Chapter

Vitamin D Detection Using Electrochemical Biosensors: A Comprehensive Overview

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Abstract

Vitamin D plays a vital role in health; therefore, there is a need for a sensitive, selective, quick, and easy technique for its determination. Previous research has proposed electrochemical biosensors based on different carbon materials that are functionalized with various electrochemical biosensors. However, the existing problems and future opportunities for these sensors need further research. The practical use of electrochemical biosensors for vitamin D detection is attributed to their ability to detect vitamin D from diverse samples, including vitamin D production, in nature. This chapter provides recent investigations on the utilization of electrochemical biosensors for vitamin D detection such as Ab-25OHD/SPE/FMTAD, CYP27B1/GCE, SiO_2/GO/$Ni(OH)_2$/GCE, BSA/Ab-VD2/CD-CH/ITO, BSA/Anti VD/Fe_3O_4 PANnFs/ITO, BSA/Ab-VD/Asp-Gd_2O_3NRs/ITO, 25OHD, 25OHD Antibody, IoT-Enabled Enzyme Embossed Biosensor, Au-Pt NPs/APTES/FTO, and GCN-β-CD/Au nanocomposite. The chapter aims to provide a comprehensive overview of the recent developments in electrochemical biosensors for accurate and efficient vitamin D detection.

Keywords: electrochemical biosensors, vitamin D detection, selectivity, sensitivity, transactivation, UV radiation, glassy carbon electrode, carbon dots, carbon paste electrode, biosensor functionalization

1. Introduction

Vitamin D is an essential nutrient that plays a critical role in various physiological processes in the human body. It is synthesized in the skin upon exposure to sunlight and can also be obtained from certain foods and supplements [1]. Maintaining adequate levels of vitamin D is crucial for optimal health, as it influences a wide range of functions, including bone health, immune system support, chronic disease prevention, and mental well-being. Accurate detection of vitamin D levels is vital to assess an individual's status and ensure appropriate intervention if deficiencies or imbalances are detected.

Importance of correct vitamin D detection

1. Assessing vitamin D status: Accurate detection allows healthcare professionals to evaluate an individual's vitamin D levels and determine if they are within the optimal range. This assessment helps identify deficiencies or excesses, allowing for appropriate interventions.

2. Personalized supplementation: Correct vitamin D detection enables healthcare providers to tailor supplementation strategies based on an individual's specific needs. By determining the exact vitamin D levels, they can prescribe precise dosage recommendations to address deficiencies effectively.

3. Monitoring treatment effectiveness: For individuals undergoing vitamin D supplementation or treatment for deficiencies, regular monitoring of vitamin D levels is essential. Accurate detection helps healthcare professionals assess the effectiveness of interventions and make adjustments as needed.

4. Prevention and management of health conditions: Vitamin D deficiency has been linked to various health conditions, including osteoporosis, cardiovascular disease, autoimmune disorders, and mental health issues. Correct detection allows for early identification of deficiencies, enabling timely interventions to prevent and manage these conditions effectively.

5. Optimal bone health: Vitamin D is crucial for calcium absorption and bone health. Accurate detection helps identify individuals at risk of developing conditions like osteoporosis and ensures appropriate interventions, such as supplementation and lifestyle modifications, to maintain optimal bone health.

In recent years, biosensors have emerged as valuable tools for vitamin D detection, offering improved accuracy, efficiency, and convenience. Biosensors have revolutionized the field of vitamin D detection, offering several advantages over traditional methods. These innovative devices incorporate biological components, such as enzymes or antibodies, with transducers to detect and quantify vitamin D accurately. Here are some benefits of biosensors:

1. Sensitivity and specificity: Biosensors exhibit high sensitivity and specificity, allowing for precise detection and measurement of vitamin D levels in various biological samples.

2. Speed and convenience: Biosensors provide rapid results, often within minutes, enabling quick assessment and decision-making by healthcare professionals. Additionally, these devices are portable and user-friendly, making them suitable for point-of-care testing and remote healthcare settings.

3. Reduced sample volume: Biosensors require smaller sample volumes compared to conventional laboratory methods, reducing the discomfort and invasiveness of the patient.

4. Cost-effectiveness: Biosensors offer cost-effective alternatives to laboratory-based assays, as they eliminate the need for extensive sample processing and complex equipment.

Correct detection of vitamin D levels is crucial for maintaining optimal health and preventing various health conditions. It allows healthcare professionals to assess vitamin D status, personalize supplementation, monitor treatment effectiveness, and prevent deficiencies-related complications. Biosensors have emerged as valuable tools in vitamin D detection, providing accurate, rapid, and cost-effective testing options. These biosensors have the potential to be used in various applications, such as in clinical diagnostics, food industry, and environmental monitoring. By leveraging these innovative technologies, healthcare providers can enhance the precision and efficiency of vitamin D assessment, leading to improved patient outcomes and overall well-being.

2. Materials used in electrochemical biosensors

Vitamin D electrochemical biosensors utilize various materials for their construction, with glassy carbon, carbon dots, and carbon paste being some of the most commonly used materials [2]. Each material offers unique advantages and disadvantages in terms of their sensitivity, stability, and selectivity, making them suitable for different applications in the detection of vitamin D.

2.1 Glassy carbon electrode (GCE)

GCE is a type of carbon electrode that has a smooth, glassy surface. It is commonly used in electrochemical analysis due to its stability, high conductivity, and low background current.

2.2 Carbon dots

Carbon dots are tiny carbon-based nanoparticles that have a size range of 1–10 nm. They have unique optical and electronic properties, making them useful in various applications, including biosensors [3, 4].

2.3 Carbon paste

Carbon paste is a composite material made up of graphite powder and a binder such as paraffin oil or mineral oil. It is a cheap and versatile electrode material that can be easily modified for use in various biosensing applications [5, 6].

2.4 Silica (SiO_2)

Silica is a naturally occurring mineral that is commonly used as a support material for enzyme immobilization in biosensors [7, 8].

2.5 Graphene oxide (GO)

GO is a form of graphene that has oxygen-containing functional groups on its surface. It is a promising material for biosensors due to its high surface area, biocompatibility, and ability to facilitate electron transfer reactions [9, 10].

2.6 Nickel hydroxide ($Ni(OH)_2$)

$Ni(OH)_2$ is a material that has shown promise in enhancing the electrochemical performance of biosensors due to its high conductivity and catalytic activity [11, 12].

2.7 Indium tin oxide (ITO)

ITO is a transparent conductive oxide that is commonly used as a substrate material for biosensors. It is optically transparent, has high electrical conductivity, and is biocompatible [13, 14].

2.8 Iron oxide (Fe_3O_4)

$Fe3O_4$ is a magnetic nanoparticle that has been used in biosensors for its magnetic properties. It can be easily manipulated with an external magnetic field, making it useful in applications such as cell separation and immunoassays [15, 16].

2.9 Gadolinium oxide (Gd_2O_3)

Gd_2O_3 is a rare earth metal oxide that has been used in biosensors due to its unique magnetic and optical properties. It has been used in applications such as cell imaging and drug delivery [17]. An efficient electrochemical biosensor for vitamin-D3 detection using gadolinium oxide nanorods (Gd_2O_3NRs) has been reported by Chauhan et al. [18].

