametric biosensors.

To find a solution to the challenge of studying the concentration of growth hormone in serum samples different types of electrochemical biosensors based on NPs and exploiting different electrochemical analytical methods have been developed.

Serafín *et al.* in 2012 and later in 2014 have developed two different types of NP-based electrochemical biosensors for the detection and quantification of GH in serum or saliva samples. In 2012 they developed a biosensor able to detect GH by SWV while in 2014 they developed biosensors for the analysis of GH and prolactin by DPV in serum and saliva.[89, 180] In 2012 they developed an electrochemical biosensor based on magnetic beads functionalized with anti-hGH (human growth hormone) antibodies, when in contact with GH the beads trap the GH and become targets for the interaction with another aliquot of antibody molecules on which a third functionalized antibody is connected. The functionalization on the last antibody produces a signal when exposed to SWV, which is proportional to the amount of GH trapped by the magnetic beads. The magnetic properties of the beads are used to drive the nanoparticles with attached antibodies complex to the surface of the electrode.[89]

In 2014 Serafín *et al.* realized an electrochemical biosensor based on a carbon printed electrode modified with carbon nanotubes on which is deposited a layer of poly(3,4-ethylenedioxythiophene) (PEDOT) NPs. On the surface of the modified electrode a layer of AuNPs is deposited and antibodies for hGH and prolactin are attached. In presence of either hGH or prolactine these electrodes can interact with dopamine generating a detectable change on the peaks shape of the DPV cycle.[180] This system was used to detect hGH and prolactin both in serum and in saliva with similar results.

3. Wearable biosensors for hormones detection

The development of comfortable, non-invasive biosensors to detect hormones in real time is becoming a real need. Real-time monitoring of hormones is possible with the development of wearable biosensors which allow their continuous detection in epidermal biofluids, such as ISF and sweat. Therefore, wearable biosensors technology received a great expansion in the last decade.[181-185] However, so far not many studies are available in this area.[186-189]

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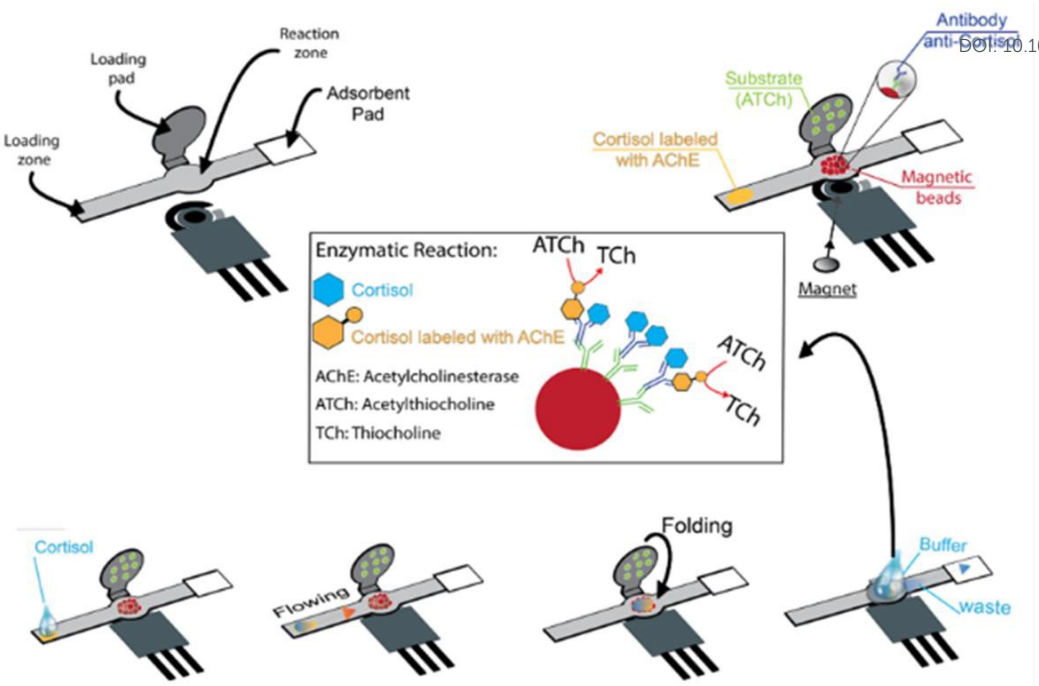

Fig. 14 Mechanism of action of an electrochemical biosensor with printed electrode modified with carbon black/Prussian blue nanoparticles.[192]

The main common issue to overcome in wearable device biosensors is the difficult electrode surface regeneration, which may cause poor reproducibility, thus affecting the possibility of continuous monitoring. Surface contamination, due to the accumulation of proteins and cells during continuous usage, may decrease the sensor sensitivity with consequent signal loss. Another issue is the use of nanomaterials which is strictly dependent on their biocompatibility and biological cell safety. It is necessary that the NPs employed are totally biocompatible, as cytotoxicity could represent a severe hazard for clinical applications, due to the direct contact between the biosensor surface and the human skin.

Four works have been recently published in literature regarding hormones detection with wearable devices.

In the first work, Mugo *et al.* developed a wearable textile electrochemical biosensor fabricated on a flexible cotton textile substrate, on which a layer of polyaniline decorated with carbon nanotubes and cellulose nanocrystals is deposed, to improve the electrical conductivity of the substrate. On this layer AuNPs and a MIP layer are formed and ensure selective capture of the cortisol molecule. With this set up Mugo *et al.* have achieved a LOD of 22.1 nM and a linear range between 27 nM and 136.6 nM in human sweat, while being wearable as a patch and can be reused for 15 measurements in a 30 days period.