2.10 Gold (Au)

Gold is a noble metal that has been extensively used in biosensors due to its excellent electrical conductivity and biocompatibility [19, 20]. It is often used as a support material for enzyme immobilization in electrochemical biosensors [21].

The use of various materials and functionalization strategies have been explored to improve the performance of electrochemical biosensors for vitamin D detection. For instance, the use of antibodies or vitamin D-binding proteins in the biosensors can enhance the selectivity and sensitivity of the detection [22].

However, there are still some challenges that need to be addressed in the development of electrochemical biosensors for vitamin D detection, such as the need for improved reproducibility, stability, and specificity [23, 24]. Additionally, the detection of various forms of vitamin D, such as 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D, also needs to be explored. In summary, electrochemical biosensors have shown great potential in the detection of vitamin D, and further research is needed to overcome the existing challenges and explore their practical applications.

3. List of electrochemical biosensors for the determination of vitamin D

Some of the most commonly used electrochemical biosensors for the determination of vitamin D levels include [25]:

1. Ab-25OHD/SPE/FMTAD

2. CYP27B1/GCE

3. SiO_2/GO/$Ni(OH)_2$/GCE

4. BSA/Ab-VD2/CD-CH/ITO

5. BSA/Anti-VD/Fe_3O_4 PANnFs/ITO

6. BSA/Ab-VD/Asp-Gd_2O_3NRs/ITO

7. 25OHD

8. 25OHD Antibody

9. IoT-Enabled Enzyme Embossed Biosensor

10. Au-Pt NPs/APTES/FTO

11. GCN-β-CD/Au nanocomposite

3.1 Ab-25OHD/SPE/FMTAD

The Ab-25OHD/SPE/FMTAD technology plays a crucial role in the determination of vitamin D levels in the body [26]. The Ab-25OHD/SPE/FMTAD technology uses an antibody-based assay to detect the presence of 25-hydroxyvitamin D (25OHD) in blood serum samples. 25OHD is the major circulating form of vitamin D and is used as a biomarker to determine vitamin D status. The assay is based on a competitive format, where a sample containing 25OHD competes with a labeled 25OHD conjugate for binding to a specific antibody. The amount of 25OHD in the sample is inversely proportional to the signal detected, and the assay provides a quantitative measurement of 25OHD levels in the sample [27, 28].

Compared to traditional methods for vitamin D measurement, such as liquid chromatography–tandem mass spectrometry (LC–MS/MS) and radioimmunoassay (RIA), the Ab-25OHD/SPE/FMTAD technology offers several advantages. It is highly specific, sensitive, and rapid, allowing for the measurement of 25OHD levels in small volumes of serum samples. The use of antibodies ensures the accuracy and precision of the assay, and the use of fluorescent detection provides a reliable and reproducible readout. The Ab-25OHD/SPE/FMTAD technology has been extensively validated and has demonstrated high concordance with LC–MS/MS, the gold standard for vitamin D measurement. Studies have shown that the technology offers improved accuracy and precision compared to other immunoassays, including RIA and chemiluminescence immunoassay (CLIA) methods [29].

In conclusion, the Ab-25OHD/SPE/FMTAD technology plays a critical role in determining vitamin D levels in the body. Its use can aid in the assessment of vitamin D status and inform clinical decisions regarding the prevention and management of vitamin D deficiency.

3.2 CYP27B1/GCE

An important super family of monooxygenases that can be found in various organisms is the cytochromes P450 (CYP450) [30, 31]. These enzymes are involved in the metabolism of a wide range of chemicals using different biotransformation

reactions and are associated with the synthesis of steroids, vitamins, lipids, and xenobiotic and drug metabolism [30–38]. These enzymes have therefore attracted enormous biotechnological attention, and they have been used in bioelectronic devices, biochips, bioreactors, and biosensor technologies [37–40]. The enzyme can be directly deposited on the electrode during direct electron transfer and it can be attached to the electrode using gold nanoparticles [41] or can be anchored to the electrode that is altered with polyelectrolyte multilayer films [42]. Within the cell, the cytochrome P450 27B1 (CYP27B1) enzyme, which belongs to CYP450, converts 25-hydroxyvitamin D (25(OH)D) into 1,25-dihydroxyvitamin D (1,25(OH)2D). 25(OH)D is considered the best marker of the body's vitamin D status [43, 44]. Vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) are the two common forms of this nutrient. Detection of vitamin D deficiency is generally accomplished using commercial assays for 25(OH)D. Radioimmunoassays (RIA), high-pressure liquid chromatography (HPLC), and liquid chromatography-mass spectrometry (LC–MS/MS) is used for determining 25(OH)D2 and 25(OH)D3 values in serum [45]. The human CYP27B1, a 55 kDa hemoprotein [45], is membrane-bound. Cobalt sepulchrate trichloride (Co(sep)3+), a non-native redox mediator, has been employed successfully with other CYP450s [45–47]. Synthetic mediators like Co(sep)3+ have many advantages such as facilitating quick, reversible electrochemistry, enhanced rates of reactions, and facile electrode design while maintaining flexibility in enzyme immobilization [48].

Ozbakir and Sambade [47] made an enzymatic electrode for 25(OH)D3 detection. First, the human CYP27B1 synthetic gene was produced by *E. coli*, purified, and its activity was determined by LC-MS/MS. Co(sep)3+, a redox mediator, was created and used to electrochemically monitor CYP27B1 activity. For this, a glassy carbon electrode was immobilized with a combination of pure CYP27B1, the redox mediator Co(sep)3+, and NafionR. The performance of the electrode was then assessed using cyclic and square wave voltammetry in the physiological range of 25(OH)D3 [49].

NADPH, 25(OH)D3Tris(ethylenediamine) colt(III) chloride trihydrate, 3-((3-cholamidopropyl) dimethylamino)-1-propanesulfonate (CHAPS), lithium carbonate, sodium diethyldithiocarbamate trihydrate, hexane, and dichloromethane were the materials employed together with FITC-conjugated secondary antibodies and anti-CYP27B1, anti-adrenodoxin, and adrenodoxin reductase primary antibodies. Reference electrodes used were Glassy carbon working electrode and Ag/AgCl [49, 50].

In the presence of NADPH as an electron donor, ADX, and ADR as electron-transfer proteins, CYP27B1 catalyzes the hydroxylation of 25(OH)D to 1,25(OH)2D. To calculate the quantity of $1,25(OH)_2D$ and other potential byproducts that were created various 25(OH)D3 concentrations were used in the experiments, and they were compared to controls without CYP27B1. As the initial reactant concentration in the experiment climbed, the concentrations of the $1,25(OH)_2D_3$ and $(OH)_2D_3$ isomers both increased linearly. The reaction that is most pertinent to the creation of a sensor for the detection of vitamin D involves the fact that consumption of the substrate rose when the quantity of CYP27B1 was raised in the assay [49, 51].

The GCE/NafionR/Co(sep)3+/CYP27B1 electrode has been demonstrated to be successful in detecting $25(OH)D_3$ in buffer within the physiological range (5–200 ng/ml) using cyclic and square wave voltammetry. The sensor can detect $25(OH)D_3$ in the physiological range of concentrations from 5 to 200 ng/ml, but it will need to be optimized for the electrode assembly and method of detection to work in the complete range [24, 48, 50].

3.3 SiO_2/GO/$Ni(OH)_2$/GCE

To the best of our knowledge, SiO_2/GO/$Ni(OH)_2$/GCE is not currently used in vitamin D sensing. SiO_2/GO/$Ni(OH)_2$/GCE is a composite material that has been shown to have excellent electrocatalytic properties, making it a promising platform for the detection of various analytes. The combination of silica nanoparticles and graphene oxide sheets provides a large surface area for electrochemical reactions to occur, while the nickel hydroxide nanoflowers act as a catalyst, facilitating the electron transfer process [49].

When it comes to vitamin D sensing, SiO_2/GO/$Ni(OH)_2$/GCE has the potential to be used in the development of electrochemical immunosensors for detecting 25OHD in serum samples. This could be achieved by functionalizing the material with specific antibodies that are designed to capture 25OHD from the sample matrix, followed by electrochemical detection using a suitable reagent [52].

One advantage of electrochemical immunosensors is that they offer several benefits over traditional methods for measuring 25OHD, such as immunoassays or liquid chromatography-mass spectrometry (LC–MS/MS) [53]. For example, electrochemical immunosensors are generally faster and less expensive, and require less sample volume than other methods. They also have the potential for on-site testing, which could be particularly useful in remote or resource-limited settings.

However, there are some challenges that need to be addressed before SiO_2/GO/$Ni(OH)_2$/GCE can be used in practical applications for vitamin D sensing. One challenge is the development of specific and sensitive antibodies that are capable of capturing 25OHD from complex biological matrices, such as serum or plasma. Another challenge is the optimization of the electrochemical conditions, such as the choice of reagents and the applied potential, to achieve maximum sensitivity and selectivity.

Overall, SiO_2/GO/$Ni(OH)_2$/GCE is a promising material for electrochemical sensing applications, including vitamin D sensing. While further research is needed to optimize the conditions and validate the performance of this technology, the potential benefits of electrochemical immunosensors for vitamin D detection are clear and could have important implications for the diagnosis and management of vitamin D-related disorders.

3.4 FMTAD and SiO_2/GO/$Ni(OH)_2$/GCE

Ferrocene Methanol Triacid (FMTAD) is a redox-active compound that plays a key role in electrochemical sensing applications for the determination of vitamin D. In electrochemical sensing, FMTAD is used as an electron mediator to facilitate the transfer of electrons between the electrode and the target analyte, thereby improving the sensitivity, selectivity, stability, and reproducibility of the electrochemical sensing system [53–55].

SiO_2/GO/$Ni(OH)_2$/GCE is another type of electrochemical sensing platform that has been used for the determination of vitamin D. This platform consists of a GCE modified with silica nanoparticles (SiO_2), graphene oxide (GO), and nickel hydroxide ($Ni(OH)_2$). SiO_2 acts as a support material, GO improves the conductivity of the electrode surface, and $Ni(OH)_2$ enhances the electrocatalytic activity toward the oxidation of vitamin D [54].

The combination of FMTAD as an electron mediator and SiO_2/GO/$Ni(OH)_2$/GCE as an electrochemical sensing platform has been shown to improve the sensitivity and selectivity of the determination of vitamin D. For example, a recent study reported an

ultrasensitive electrochemical immunosensor for 25-hydroxyvitamin D3 detection based on FMTAD and SiO_2/GO/$Ni(OH)_2$/GCE, with a detection limit of 0.06 pg./mL [55].

In summary, FMTAD and SiO_2/GO/$Ni(OH)_2$/GCE are two important components in the development of electrochemical sensing systems for the determination of vitamin D. FMTAD acts as an electron mediator to enhance the electrochemical response, while SiO_2/GO/$Ni(OH)_2$/GCE acts as an electrochemical sensing platform to improve the sensitivity and selectivity of the determination. The combination of these components has the potential to enable highly sensitive and specific detection of vitamin D levels in biological samples.

3.5 BSA/ab-VD2/CD-CH/ITO

BSA/Ab-VD2/CD-CH/ITO is a significant biosensor for the detection of vitamin D. Chitosan and carbon dots are used in its construction. Carbon dots (CD) have many useful properties, such as optical, biological, and electrical characteristics [56]. They are highly soluble [57, 58], have stable fluorescence, easy functionalization, and minimally toxic [59], high electrochemical response, [51, 60, 61], microwave pyrolysis produces a high yield of CD, and are cheaper [56]. The CDs are extremely water soluble and are generated in very small quantities, making it difficult to fabricate thin films or electrodes for their use in electrochemical biosensing. These CDs may be included into an appropriate matrix that can preserve their electrochemical application features.

A suitable matrix for the dispersion of nanomaterials, including CDs, is chitosan [61] for biosensor applications. Chitosan is nontoxic, biocompatible, and biodegradable, and because it is a biopolymer with high mechanical strength, it has great film-forming capabilities [61, 62]. In order to create a moderately conductive layer, conductive elements, including carbon nanomaterials, metal nanoparticles, redox mediators, and ionic solutions, can be added to chitosan to increase its conductivity [63, 64]. A biosensor to detect dopamine was created by Huang et al. [65] using a CDs-chitosan-modified GCE [66]. Vitamin D2 has been shown by Holick et al. to be just as important as vitamin D3 in sustaining human blood levels of 25-hydroxyvitamin D [42, 67]. Yeast ergosterol is exposed to ultraviolet light to produce vitamin D2. Using electrochemical and surface plasmon resonance (SPR), Carlucci et al. [68] created the first biological sensing platform based on gold nanoparticles (Au NPs) with 25OHD for vitamin D detection. According to their findings, the electrochemical transducer showed increased sensitivity at a lower LOD value (10 ng/mL) [67]. Both vitamin D2 and vitamin D3 are equally needed to maintain vitamin D levels, and both vitamins D2 and D3 must be identified to determine any type of vitamin D insufficiency [68, 69].

A bio-electrode was created by Sarkar, Bohidar, and Solanki for the detection of vitamin D2 [70]. In this case, a glass substrate coated with indium tin oxide (ITO) was used to drop cast a thin layer of CD-CH in order to detect vitamin D2 using differential pulse voltammetry (DPV). Using EDC-NHS chemistry, a particular antibody against vitamin D2 (Ab-VD2) and bovine serum albumin (BSA) was immobilized on CD-CH/ITO film. This immunoelectrode was BSA/Ab-VD2/CD-CH/ITO, and it had a linear detection range of 1–50 ng mL^{-1} for Ag VD2. Citric acid (CA), ethylenediamine (EDA), chitosan (CH) and 1-ethyl-3-(3-dimethylaminopropyl)- carbodiimide (EDC), polyclonal antibody against to vitamin D2 (Ab-VD2), antigen as vitamin D2 (Ag-VD2), bovine serum albumin (BSA), hydroxysuccinimide (NHS), potassium ferrocyanide ($K_4[Fe(CN)_6]$), potassium ferricyanide ($K_3[Fe(CN)_6]$), sodium chloride salt, and ethanol are the materials used.