To ensure complete removal of cortisol in order to allow repeated measurement, 45 CV cycles were applied (−1.0 - 1.0 V range, 0.1 V/s scan rate), and the phosphate buffer was replaced every 15 cycles.[190]

The integration of microfluidic technologies and wearable biosensors have also been utilized in laboratory medicine applications. A recent study by Lee and co-workers depicted a wearable lab-on-a-patch (LOP) platform comprising of a stretchable, label-free, impedimetric biosensor and a stretchable microfluidic device for on body non-invasive immunodetection of cortisol. This device utilized a 3D nanostructured Au working electrode and could detect cortisol in sweat at pM levels.[191]

Fiore *et al.* (**Figure 14**) used a printed electrode modified with carbon black/Prussian blue NPs (CB/PB-NPs) as support for their electrochemical biosensor. On the printed electrode they deposited magnetic beads modified with a layer of antibodies supporting a layer of monoclonal cortisol antibodies, in order to have a functional distance from the beads and the antibody. The test devised by Fiore *et al.* works a competitive assay in which enough cortisol modified with acetylcholinesterase to saturate the magnetic beads, in presence of unmodified cortisol from the sweat sample the marked cortisol is displaced and the signal generated by the system reduced. With this system Fiore and collaborators reached an LOD of 8.3 nM and a linear range between 27.6 nM and 386.2 nM. Compared with the other biosensors of this section Fiore L. *et al.* are the least sensitive, but they are still sensitive enough to detect sweat cortisol levels of 8 ng/mL to 140 ng/mL, as a trade off the result of the analysis can be read directly from a wearable patch on the patient using a near field communication (NFC) capable smartphone and a dedicated app.[192]

There is only one paper regarding a wearable nanobiosensor for real time oestradiol monitoring in sweat (Ye et al). The device is based on aptamers properly attached to a MXene surface modified with AuNPs and bind to single-stranded DNA molecules tagged with a molecule that can directly donate or accept electrons under certain conditions. When an aptamer binds to an oestradiol molecule, it releases the redox molecule. That molecule is then recaptured by a nearby electrode, generating an electrical signal that correlates with the oestradiol level. The device is able to wirelessly transmit the

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data it collects to an app that runs on a smart phone, providing a simple interface for the users. Another innovation of this device was the design of the microfluidics that collect sweat and channel it into the sensor. Tiny automatic valves incorporated in the microfluidics allow only a small, fixed amount of sweat into the sensor and then prevent additional sweat from entering. The design enables stable estradiol analysis without additional sweat disturbing the process. Moreover, to account for difference in sweat composition, the device also collects information about sweat pH, sweat salt levels, and skin temperature, and uses them for real-time calibration. The biosensor offers extraordinary sensitivity with an ultra-low limit of detection of 0.14 pM.[193]

Despite the great research efforts made in several laboratories, the wearable devices developed in this field are still proof-of-concepts, and further research is still needed.

4. Conclusions and future perspectives

In this review, recent advances in NP-based biosensors for hormone detection are discussed, focusing on principles, techniques, and biological fluids of detection. Various electrochemical platforms and microfabrication techniques are described and compared, in order to improve the performance of biosensors, in terms of sensitivity, power output and reproducibility.

As shown in **Tables 2-6**, compared to the voltammetric biosensors reported for all hormones, the performance in terms of linear range and LOD of the reported impedimetric biosensors was weaker in these two aspects. As far as the biological fluid of detection, all hormones have been detected in serum. Cortisol, insulin, testosterone, and GH were also determined in saliva, whereas urine was chosen in several works for sex hormones biosensors testing. It is interesting to note that TSH was detected only via EIS in serum samples and that cortisol is the only hormone to be detected in human sweat, both with voltammetric and impedimetric transduction, but once again the voltammetric mode gave lower LODs, in the fempto-aptomolar range (**Table 2**).

The combination of biorecognition elements with innovative NPs held much promise for enhancing hormones detection. However, there are still limitation and challenges to overcome for further application and commercialization.

Although NPs-based biosensors for hormones detection are well developed, no commercially available device for POC use still exists and the path towards commercialization of wearable technologies is even tougher.

Effectively, only very few start-up companies around the world have commercialized microneedles-based devices, like Caura (UK) for the simultaneous glucose and heart rate detection and Nutromics (Australia) for the continuous measurement of a range of micronutrients.

The main challenges for the development of microneedles-based platforms are the followings: i) the biofouling effect with consequent decrease of sensor reproducibility and lifetime; ii) the low biocompatibility of the device which can cause local skin inflammation; iii) the deficiencies in the calibration and validation protocols which limit the accuracy of the device. Of course, the intense scientific work in this area will show great promise but the major problem to overcome toward their possible marketization remains the gap existing between academic research and industry, together with the difficulties related to obtaining formal approvals for *in vivo* research studies. Moreover, the clinical significance of wearable testing data is another important issue which needs to be addressed.

However, it is very important to keep in mind that the successful realization of these challenging targets will be reached only by a multidisciplinary work between electrochemistry, nano- and bio-engineering, electronics, and medical communities.

For the successful translation of "proof-of-concepts" wearable biosensors into marketable PoC devices, a proper interface with smartphone-based wireless devices and algorithm-based applications has to be developed. At present, two technologies are used for real-time data streaming and analysis in wearable sensing devices: low-energy Bluetooth and near field communication (NFC). Unfortunately, both of them have obvious drawbacks, for example, NFC requires to be close to the receiver electronics for a good working. Therefore, a transmission system that achieves the ideal connection has yet to be developed. The integration of machine learning algorithms with smartphone-based sensors has the potential to lead to highly accurate analytical results.

In a near future, the development of all these technologies will be certainly scaled-up to the industrial level and the amalgamation hormone biosensors/wireless communication/ data analysis platforms presents a great potential for real-time remote health monitoring and personalized medicine development.

Author Contributions

NTKT and RA: conceptualised, writing, edit, and review the MS, FR wrote the sections about cortisol and GSH and created some of the images, TT wrote the sections about insulin, VG wrote the sections about sex hormones and TSH and created some images, CT wrote the introduction about the electrochemical biosensors, AI and AL reviewed the introduction section.