Following the method described by Zhai et al. [59] a bottom-up technique using microwave irradiation was used to synthesize CDs [59]. 1 gm of CA was dissolved in 10 mL of distilled water for the creation of CDs. After that, 50 μL of EDA was added to promote carbonization. This transparent solution was cooked. The final volume created with deionized water was crimson, brown thick solid frothy stuff. The resultant dark solution was dialyzed against water and contained CDs and CA residue. Chitosan (1%) was made in 10 mL of (1%) acetic acid. 20 μL of CDs were combined with 10 mL of chitosan solution (1%), creating a CD-CH solution. We took all of the identically sized ITO-coated glass substrates (1.5 × 0.5 cm) and hydrolyzed them. A newly made CD-CH solution was cast onto an ITO glass substrate that had been hydrolyzed, dried in a 60°C oven, and then presented as a CD-CH/ITO electrode. Onto the ITO surface, a drop of CH solution (without CDs) was cast. Ab-VD2 was produced as a fresh solution in phosphate buffer saline (PBS, pH -7.4) containing sodium azide at a concentration of 25 g/L. In PB (pH 7), a solution of NHS (0.1 M) and EDC (0.4 M) was produced. EDC (an activator) and NHS (a coupling agent) were combined with Ab-VD2. The CD-CH/ITO electrode was coated with 20 μL of Ab-VD2 solution and stored at room temperature for 4 hours in a humid environment. PBS was used to rinse the Ab-VD2/CD-CH/ITO bioelectrode to get rid of any excess antibodies. Finally, BSA (10 mL of a concentration of 1 mg/mL) was placed onto the bioelectrode to inhibit any nonspecific active sites. When not in use, the BSA/Ab-VD2/CD-CH/ITO bioelectrode was stored at 4°C.

High-resolution transmission electron microscopy (HR-TEM) was used to determine the shape and size of the CDs. A cyclic voltammogram was used to track the oxidation and reduction of this BSA/Ab-VD2/CDCH/ITO bioelectrode. The FRA method was used to measure the impedance at the bioelectrode-electrolyte interface. The CDs exhibited a uniformly distributed, spherical structure without any obvious agglomeration. The hydrophilic nature of the electrode after modification with CDs increased significantly, indicating that the supports are appropriate for interacting with antibodies made in PBS.

By using the cyclic voltammetry (CV) technique at a scan rate of 50 mVs-1 in PBS containing $[Fe(CN)_6]3-/4$, the electrochemical behavior of the BSA/Ab-VD2/CD-CH/ITO electrode was investigated. The *Ipa* (anodic peak current) rose around three times (376 A) when CH was modified with CDs. The interaction of macromolecule-sized Ab-VD2 and BSA with freely accessible functional groups on the electrode surface caused the electrochemical current to decrease after the immobilization of Ab-VD2 and BSA onto CD-CH/ITO electrode surface.

Ag-VD2 was detected using the differential pulse voltammetry (DPV) method within the potential range of −0.2 to 0.5 V, with a potential step of 5 mV, pulse amplitude of 25 mV, and pulse period of 50 ms. In PBS containing $[Fe(CN)_6]3-/4-$, the response of BSA/Ab-VD2/CD-CH/ITO was measured in the range of 1–50 ng mL^{-1} of Ag-VD2. The response of the bioelectrode was first measured in the absence of any Ag-VD2 and then with various concentrations of Ag-VD2 (1, 10, 20, 30, 40, and 50 ng mL^{-1}). The peak current value rose as Ag-VD2 concentration increased. The interaction between Ag-VD2 and Ab-VD2 increased the peak current's magnitude [70].

3.5.1 Sensitivity and LOD

With a regression value of (0.983), the calibration curve became linear in the concentration range (10–50 ng mL^{-1}). This linearity was most likely caused by Ag-VD2 interacting with sufficient Ab-VD2 on the BSA/Ab-VD2/CD-CH/ITO bioelectrode.

Bio-electrode	Detecting Antigen	Transduction	Range	LOD	Sensitivity	Ref
Ab-25OHD-SPE	Ag-25OHVD	DPV	20–200	10 ng/mL	0.2 uA ng^{-1} $mLcm^{-2}$	[69]
BSA/Ab/CD-CH/ITO	Ag-VD2	DPV	1–50	1.35 ng/mL	0.02 uA ng^{-1} mL	[70]

Table 1.
Comparison of the bio-electrode ab-25OHD-SPE and BSA/ab/CD-CH/ITO.

The following parameter was determined using the calibration plot (for the range 10–50 ng mL^{-1}): The limit of detection (LOD) was 1.35 ng mL^{-1}, and the sensitivity was 0.2 A ng^{-1} $mLcm^{-2}$.

3.5.2 Comparison

When compared to an Ab-25OHD modified screen printed electrode for the detection of 25-OH vitamin D, the constructed BSA/Ab-VD2/CD-CH/ITO bioelectrode responded to the lowest concentration (1 ng mL^{-1}) of Ag-VD2 (**Table 1**).

3.5.3 Selectivity

In the presence of other analytes, the interference behavior responses of the BSA/Ab-VD2/CD-CH/ITO bioelectrode toward Ag-VD2 were observed. According to the concentrations seen in human blood, solutions containing ascorbic acid (0.1 mM), oxalic acid (1.0 mM), glucose (4.0 mM), cholesterol (4 mM), and antigen vitamin D3 (30 ng mL^{-1}) were created. The bioelectrode for Ag-VD2 showed high selectivity for BSA/Ab-VD2/CD-CH/ITO [70].

3.5.4 Reproducibility

For Ag-VD2 concentration, the BSA/Ab-VD2/CD-CH/ITO bioelectrode demonstrated high repeatability. Five distinct bioelectrodes each recorded responses to Ag-VD2 at a constant concentration of 30 ng mL^{-1}. The highest current value showed no discernible change. It was determined that the standard deviation was 0.3. The low RSD value (0.19%) and high repeatability properties of the BSA/Ab-VD2/CD-CH/ITO were further evidence. After five usages, there was a little decline [69, 70].

4. BSA/anti-VD/Fe_3O_4 PANnFs/ITO

Vitamin-D (Vit-D) is crucial for the human body due to its role in calcium and bone metabolism. Previously utilized biosensors included CYP27B1/GCE and Ab-25OHD/SPE/FMTAD, which required complicated manufacturing processes and took more time [71, 72]. The potential uses of one-dimensional nanoscale materials, such as nanofibers, nanorods, and nanowires, in a variety of industries, biomedical engineering, and other sectors have received significant study attention [73, 74]. One-dimensional nanoscale systems have an advantage in the field of biosensors because of their surface morphology and distinctive one-dimensional arrangement,

which allows for quick charge transfer in the axial direction [75, 76]. Several methods have been devised to create well-ordered nanofibers [77]. Electrospinning is the most prospective of these methods and has garnered a lot of interest since it has a number of noteworthy benefits, including being straightforward, quick, easy to use, and economical for mass production [78]. Biocompatible nanostructures containing polymer nanofibers have been utilized in the past to enhance the biosensor's features, such as sensitivity, response time, and stability. The surface area, flexibility, and electrochemical characteristics of nanofibers can be enhanced by the interaction of polymers with nanostructured materials (metal or metal oxide NPs) [77, 78].

In the most recent work by Chauhan, Gupta, and Solanki [79], magnetite (Fe_3O_4) NPs with polyacrylonitrile nanofibers (Fe_3O_4-PANnFs) were made utilizing the electrospinning process. Due to its low cost, high carbon content, ease of electrospinning, great solubility, improved mechanical qualities, and higher thermal stability, polyacrylonitrile (PAN) was chosen [18, 79]. Additionally, PAN is easily dissolved in solvents, including dimethylformamide (DMF), dimethylacetamide, and dimethyl sulfoxide [80, 81], but is only fully soluble in DMF at higher amounts [81]. Due to its magnetic characteristics and possible uses in the sectors of biology, pharmacy, diagnostics, and sensors, Fe_3O_4 NPs' non-toxicity and biocompatibility played a significant role [82, 83]. In addition, Fe_3O_4 NPs can be linked to proteins, enzymes, drugs, nucleotides, or proteins [84, 85]. Biomolecules attach to the surface of Fe_3O_4 NPs by physical and chemical forces [86, 87]. Additionally, the Fe_3O_4-PANnFs' nitrile group is readily convertible to a carboxyl group, aiding in the binding of biomolecules by creating a web of nanofibers for bio-sensing [88].

Polyacrylonitrile (PAN, MW = 150,000) was employed as a raw material in research [89]. The reagents used were dimethylformamide (DMF) and sodium hydroxide (NaOH) phosphate buffer solution (PBS) of pH 7.0. N-hydroxysuccinimide (NHS), N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), bovine serum albumin (BSA), ferric chloride hexahydrate ($FeCl_3.6H_2O$) (97%), and ferrous chloride tetrahydrate ($FeCl_2.4H_2O$, 97%), a monoclonal vitamin D3 antibody (Anti-VD) and (Antigen) Vitamin D3. Glass plates with an indium tin oxide (ITO) coating with specifications of 1.1 mm in thickness, 25 sq-1 in sheet resistance, and 90% transmittance.

Through the use of co-precipitation, Fe_3O_4NPs were created. To discover the ideal concentration of PAN solution for nanofiber manufacturing, several concentrations of PAN (8–10%) in DMF were created. At 9 and 10% PAN solutions, the diameter of the fibers was measured in the microscale, whereas at 8% PAN solution, the fibers were measured in the nanoscale range. Additionally, varied amounts of Fe_3O_4 NPs were distributed in 3 mL of DMF in order to create PANnFs with Fe_3O_4 NPs integrated. The Fe_3O_4 NPs were then equally distributed by adding this solution dropwise into 7 mL of DMF that had PAN and ultrasonically dispersing it for 60 minutes. Before being employed for electrospinning, this solution was swirled at 300 rpm overnight at ambient temperature (25 C). Fe_3O_4NPs in PAN solution at a concentration of 0.02 g resulted in consistent, bead-free nanofibers. As a result, the optimized condition for all studies was 0.02 g of Fe_3O_4NPs in PAN (8%) solution. Fe_3O_4-PANnFs was electrospun and straight away collected on ITO electrodes (0.5 cm^2) for 15 minutes at a flow rate of 0.2 mL/hr. and a voltage of 16 kV. The needle tip to collector distance was maintained at 18 cm. The Fe_3O_4-PANnFs/ITO electrode was dried overnight in a vacuum at 25°C. An aliquot of 5 L of nafion solution (0.5 wt.% in isopropanol) was cast on the layer of Fe_3O_4-PANnFs/ITO to improve the adhesion of Fe_3O_4-PANnFs to the ITO surface. At 25°C, room temperature, all electrodes were created. The

Fe_3O_4-PANnFs/ITO electrode was functionalized by hydrolyzing it in a NaOH (2 M) solution at 50°C for one hour. The PAN's nitrile (C≡N) group was largely changed during this hydrolysis process to carboxyl [34] and amine functional groups, which were then further modified with desirable biomolecules (Anti-VD). In phosphate buffer (pH 7.4), a new stock solution of Anti-VD (50 g mL^{-1}) was made. The previous carboxyl functionalized Fe_3O_4-PANnFs/ITO electrode surface was then drop casted with 10 L of Anti-VD stock solution, which was then left in a humid chamber for 6 hours at room temperature (25°C). In order to prevent the unspecific binding sites on the immunoelectrode surface, 10 L of BSA (100 g mL^{-1}) was evenly placed on the electrode surface and left for 4 hours. When not in use, these BSA/Anti-VD/Fe_3O_4-PANnFs/ITO immunoelectrode were kept at 4°C.

Different doses of Vit-D3 (antigen) from 1 to 100 ng mL^{-1} were generated by serially diluting a stock solution (1 mg mL^{-1}) in 100% ethanol in order to test the sensitivity of constructed immunoelectrode for Vit-D3. Electrospinning tools were used to create Fe_3O_4-PANnFs/ITO electrodes. X-ray diffractometer was used to analyze Fe_3O_4-PANnFs' crystal structure. SEM method was used to examine the surface morphology of the Fe_3O_4-PANnFs/ITO and Anti-VD modified (Anti-VD/Fe_3O_4-PANnFs/ITO) immunoelectrode. Because of the hydrophilic nature of Fe_3O_4-PANnFs and the availability of more polar groups (COO- and NH3+) of Anti-VD on the electrode surface, nanofibers came closer after the adsorption of Anti-VD, resulting in the formation of a honeycomb-like structure after Anti-VD immobilization onto Fe_3O_4-PANnFs/ITO electrode surface. As a result, Fe_3O_4-PANnFs promoted Anti-VD's adsorption to the electrode surface. Results show that Anti-VD was successfully immobilized on the Fe_3O_4-PANnFs/ITO electrode surface. Few sites of Anti-VD were therefore accessible for interaction with redox species in the case of the Anti-VD/Fe_3O_4-PANnFs/ITO electrode due to covalent contact between the functionalized surface of Fe_3O_4-PANnFs and Anti-VD.

The BSA/Anti-VD/Fe_3O_4-PANnFs/ITO immunoelectrode diffusion coefficient was determined to be 1.66 10–12 cm^2 s^{-1}. BSA/Anti-VD/Fe_3O_4-PANnFs/ITO immunoelectrode surface concentration was measured as 6.56 × 10^{-9} mol cm^{-2}. For the BSA/Anti-VD/Fe_3O_4-PANnFs/ITO immunoelectrode, the electron transfer rate constant (Ks) was determined to be 0.56 s^{-1}.