Conflicts of interest

There are no conflicts to declare.

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References

1. N. Haroon and K. J. Stine, *Coatings*, 2023, **13**, 2040.
2. T. Zhang, X. Du and Z. Zhang, *Frontiers in Bioengineering and Biotechnology*, 2022, **10**.

3. E. O. Adegoke, M. S. Rahman, Y. J. Park, Y. J. Kim and M. G. Pang, *Int J Mol Sci*, 2021, **22**.
4. T. Ozgocer, S. Yildiz and C. Uçar, *J Immunoassay Immunochem*, 2017, **38**, 147-164.
5. H. Zhang, Z. Cui, B. Yang, D. Fang, Y. Liu and Z. Wang, *Sci Total Environ*, 2021, **773**, 145569.
6. J. O. Abdulsattar and G. M. Greenway, *Journal of Analytical Science and Technology*, 2019, **10**, 34.
7. I. R. Suhito, K. M. Koo and T. H. Kim, *Biomedicines*, 2020, **9**.
8. K. Ghoshal, in *Advanced Sensor Technology*, eds. A. Barhoum and Z. Altintas, Elsevier, 2023, DOI: https://doi.org/10.1016/B978-0-323-90222-9.00012-1, pp. 261-295.
9. E. B. Bahadır and M. K. Sezgintürk, *Biosens Bioelectron*, 2015, **68**, 62-71.
10. S. Campuzano, P. Yáñez-Sedeño and J. M. Pingarrón, *Sensors*, 2020, **20**, 5125.
11. A. Núñez-De La Mora, G. R. Bentley, O. A. Choudhury, D. A. Napolitano and R. T. Chatterton, *Am J Hum Biol*, 2008, **20**, 2-14.
12. N. Gavrilova and S. T. Lindau, *J Gerontol B Psychol Sci Soc Sci*, 2009, **64 Suppl 1**, i94-105.
13. G. Maskarinec, F. Beckford, Y. Morimoto, A. A. Franke and F. Z. Stanczyk, *Biomark Med*, 2015, **9**, 417-424.
14. J. D. Meeker, S. R. Ravi, D. B. Barr and R. Hauser, *Reprod Toxicol*, 2008, **25**, 184-191.
15. H. T. Depypere, S. Bolca, M. Bracke, J. Delanghe, F. Comhaire and P. Blondeel, *Maturitas*, 2015, **81**, 42-45.
16. G. Jasienska and M. Jasienski, *Am J Hum Biol*, 2008, **20**, 35-42.
17. B. Nagy, J. Szekeres-Barthó, G. L. Kovács, E. Sulyok, B. Farkas, Á. Várnagy, V. Vértes, K. Kovács and J. Bódis, *Int J Mol Sci*, 2021, **22**.
18. M. Oettel and A. K. Mukhopadhyay, *Aging Male*, 2004, **7**, 236-257.
19. J. Flegr, J. Lindová and P. Kodym, *Parasitology*, 2008, **135**, 427-431.
20. W. Futterweit, N. L. McNiven, R. Guerra-Garcia, N. Gibree, M. Drosdowsky, G. L. Siegel, L. J. Soffer, I. M. Rosenthal and R. I. Dorfman, *Steroids*, 1964, **4**, 137-145.
21. G. D. Braunstein, R. E. Reitz, A. Buch, D. Schnell and M. P. Caulfield, *J Sex Med*, 2011, **8**, 2924-2934.
22. T. G. Travison, H. W. Vesper, E. Orwoll, F. Wu, J. M. Kaufman, Y. Wang, B. Lapauw, T. Fiers, A. M. Matsumoto and S. Bhasin, *J Clin Endocrinol Metab*, 2017, **102**, 1161-1173.
23. M. Zea, F. G. Bellagambi, H. Ben Halima, N. Zine, N. Jaffrezic-Renault, R. Villa, G. Gabriel and A. Errachid, *TrAC Trends in Analytical Chemistry*, 2020, **132**, 116058.
24. M. Stachowicz and A. Lebiedzińska, *European Food Research and Technology*, 2016, **242**, 2001-2009.
25. O. Stojadinovic, K. A. Gordon, E. Lebrun and M. Tomic-Canic, *Adv Wound Care (New Rochelle)*, 2012, **1**, 29-35.
26. A. S. Zainol Abidin, R. A. Rahim, M. K. Md Arshad, M. F. Fatin Nabilah, C. H. Voon, T. H. Tang and M. Citartan, *Sensors (Basel)*, 2017, **17**.
27. Y. Zhang, Q. Lai, W. Chen, C. Zhang, L. Mo and Z. Liu, *Chemosensors*, 2023, **11**, 90.