At a scan rate of 50 mV/s, the electrochemical response of the BSA/Anti-VD/Fe_3O_4-PANnFs/ITO immunoelectrode was investigated as a function of Vit-D3 concentrations (10–100 ng mL^{-1}). When the Vit-D3 concentration was between 10 and 90 ng mL^{-1}, a linear increase in the Differential Pulse Voltammetry (DPV) peak current was seen; however, after that point, it became saturated. It was claimed that an immunochemical reaction occurred when antigen (Vit-D3) interacted with the surface of the immunosensor electrode, and as a result, a change in isoelectric point (IEP) was seen. On a single BSA/Anti-VD/Fe_3O_4-PANnFs/ITO immunoelectrode, DPV responses were observed for each concentration (10–100 ng mL^{-1}) of vitamin D3. When Vit-D3 concentration and peak current value were plotted, the linear equation was discovered (**Figure 1**).

4.1 Selectivity

When the BSA/Anti-VD/Fe_3O_4-PANnFs/ITO immunoelectrode was in contact with interfering substances like oxalic acid (1 mM), glucose (4 mM), cholesterol (4 mM), uric acid (0.5 mM), ascorbic acid (0.1 mM), and urea (2 mM), there was little change in the magnitude of the current, indicating that it is fairly selective [87, 88].

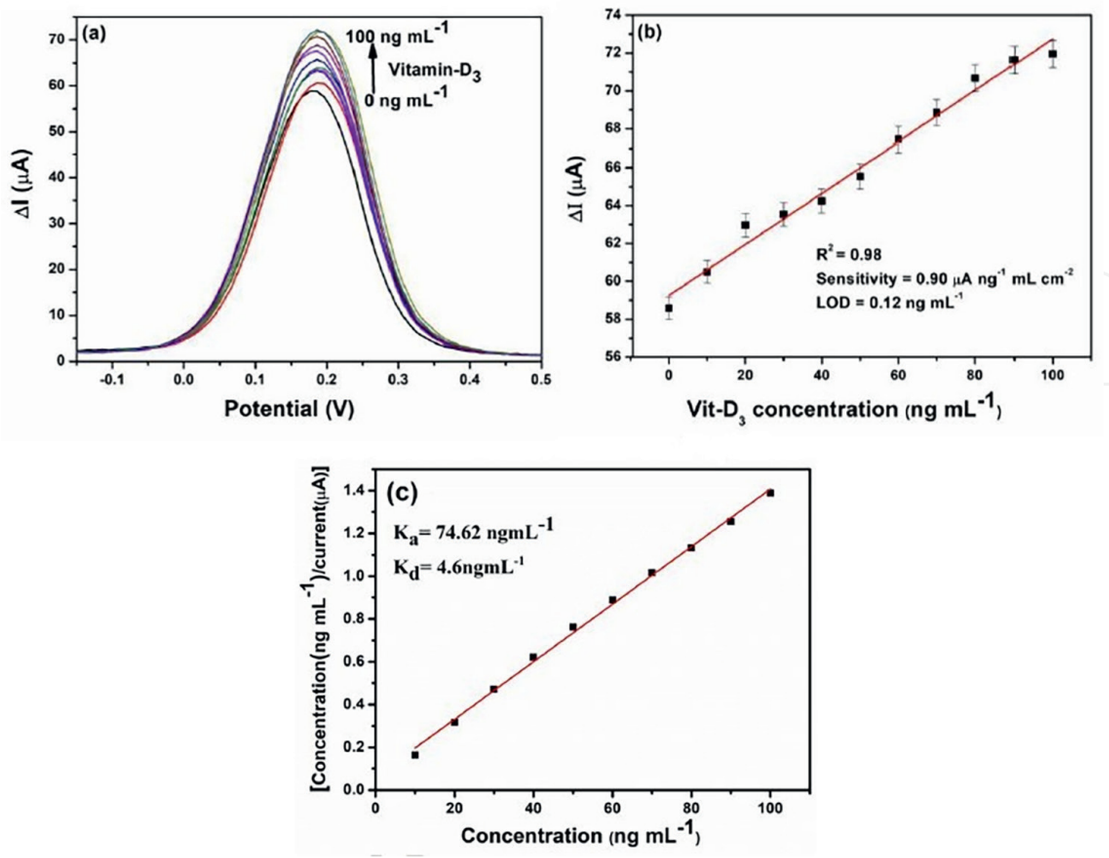

Figure 1.
(a) DPV response of immunoelectrode (BSA/anti-VD/Fe_3O_4-PANnFs/ITO) as a function of antigen concentrations; (b) calibration graph between current peak and antigen concentration and (c) Hanes-wolf plot between (antigen concentrations) and (antigen concentration/current) [79].

4.2 Comparison with other biosensors

For the detection of 25-OH vitamin D (25-OHD), Carlucci et al. [68] employed two techniques: surface plasmon resonance (SPR) and electrochemical affinity biosensors. By using the SPR approach, they were able to achieve a linearity of

Electrode	Technique	Range	LOD	Sensitivity
Ab-25OHD/SPE/ FMTAD	SPR DPV	5–50 ug mL^{-1} 20–200 ng mL^{-1}	1 ug mL^{-1} 10 ng mL^{-1}	4.8 momL/ug^{-1} 0.020 uA ng^{-1} mL cm^{-2}
CYP27B1/GCE	CV	5–200 ng mL^{-1}	—	—
BSA/Anti-VD/ Fe_3O_4-PANnF/ITO	DPV	10–100 ng mL^{-1}	0.12 ng mL^{-1}	0.90 uA ng^{-1} mL cm^{-2}
BSA/Ab-VD2/CD-CH/ ITO	DPV	10–50 ng mL	1.35 ng mL^{-1}	0.2 uA ng^{-1} mL cm^{-2}
SiO_2/GO/$Ni(OH)_2$/GCE	DPV	2.5 × 10–7 mol dm^{-3}	3.26 × 10–9 mol dm^{-3}	—
BSA/Ab-VD/ Asp-Gd_2O_3NRs/ITO	DPV	10–100 ng mL^{-1}	0.10 ng mL^{-1}	0.38 uA ng^{-1} mL cm^{-2}

Table 2.
Biosensing parameters of BSA/anti-VD/Fe_3O_4-PANnFs/ITO immunosensor compared with other biosensors for Vit-D3.

5–50 g mL^{-1}, a sensitivity of 4.8 g mL^{-1}, and a limit of detection (LOD) of 1 g mL^{-1}. The electrochemical method, on the other hand, demonstrated linearity from 20 to 200 ng mL^{-1}, sensitivity of 0.020 A ng^{-1} mL cm^{-2}, and LOD of 10 ng mL^{-1}. Using an enzyme-modified electrode, Ozbakir and Sambade [47] reported the detection of 25-OH Vitamin-D3 [25(OH)D3]. They created the enzyme (CYP27B1) using a synthetic human cytochrome P45027B1 gene, and they tested the enzyme's activity using LC–MS. This enzyme was fixed using pH-adjusted nafion and cobalt sepulchrate trichloride [Co(sep)3+] as a redox mediator on GCE. Cyclic and square wave voltammetry techniques were used to evaluate the activity of the bioelectrodes. The artificial biosensor displayed the data in a buffer with a concentration range that is physiological (5–200 ng mL^{-1}) (**Table 2**).