28. T. Iqbal, A. Elahi, W. Wijns and A. Shahzad, *Health Sciences Review*, 2023, **6**, 100079.
29. P. Pearlmutter, G. DeRose, C. Samson, N. Linehan, Y. Cen, L. Begdache, D. Won and A. Koh, *Scientific Reports*, 2020, **10**, 19050.
30. D. Gonzalez, D. Jacobsen, C. Ibar, C. Pavan, J. Monti, N. Fernandez Machulsky, A. Balbi, A. Fritzler, J. Jamardo, E. M. Repetto, G. Berg and B. Fabre, *Scientific Reports*, 2019, **9**, 8213.
31. J. Marcos, N. Renau, G. Casals, J. Segura, R. Ventura and O. J. Pozo, *Anal Chim Acta*, 2014, **812**, 92-104.
32. S. Pradhan, B. D. Nicholson, S. Albin, R. L. Heise and V. K. Yadavalli, *Biosensors and Bioelectronics: X*, 2022, **12**, 100280.
33. M. Venugopal, S. K. Arya, G. Chornokur and S. Bhansali, *Sensors and Actuators A: Physical*, 2011, **172**, 154-160.
34. M. Jia, W. M. Chew, Y. Feinstein, P. Skeath and E. M. Sternberg, *Analyst*, 2016, **141**, 2053-2060.
35. H. Raff and J. M. Phillips, *J Endocr Soc*, 2019, **3**, 1631-1640.
36. Beckman Coulter, Cortisol RIA KIT, December 2022, Accessed: April 2024, [online], Available: https://www.demeditec.com/en/products/cortisol-ria-it1841/ifu-it1841-cortisol-ria-ce2797-01-12-22-e.pdf
37. C. E. Karachaliou, G. Koukouvinos, D. Goustouridis, I. Raptis, S. Kakabakos, P. Petrou and E. Livaniou, *Biosensors (Basel)*, 2023, **13**.
38. A. Boolani, D. Channaveerappa, E. J. Dupree, M. Jayathirtha, R. Aslebagh, S. Grobe, T. Wilkinson and C. C. Darie, *Adv Exp Med Biol*, 2019, **1140**, 649-664.
39. S. Khumngern and I. Jeerapan, *Analytical and Bioanalytical Chemistry*, 2023, **415**, 3863-3877.
40. J. Y. Moon, M. H. Choi and J. Kim, *Endocr Relat Cancer*, 2016, **23**, R455-467.
41. L. Goedeke and C. Fernández-Hernando, *Cellular and Molecular Life Sciences*, 2012, **69**, 915-930.
42. M. Alemany, *International journal of molecular sciences*, 2022, **23**, 11952.
43. R. Lauretta, M. Sansone, A. Sansone, F. Romanelli and M. Appetecchia, *International journal of endocrinology*, 2018, **2018**.
44. I. W. Craig, E. Harper and C. S. Loat, *Ann Hum Genet*, 2004, **68**, 269-284.
45. G. Baggio, A. Corsini, A. Floreani, S. Giannini and V. Zagonel, *Clin Chem Lab Med*, 2013, **51**, 713-727.
46. I. Barsoum and H. H. Yao, *Trends Endocrinol Metab*, 2006, **17**, 223-228.
47. W. E. Stumpf, *Experientia*, 1990, **46**, 13-25.
48. J. Cui, Y. Shen and R. Li, *Trends Mol Med*, 2013, **19**, 197-209.
49. M. L. Rao and H. Kölsch, *Psychoneuroendocrinology*, 2003, **28**, 83-96.
50. Y. Zhang, X. Xiao, X. M. Zhang, Z. Q. Zhao and Y. Q. Zhang, *J Biol Chem*, 2012, **287**, 33268-33281.
51. S. Zárate, G. Jaita, J. Ferraris, G. Eijo, M. L. Magri, D. Pisera and A. Seilicovich, *PLoS One*, 2012, **7**, e41299.
52. M. Birkhäuser, *Arch Gynecol Obstet*, 1996, **259 Suppl 1**, S74-79.
53. A. Kotov, J. L. Falany, J. Wang and C. N. Falany, *J Steroid Biochem Mol Biol*, 1999, **68**, 137-144.
54. H. Guo, Y. Zhang, D. A. Brockman, W. Hahn, D. A. Bernlohr and X. Chen, *Endocrinology*, 2012, **153**, 1183-1193.
55. L. Kolatorova, J. Vitku, J. Suchopar, M. Hill and A. Parizek, *Int J Mol Sci*, 2022, **23**.
56. V. Henderson, *Climacteric*, 2018, **21**, 333-340.

57. I. Sundström-Poromaa, E. Comasco, R. Sumner and E. Luders, *Frontiers in Neuroendocrinology*, 2020, **59**, 100856.
58. Y. Zhang, M. Nadeau, F. Faucher, O. Lescelleur, S. Biron, M. Daris, C. Rhéaume, V. Luu-The and A. Tchernof, *Molecular and Cellular Endocrinology*, 2009, **298**, 76-83.
59. M. Rossato, A. Nogara, M. Merico, A. Ferlin and C. Foresta, *Steroids*, 1999, **64**, 168-175.
60. B. Stoffel-Wagner, *Eur J Endocrinol*, 2001, **145**, 669-679.
61. R. Naamneh Elzenaty, T. du Toit and C. E. Flück, *Best Pract Res Clin Endocrinol Metab*, 2022, **36**, 101665.
62. T. W. Kelsey, L. Q. Li, R. T. Mitchell, A. Whelan, R. A. Anderson and W. H. Wallace, *PLoS One*, 2014, **9**, e109346.
63. F. Fanelli, F. Baronio, R. Ortolano, M. Mezzullo, A. Cassio, U. Pagotto and A. Balsamo, *Sex Dev*, 2018, **12**, 50-94.
64. A. E. Kulle, F. G. Riepe, D. Melchior, O. Hiort and P. M. Holterhus, *J Clin Endocrinol Metab*, 2010, **95**, 2399-2409.
65. H. A. Feldman, C. Longcope, C. A. Derby, C. B. Johannes, A. B. Araujo, A. D. Coviello, W. J. Bremner and J. B. McKinlay, *The Journal of Clinical Endocrinology & Metabolism*, 2002, **87**, 589-598.
66. S. Bhasin, J. P. Brito, G. R. Cunningham, F. J. Hayes, H. N. Hodis, A. M. Matsumoto, P. J. Snyder, R. S. Swerdloff, F. C. Wu and M. A. Yialamas, *J Clin Endocrinol Metab*, 2018, **103**, 1715-1744.
67. G. Casals, R. F. Costa, E. U. Rull, H. F. Escobar-Morreale, J. Argente, G. Sesmilo and B. Biagetti, *Journal*, 2023, **4**, 52-69.
68. Z. Liu, in *Cellular Endocrinology in Health and Disease (Second Edition)*, eds. A. Ulloa-Aguirre and Y.-X. Tao, Academic Press, Boston, 2021, DOI: https://doi.org/10.1016/B978-0-12-819801-8.00015-6, pp. 315-331.
69. G. Litwack, in *Hormones (Fourth Edition)*, ed. G. Litwack, Academic Press, 2022, DOI: https://doi.org/10.1016/B978-0-323-90262-5.00022-6, pp. 123-157.
70. Q. Hua, *Protein Cell*, 2010, **1**, 537-551.
71. S. E. Kahn, *Diabetologia*, 2003, **46**, 3-19.
72. A. Katsarou, S. Gudbjörnsdottir, A. Rawshani, D. Dabelea, E. Bonifacio, B. J. Anderson, L. M. Jacobsen, D. A. Schatz and Å. Lernmark, *Nature Reviews Disease Primers*, 2017, **3**, 17016.
73. V. Ormazabal, S. Nair, O. Elfeky, C. Aguayo, C. Salomon and F. A. Zuñiga, *Cardiovascular Diabetology*, 2018, **17**, 122.
74. F. Aun, M. M. Meguid, J. S. Soeldner and N. A. Stolf, *Postgrad Med J*, 1975, **51**, 622-626.
75. A. H. Rubenstein, C. Lowy, T. A. Welborn and T. R. Fraser, *Metabolism*, 1967, **16**, 234-244.
76. I. Spitz, A. Rubenstein, I. Bersohn, A. Wright and C. Lowy, *The Journal of Laboratory and Clinical Medicine*, 1970, **75**, 998-1005.
77. K. Lian, H. Feng, S. Liu, K. Wang, Q. Liu, L. Deng, G. Wang, Y. Chen and G. Liu, *Biosensors and Bioelectronics*, 2022, **203**, 114029.
78. S. Gaillard and F. E. Wondisford, in *Clinical Management of Thyroid Disease*, eds. F. E. Wondisford and S. Radovick, W.B. Saunders, Philadelphia, 2009, DOI: https://doi.org/10.1016/B978-1-4160-4745-2.00007-9, pp. 81-101.
79. K. Yoshida, T. Sakurada, K. Kaise, N. Kaise, T. Nomura, Y. Itagaki, M. Yamamoto, S. Saito and K. Yoshinaga, *Endocrinol Jpn*, 1988, **35**, 733-739.