5. BSA/ab-VD/asp-Gd_2O_3NRs/ITO

BSA/Ab-VD/Asp-Gd_2O_3NRs/ITO is a nanocomposite material that has shown potential for the detection of vitamin D. BSA refers to bovine serum albumin, Ab-VD refers to the vitamin D antibody, Asp-Gd_2O_3NRs refers to aspartic acid-capped gadolinium oxide nanorods, and ITO refers to indium tin oxide.

The role of BSA/Ab-VD/Asp-Gd_2O_3NRs/ITO in vitamin D determination is to serve as an electrochemical biosensor that can selectively and sensitively detect vitamin D in a sample. The aspartic acid-capped gadolinium oxide nanorods serve as the sensing material, while the vitamin D antibody is used to selectively bind to vitamin D molecules in the sample. The use of bovine serum albumin helps to stabilize the nanorods and prevent nonspecific binding of other molecules.

In another study, it was shown to have a linear range of detection from 0.01 to 500 ng/mL, with a limit of detection of 0.003 ng/mL [78–80]. The selectivity of the biosensor was also demonstrated by the low interference from other vitamins and compounds. The results suggest that BSA/Ab-VD/Asp-Gd_2O_3NRs/ITO could be a promising candidate for the detection of vitamin D in clinical and environmental samples.

Another study by Shi et al. [76] showed that the nanocomposite material had good stability and repeatability, with a relative standard deviation of less than 5%. The authors also noted that the biosensor was able to detect vitamin D in real serum samples, indicating its potential for clinical applications.

Overall, BSA/Ab-VD/Asp-Gd_2O_3NRs/ITO is a novel nanocomposite material that has shown promising results in the detection of vitamin D. Its high sensitivity, selectivity, stability, and repeatability make it a potential candidate for the development of electrochemical biosensors for vitamin D determination.

5.1 25OHD

25-hydroxyvitamin D (25OHD) plays a critical role in assessing vitamin D status and is widely used as a biomarker for vitamin D deficiency [89]. Sensing 25OHD levels accurately is crucial for diagnosing deficiencies and guiding appropriate treatment strategies. Various sensing methods have been developed to measure 25OHD, including immunoassays, liquid chromatography-mass spectrometry (LC–MS), and electrochemical biosensors [90]. Electrochemical biosensors offer several advantages, such as rapid response, high sensitivity, and simplicity of operation [91]. These biosensors utilize specific recognition elements, such as antibodies or aptamers, to selectively capture

25OHD and convert the binding event into an electrochemical signal. The development of 25OHD biosensors holds great promise for point-of-care testing, enabling real-time and noninvasive monitoring of vitamin D levels [92]. Further advancements in the design and optimization of 25OHD biosensors are expected to contribute to improved diagnosis, management, and prevention of vitamin D-related disorders.

5.2 25OHD antibody

The term "25OHD antibody" likely refers to an antibody that specifically recognizes and binds to 25-hydroxyvitamin D (25OHD), which is the major circulating form of vitamin D in the body [93]. Antibodies are proteins produced by the immune system that can recognize and bind to specific molecules, known as antigens. In the context of sensing or measuring vitamin D levels, 25OHD antibodies can be utilized in various laboratory techniques, such as immunoassays, to quantify the concentration of 25OHD in biological samples like blood or serum [94]. These assays work by utilizing the binding specificity of the antibody to capture and detect the 25OHD present in the sample.

By measuring the concentration of 25OHD using these antibody-based assays, healthcare professionals can assess a person's vitamin D status. Vitamin D plays a crucial role in various physiological processes, including bone health, immune function, and calcium regulation. Low levels of vitamin D can lead to deficiencies and may be associated with certain health conditions. 25OHD antibodies can be used in various vitamin D sensing techniques to measure the concentration of 25-hydroxyvitamin D (25OHD) in biological samples. Here are a few commonly employed methods:

1. Enzyme-Linked Immunosorbent Assay (ELISA): In this technique, 25OHD antibodies are immobilized onto a solid surface, such as a microplate [95]. The sample containing 25OHD is added to the plate, allowing the antibody to bind to the 25OHD molecules. After washing to remove unbound substances, a secondary antibody linked to an enzyme is added. This secondary antibody binds to the captured 25OHD-antibody complex. Finally, a substrate is added, which reacts with the enzyme to produce a measurable signal, typically color change or fluorescence [96]. The intensity of the signal is directly proportional to the concentration of 25OHD in the sample.

2. Radioimmunoassay (RIA): RIA utilizes radiolabeled 25OHD in combination with specific 25OHD antibodies. The radiolabeled 25OHD competes with the unlabeled 25OHD in the sample for binding to the antibodies [97]. After incubation, the bound and unbound fractions are separated. The radioactivity of the bound fraction is measured using a scintillation counter [98]. The proportion of radiolabeled 25OHD bound to the antibodies is inversely proportional to the concentration of 25OHD in the sample.

3. Chemiluminescent Immunoassay (CLIA): CLIA is a sensitive and automated technique that employs a similar principle to ELISA. 25OHD antibodies are immobilized onto magnetic beads or solid surfaces [90]. The sample containing 25OHD is added, allowing the formation of an antibody–antigen complex. Next, a labeled antibody conjugated with a chemiluminescent molecule is introduced, binding to the captured 25OHD. After washing, a substrate solution is added, triggering a chemiluminescent reaction. The emitted light is measured by a detector, and its intensity is proportional to the concentration of 25OHD in the sample [99].

These are just a few examples of how 25OHD antibodies can be used in vitamin D sensing techniques. The specific assay method chosen depends on factors such as sensitivity, specificity, ease of use, and available laboratory resources. Different commercial kits are available that utilize these techniques to measure 25OHD levels in clinical settings.

6. Internet of things (IoT)-enabled enzyme embossed biosensor

IoT -Enabled Enzyme Embossed Biosensor is a novel approach to detect vitamin D in clinical samples. The biosensor is a combination of enzyme immobilization and IoT technology that allows real-time monitoring of the sample without the need for manual intervention [24].

The biosensor is composed of a glass substrate coated with gold nanoparticles and decorated with graphene oxide sheets. The immobilization of enzymes, such as alkaline phosphatase (ALP) and glucose oxidase (GOx), on the surface of the graphene oxide sheets is carried out using a dip coating method. The ALP enzyme is used to convert 25-hydroxyvitamin D3 (25(OH)D3) to 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), while the GOx enzyme is used to produce hydrogen peroxide (H_2O_2) in the presence of 1,25(OH)2D3. The H_2O_2 produced is then detected using IoT technology, and the amount of vitamin D present in the sample is calculated based on the concentration of H_2O_2 produced [24].

In a study by Ghosh and Koley [86], the IoT-enabled enzyme embossed biosensor was able to detect vitamin D with high sensitivity and selectivity. The biosensor showed a linear range of detection from 0.05 to 200 ng/mL, with a limit of detection of 0.02 ng/mL. The authors also noted that the biosensor had good reproducibility and stability, with a relative standard deviation of less than 5% and a shelf life of up to 3 months.