80. R. W. Flynn, S. R. Bonellie, R. T. Jung, T. M. MacDonald, A. D. Morris and G. P. Leese, *J Clin Endocrinol Metab*, 2010, **95**, 186-193.
81. S. B. Soh and T. C. Aw, *Ann Lab Med*, 2019, **39**, 3-14.
82. S. Ranabir and K. Reetu, *Indian journal of endocrinology and metabolism*, 2011, **15**, 18-22.
83. M. Caputo, S. Pigni, E. Agosti, T. Daffara, A. Ferrero, N. Filigheddu and F. Prodam, *Cells*, 2021, **10**.
84. C. C. Chernecky and B. J. Berger, *Laboratory Tests and Diagnostic Procedures*, Elsevier Health Sciences, 2012.
85. J. J. Kopchick, D. E. Berryman, V. Puri, K. Y. Lee and J. O. L. Jorgensen, *Nat Rev Endocrinol*, 2020, **16**, 135-146.
86. K. Berneis and U. Keller, *Baillieres Clin Endocrinol Metab*, 1996, **10**, 337-352.
87. U. Kumar, *Int J Mol Sci*, 2023, **25**.
88. J. Ayuk and M. C. Sheppard, *Postgrad Med J*, 2006, **82**, 24-30.
89. V. Serafín, N. Úbeda, L. Agüí, P. Yáñez-Sedeño and J. M. Pingarrón, *Anal Bioanal Chem*, 2012, **403**, 939-946.
90. L. Gough, L. M. Castell, R. Gatti and R. J. Godfrey, *Sports Med Open*, 2015, **2**, 30.
91. M. Bidlingmaier and P. U. Freda, *Growth Horm IGF Res*, 2010, **20**, 19-25.
92. D. Liu, J. Wang, L. Wu, Y. Huang, Y. Zhang, M. Zhu, Y. Wang, Z. Zhu and C. Yang, *TrAC Trends in Analytical Chemistry*, 2020, **122**, 115701.
93. A. Haleem, M. Javaid, R. P. Singh, R. Suman and S. Rab, *Sensors International*, 2021, **2**, 100100.
94. W. Wang and S. Gunasekaran, *TrAC Trends in Analytical Chemistry*, 2020, **126**, 115841.
95. M. Lv, Y. Liu, J. Geng, X. Kou, Z. Xin and D. Yang, *Biosensors and Bioelectronics*, 2018, **106**, 122-128.
96. C. I. L. Justino, A. C. Duarte and T. A. P. Rocha-Santos, *Sensors (Basel)*, 2017, **17**.
97. S. U. Singh, S. Chatterjee, S. A. Lone, H.-H. Ho, K. Kaswan, K. Peringeth, A. Khan, Y.-W. Chiang, S. Lee and Z.-H. Lin, *Microchimica Acta*, 2022, **189**, 236.
98. B. Senf, W.-H. Yeo and J.-H. Kim, *Biosensors*, 2020, **10**, 127.
99. V. Naresh and N. Lee, *Sensors*, 2021, **21**, 1109.
100. M. B. Kulkarni, N. H. Ayachit and T. M. Aminabhavi, *Biosensors*, 2022, **12**, 543.
101. E. S. Williams and L. M. Silverman, in *Essential Concepts in Molecular Pathology (Second Edition)*, eds. W. B. Coleman and G. J. Tsongalis, Academic Press, 2020, DOI: https://doi.org/10.1016/B978-0-12-813257-9.00030-9, pp. 549-562.
102. L. C. Lopes, A. Santos and P. R. Bueno, *Sensors and Actuators Reports*, 2022, **4**, 100087.
103. A. P. F. Turner, *Science*, 2000, **290**, 1315-1317.
104. J. P. Chambers, B. P. Arulanandam, L. L. Matta, A. Weis and J. J. Valdes, *Curr Issues Mol Biol*, 2008, **10**, 1-12.
105. D. R. Thévenot, K. Toth, R. A. Durst and G. S. Wilson, *Biosensors and Bioelectronics*, 2001, **16**, 121-131.
106. M. Ramya, P. Senthil Kumar, G. Rangasamy, V. Uma shankar, G. Rajesh, K. Nirmala, A. Saravanan and A. Krishnapandi, *Chemosphere*, 2022, **308**, 136416.
107. A. Curulli, *Molecules*, 2020, **25**.
108. I.-H. Cho, D. H. Kim and S. Park, *Biomaterials Research*, 2020, **24**, 6.
109. J. L. Hammond, N. Formisano, P. Estrela, S. Carrara and J. Tkac, *Essays Biochem*, 2016, **60**, 69-80.

110. X. Liu, M. Hoene, X. Wang, P. Yin, H.-U. Häring, G. Xu and R. Lehmann, *Analytica Chimica Acta*, 2018, **1037**, 293-300.
111. J. Xu, Y. Fang and J. Chen, *Biosensors*, 2021, **11**, 245.
112. S. R. Corrie, J. W. Coffey, J. Islam, K. A. Markey and M. A. F. Kendall, *Analyst*, 2015, **140**, 4350-4364.
113. Z. Rezapoor-Fashtali, M. R. Ganjali and F. Faridbod, *Biosensors*, 2022, **12**, 720.
114. V. Sharma, T. K. Sharma and I. Kaur, *Journal of Applied Electrochemistry*, 2023, **53**, 1765-1776.
115. D. Duan, H. Lu, L. Li, Y. Ding and G. Ma, *Microchemical Journal*, 2022, **175**, 107231.
116. J. Liu, N. Xu, H. Men, S. Li, Y. Lu, S. S. Low, X. Li, L. Zhu, C. Cheng, G. Xu and Q. Liu, *Sensors (Basel)*, 2020, **20**.
117. A. Tolun and Z. Altintas, in *Advanced Sensor Technology*, eds. A. Barhoum and Z. Altintas, Elsevier, 2023, DOI: https://doi.org/10.1016/B978-0-323-90222-9.00004-2, pp. 593-646.
118. G. Deffo, T. F. Nde Tene, L. Medonbou Dongmo, S. L. Zambou Jiokeng and R. C. Tonleu Temgoua, in *Encyclopedia of Solid-Liquid Interfaces (First Edition)*, eds. K. Wandelt and G. Bussetti, Elsevier, Oxford, 2024, DOI: https://doi.org/10.1016/B978-0-323-85669-0.00040-4, pp. 409-417.
119. S. Yeasmin, B. Wu, Y. Liu, A. Ullah and L.-J. Cheng, *Biosensors and Bioelectronics*, 2022, **206**, 114142.
120. B. Yang, H. Li, C. Nong, X. Li and S. Feng, *Analytical Biochemistry*, 2023, **669**, 115117.
121. N. K. Bakirhan, B. Uslu and S. A. Ozkan, in *Nanostructures for Antimicrobial Therapy*, eds. A. Ficai and A. M. Grumezescu, Elsevier, 2017, DOI: https://doi.org/10.1016/B978-0-323-46152-8.00003-2, pp. 55-83.
122. C. Nong, B. Yang, X. Li, S. Feng and H. Cui, *Microchemical Journal*, 2022, **179**, 107434.
123. P. K. Vabbina, A. Kaushik, N. Pokhrel, S. Bhansali and N. Pala, *Biosensors and Bioelectronics*, 2015, **63**, 124-130.
124. L. Cantelli, W. J. Paschoalino, S. Kogikosky, T. M. Pessanha and L. T. Kubota, *Biosensors and Bioelectronics: X*, 2022, **12**, 100228.
125. Q. Liu, W. Shi, L. Tian, M. Su, M. Jiang, J. Li, H. Gu and C. Yu, *Anal Chim Acta*, 2021, **1184**, 339010.
126. Z. Huang, H. Chen, H. Ye, Z. Chen, N. Jaffrezic-Renault and Z. Guo, *Biosens Bioelectron*, 2021, **190**, 113451.
127. M. Sekar, M. Pandiaraj, S. Bhansali, N. Ponpandian and C. Viswanathan, *Scientific Reports*, 2019, **9**, 403.
128. Q. Zhou, P. Kannan, B. Natarajan, T. Maiyalagan, P. Subramanian, Z. Jiang and S. Mao, *Sensors and Actuators B: Chemical*, 2020, **317**, 128134.
129. R. D. Munje, S. Muthukumar, A. Panneer Selvam and S. Prasad, *Scientific Reports*, 2015, **5**, 14586.
130. A. Kaushik, A. Vasudev, S. K. Arya and S. Bhansali, *Biosens Bioelectron*, 2013, **50**, 35-41.
131. B. Sun, Y. Gou, Y. Ma, X. Zheng, R. Bai, A. A. Ahmed Abdelmoaty and F. Hu, *Biosens Bioelectron*, 2017, **88**, 55-62.
132. X. Liu, R. Zhao, W. Mao, H. Feng, X. Liu and D. K. Y. Wong, *Analyst*, 2011, **136**, 5204-5210.
133. S. Sanli, H. Moulahoum, F. Ghorbanizamani, Z. P. Gumus and S. Timur, *ChemistrySelect*, 2020, **5**, 14911-14916.
134. K.-J. Huang, Y.-J. Liu and J.-Z. Zhang, *Microchimica Acta*, 2015, **182**, 409-417.
135. E. Povedano, F. H. Cincotto, C. Parrado, P. Díez, A. Sánchez, T. C. Canevari, S. A. S. Machado, J. M. Pingarrón and R. Villalonga, *Biosensors and Bioelectronics*, 2017, **89**, 343-351.
136. K.-J. Huang, Y.-J. Liu, J.-Z. Zhang, J.-T. Cao and Y.-M. Liu, *Biosensors and Bioelectronics*, 2015, **67**, 184-191.
137. F. H. Cincotto, G. Martínez-García, P. Yáñez-Sedeño, T. C. Canevari, S. A. S. Machado and J. M. Pingarrón, *Talanta*, 2016, **147**, 328-334.
138. M. Ghanbarzadeh, A. Ghaffarinejad and F. Shahdost-Fard, *Talanta*, 2024, **273**, 125927.
139. J. Radecki and H. Radecka, in *Handbook of Bioanalytics*, eds. B. Buszewski and I. Baranowska, Springer International Publishing, Cham, 2022, DOI: 10.1007/978-3-030-95660-8_34, pp. 747-760.
140. X. Liu, K. Deng, H. Wang, C. Li, S. Zhang and H. Huang, *Microchimica Acta*, 2019, **186**, 347.
141. Y. Wang, J. Luo, J. Liu, X. Li, Z. Kong, H. Jin and X. Cai, *Biosens Bioelectron*, 2018, **107**, 47-53.
142. M. J. Monerris, F. J. Arévalo, H. Fernández, M. A. Zon and P. G. Molina, *Sensors and Actuators B: Chemical*, 2015, **208**, 525-531.
143. T. Ming, Y. Wang, J. Luo, J. Liu, S. Sun, Y. Xing, G. Xiao, H. Jin and X. Cai, *ACS Sensors*, 2019, **4**, 3186-3194.
144. Z. Zhao, H. Chen, Y. Cheng, Z. Huang, X. Wei, J. Feng, J. Cheng, S. M. Mugo, N. Jaffrezic-Renault and Z. Guo, *Microchimica Acta*, 2022, **189**, 178.
145. H. A. Samie and M. Arvand, *Bioelectrochemistry*, 2020, **133**, 107489.
146. A. Laza, A. Godoy, S. Pereira, P. R. Aranda, G. A. Messina, C. D. Garcia, J. Raba and F. A. Bertolino, *Microchemical Journal*, 2022, **183**, 108113.
147. R. S. Malon, S. Sadir, M. Balakrishnan and E. P. Córcoles, *Biomed Res Int*, 2014, **2014**, 962903.
148. E. Lerchbaum, V. Schwetz, A. Giuliani, T. R. Pieber and B. Obermayer-Pietsch, *Fertil Steril*, 2012, **98**, 1318-1325.e1311.
149. Z. Sun, Y. An, H. Li, H. Zhu and M. Lu, *International Journal of Electrochemical Science*, 2017, **12**, 11224-11234.
150. A. S. Mackay, R. J. Payne and L. R. Malins, *Journal of the American Chemical Society*, 2022, **144**, 23-41.
151. M. T. Stankovich and A. J. Bard, *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*, 1977, **85**, 173-183.
152. J. J. Berzas Nevado, J. Rodriguez Flores and G. Castañeda Peñalvo, *Fresenius' Journal of Analytical Chemistry*, 1999, **364**, 753-757.
153. A. Salimi, A. Noorbakhash, E. Sharifi and A. Semnani, *Biosensors and Bioelectronics*, 2008, **24**, 792-798.
154. A. Noorbakhsh and A. I. K. Alnajar, *Microchemical Journal*, 2016, **129**, 310-317.
155. I. Šišoláková, J. Hovancová, R. Oriňaková, A. Oriňak, L. Trnková, I. Třísková, Z. Farka, M. Pastucha and J. Radoňák, *Journal of Electroanalytical Chemistry*, 2020, **860**, 113881.
156. J. Hovancová, I. Šišoláková, R. Oriňaková and A. Oriňak, *Journal of Solid State Electrochemistry*, 2017, **21**, 2147-2166.
157. Y. Zhao, Y. Xu, M. Zhang, J. Xiang, C. Deng and H. Wu, *Analytical Biochemistry*, 2019, **573**, 30-36.
158. W. Zhu, L. Xu, C. Zhu, B. Li, H. Xiao, H. Jiang and X. Zhou, *Electrochimica Acta*, 2016, **218**, 91-100.

DOI: 10.1039/D4NR02075H

159. H. Sun, S. Wu, X. Zhou, M. Zhao, H. Wu, R. Luo and S. Ding, *Microchimica Acta*, 2018, **186**, 6.
160. R. Sakthivel, S. B. Prasanna, C.-L. Tseng, L.-Y. Lin, Y.-F. Duann, J.-H. He and R.-J. Chung, *Small*, 2022, **18**, 2202516.
161. Z. Gao, Y. Li, C. Zhang, S. Zhang, F. Li, P. Wang, H. Wang and Q. Wei, *Biosensors and Bioelectronics*, 2019, **126**, 108-114.
162. Y. Li, L. Tian, L. Liu, M. S. Khan, G. Zhao, D. Fan, W. Cao and Q. Wei, *Talanta*, 2018, **179**, 420-425.
163. F. Abazar and A. Noorbakhsh, *Sensors and Actuators B: Chemical*, 2020, **304**, 127281.
164. F. Abazar, E. Sharifi and A. Noorbakhsh, *Microchemical Journal*, 2022, **180**, 107560.
165. A. K. Yagati, Y. Choi, J. Park, J.-W. Choi, H.-S. Jun and S. Cho, *Biosensors and Bioelectronics*, 2016, **80**, 307-314.
166. J. M. Feldman and B. A. Chapman, *Clinical Chemistry*, 1973, **19**, 1250-1254.
167. J. R. Henderson, *The Lancet*, 1970, **296**, 545-547.
168. A. A. Ensafi, E. Khoddami and B. Rezaei, *Colloids Surf B Biointerfaces*, 2017, **159**, 47-53.
169. A. Garcia Cruz, I. Haq, T. Cowen, S. Di Masi, S. Trivedi, K. Alanazi, E. Piletska, A. Mujahid and S. A. Piletsky, *Biosensors and Bioelectronics*, 2020, **169**, 112536.
170. S. Liu, Z. Shen, L. Deng and G. Liu, *Biosensors and Bioelectronics*, 2022, **209**, 114251.
171. Y. Wu, B. Midinov and R. J. White, *ACS Sensors*, 2019, **4**, 498-503.
172. N. I. Wardani, T. Kangkamano, R. Wannapob, P. Kanatharana, P. Thavarungkul and W. Limbut, *Talanta*, 2023, **254**, 124137.
173. Y. Wang, H. Sha, H. Ke, X. Xiong and N. Jia, *Electrochimica Acta*, 2018, **290**.
174. R. Saxena and S. Srivastava, *Materials Today: Proceedings*, 2019, **18**, 1351-1357.
175. H. Beitollahi, S. G. Ivari and M. Torkzadeh-Mahani, *Biosensors and Bioelectronics*, 2018, **110**, 97-102.
176. R. Saxena and S. Srivastava, *Sensors and Actuators B: Chemical*, 2019, **297**, 126780.
177. B. Rezaei, T. Khayamian, N. Majidi and H. Rahmani, *Biosensors and Bioelectronics*, 2009, **25**, 395-399.
178. A. R. Allafchian, E. Moini and S. Z. Mirahmadi-Zare, *IEEE Sensors Journal*, 2018, **18**, 8979-8985.
179. S. Bohlooli, S. Kia, S. Bohlooli and R. Sariri, *Monatshefte für Chemie - Chemical Monthly*, 2022, **153**, 39-48.
180. V. Serafín, G. Martínez-García, L. Agüí, P. Yáñez-Sedeño and J. M. Pingarrón, *Analyst*, 2014, **139**, 4556-4563.
181. J. Kim, A. S. Campbell, B. E.-F. de Ávila and J. Wang, *Nature Biotechnology*, 2019, **37**, 389-406.
182. J. Min, J. R. Sempionatto, H. Teymourian, J. Wang and W. Gao, *Biosensors and Bioelectronics*, 2021, **172**, 112750.
183. A. Erdem, E. Eksin, H. Senturk, E. Yildiz and M. Maral, *TrAC Trends in Analytical Chemistry*, 2024, **171**, 117510.
184. J. J. García-Guzmán, C. Pérez-Ràfols, M. Cuartero and G. A. Crespo, *TrAC Trends in Analytical Chemistry*, 2021, **135**, 116148.
185. H. Sun, Y. Zheng, G. Shi, H. Haick and M. Zhang, *Small*, 2023, **19**, 2207539.
186. H. Teymourian, C. Moonla, F. Tehrani, E. Vargas, R. Aghavali, A. Barfidokht, T. Tangkuaram, P. P. Mercier, E. Dassau and J. Wang, *Analytical Chemistry*, 2020, **92**, 2291-2300.
187. P. Bollella, S. Sharma, A. E. G. Cass and R. Antiochia, *Biosens Bioelectron*, 2019, **123**, 152-159.
188. P. Bollella, S. Sharma, A. E. G. Cass and R. Antiochia, *Electroanalysis*, 2019, **31**, 374-382.
189. P. Bollella, S. Sharma, A. E. G. Cass, F. Tasca and R. Antiochia, *Catalysts*, 2019, **9**, 580.
190. S. M. Mugo, W. Lu and S. Robertson, *Biosensors*, 2022, **12**, 854.
191. H.-B. Lee, M. Meeseepong, T. Q. Trung, B.-Y. Kim and N.-E. Lee, *Biosensors and Bioelectronics*, 2020, **156**, 112133.
192. L. Fiore, V. Mazzaracchio, A. Serani, G. Fabiani, L. Fabiani, G. Volpe, D. Moscone, G. M. Bianco, C. Occhiuzzi, G. Marrocco and F. Arduini, *Sensors and Actuators B: Chemical*, 2023, **379**, 133258.
193. C. Ye, M. Wang, J. Min, R. Y. Tay, H. Lukas, J. R. Sempionatto, J. Li, C. Xu and W. Gao, *Nature Nanotechnology*, 2024, **19**, 330-337.

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Valeria Gigli obtained her biotechnology degree and the equivalent research master degree in industrial biotechnology from the department of "Innovation in Biological, Agri-Food and Forestry Systems" of University of Tuscia, Italy in 2021. Currently, she is doing a PhD at Sapienza University of Rome (Italy) from the department of "Experimental Medicine" under the supervision of Professor Riccarda Antiochia. Throughout her PhD studies, she embarked on a visiting PhD program at University College London, focusing on synthesizing cutting-edge metallic nanomaterials for biomedical applications. She worked under the guidance of Professor Than during this tenure. Her ongoing research initiatives revolve around the design and advancement of electrochemical biosensors tailored for monitoring hormones and their metabolites.

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Professor Nguyễn Thị Kim Thanh, MAE, FRSC, FInstP, FIMMM FRSB (http://www.ntk-thanh.co.uk) held a prestigious Royal Society University Research Fellowship (2005-2014). She was appointed a Full Professor in Nanomaterials in 2013 at University College London. She leads a very dynamic group conducting cutting edge interdisciplinary and innovative research on the design, and synthesis of magnetic and plasmonic nanomaterials mainly for biomedical applications. In 2019, she has been honoured for her achievements in the field of nanomaterials, and was awarded highly prestigious Royal Society Rosalind Franklin Medal. She was RSC Interdisciplinary Prize winner in 2022. She was awarded SCI/RSC Colloids Groups 2023 Graham Prize Lectureship to recognise an

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No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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