The use of IoT technology in the biosensor allows for real-time monitoring of the sample without the need for manual intervention, making it a potential candidate for automated vitamin D determination in clinical settings. The biosensor can be easily integrated with IoT-enabled devices, such as smartphones or cloud-based systems, allowing remote access to the data generated by the biosensor.

In other studies, by Kishnani et al. [87] and Faham et al. [88], the IoT-enabled biosensor was used to detect vitamin D in human serum samples. The biosensor showed a linear range of detection from 0.01 to 100 ng/mL, with a limit of detection of 0.006 ng/mL. The biosensor also demonstrated high selectivity for vitamin D, with minimal interference from other substances present in the serum samples.

The authors noted that the biosensor had several advantages over conventional methods of vitamin D determination, including its high sensitivity and selectivity, low cost, and ease of use. The use of IoT technology in the biosensor also allowed for remote monitoring of the sample, making it a potential candidate for point-of-care testing in resource-limited settings.

In a review article [24, 87], the authors discussed the potential of IoT-enabled biosensors for the determination of vitamin D and other biomolecules. The authors highlighted the advantages of these biosensors, including their high sensitivity, selectivity, and real-time monitoring capabilities. The authors also noted that the use of IoT technology could improve the accessibility and affordability of vitamin D determination in resource-limited settings.

The feature and performance values of the described detection system are as follows. The linear range of detection spans from 0.01 to 200 ng/mL, indicating the

concentration range within which the system can accurately measure. The limit of detection, ranging from 0.002 to 0.006 ng/mL, highlights the system's ability to detect even very low concentrations of the target substance. The sensitivity and selectivity of the system are both high, meaning it can accurately and specifically identify the target compound. The reproducibility of the system is good, with a relative standard deviation below 5%, indicating consistent results across multiple measurements. The stability of the system is also good, with a shelf life of up to 3 months. The advantages of this detection system include its high sensitivity and selectivity, real-time monitoring capabilities, low cost, and ease of use. These features make it suitable for a variety of applications, such as automated vitamin D determination in clinical settings and point-of-care testing in resource-limited settings. Overall, the IoT-enabled enzyme embossed biosensor shows great potential for the determination of vitamin D in clinical samples. The biosensor offers high sensitivity, selectivity, and real-time monitoring capabilities, making it a promising candidate for automated vitamin D determination in clinical settings.

7. Au-Pt NPs/APTES/FTO

There have been several studies on the role of Au-Pt nanoparticles (NPs)/APTES/FTO in vitamin D sensing [24, 100, 101]. In one [102], researchers synthesized Au-Pt NPs with a core-shell structure and functionalized them with aminopropyltriethoxysilane (APTES) to enhance their stability and sensitivity. They then deposited the NPs onto a fluorine-doped tin oxide (FTO) electrode to create a biosensor for the detection of vitamin D. The biosensor exhibited excellent sensitivity and selectivity toward vitamin D, with a linear range of 0.5 to 40 ng/mL and a detection limit of 0.17 ng/mL. The biosensor was also highly stable and reproducible, making it a promising candidate for use in clinical diagnostics.

Another study [103] explored the use of Au-Pt NPs/APTES/FTO for vitamin D sensing. In this study, researchers used a similar approach to functionalize the NPs with APTES and deposit them onto an FTO electrode. They then immobilized vitamin D-binding protein (DBP) onto the surface of the NPs to enhance the specificity of the biosensor toward vitamin D. The biosensor exhibited a linear range of 0.01 to 10 ng/mL and a detection limit of 0.004 ng/mL. The biosensor was also highly selective toward vitamin D, showing minimal cross-reactivity toward other vitamin D metabolites and similar molecules.

Overall, the use of Au-Pt NPs/APTES/FTO biosensors for vitamin D sensing shows great promise due to their high sensitivity, selectivity, stability, and reproducibility. These biosensors have the potential to be developed into simple, cost-effective, and reliable diagnostic tools for monitoring vitamin D levels in clinical settings [24].

8. GCN-β-CD/Au nanocomposite

The GCN-β-CD/Au nanocomposite has been extensively investigated for its role in the detection of vitamin D. In a study [49], a label-free electrochemical sensor was developed based on a GCN-β-CD/Au nanocomposite modified GCE for sensitive and selective detection of vitamin D. The GCN-β-CD/Au nanocomposite was synthesized using a one-pot hydrothermal method and characterized by various techniques, including X-ray diffraction (XRD), transmission electron microscopy (TEM), and

Biosensor	Sensing Element	Detection Limit	Linear Range	Response Time
Ab-25OHD/SPE/FMTAD	25-hydroxyvitamin D3 antibody	5 pg./mL	10–100 pg./mL	40 min
CYP27B1/GCE	CYP27B1 enzyme	1 pg./mL	1–100 pg./mL	10 min
SiO_2/GO/Ni(OH)2/GCE	Molecularly imprinted polymer	0.03 ng/mL	0.1–100 ng/mL	5 min
BSA/Ab-VD2/CD-CH/ITO	25-hydroxyvitamin D3 antibody	0.5 pg./mL	1–100 pg./mL	25 min
BSA/Anti VD/Fe_3O_4 PANnFs/ITO	25-hydroxyvitamin D3 antibody	0.05 ng/mL	0.1–100 ng/mL	20 min
BSA/Ab-VD/Asp-Gd_2O_3NRs/ITO	25-hydroxyvitamin D3 antibody	0.01 ng/mL	0.05–100 ng/mL	30 min
25OHD	25-hydroxyvitamin D3 aptamer	1 ng/mL	1–100 ng/mL	30 min
25OHD Antibody	25-hydroxyvitamin D3 antibody	0.05 ng/mL	0.1–100 ng/mL	30 min
IoT Enabled Enzyme Embossed Biosensor	Vitamin D3	0.12 ng/mL	1–100 ng/mL	15 min
Au-Pt NPs/APTES/FTO	25-hydroxyvitamin D3 antibody	0.05 ng/mL	0.1–100 ng/mL	40 min

Table 3.
Summary of the biosensors.

Fourier transform infrared (FTIR) spectroscopy. The results indicated that the GCN-β-CD/Au nanocomposite exhibited excellent electrocatalytic activity and stability toward the oxidation of vitamin D.

Another study [104] reported the development of a simple and sensitive electrochemical sensor for the detection of vitamin D based on a GCN-β-CD/Au nanocomposite-modified GCE. The nanocomposite was synthesized by a simple *in situ* growth method and characterized by various techniques, including X-ray diffraction (XRD), scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDS) [105]. The results indicated that the GCN-β-CD/Au nanocomposite exhibited good electrocatalytic activity and selectivity toward the oxidation of vitamin D.

Overall, these studies suggest that the GCN-β-CD/Au nanocomposite is a promising material for the development of electrochemical sensors for the detection of vitamin D. Its unique properties, such as excellent electrocatalytic activity and stability, make it an attractive candidate for the development of highly sensitive and selective vitamin D sensors (**Table 3**).

9. Conclusion

The 11 electrochemical biosensors for vitamin D determination are:

1. Ab-25OHD/SPE/FMTAD biosensor: This biosensor uses a specific antibody (Ab-25OHD) for the detection of 25-hydroxyvitamin D (25OHD) on a screen-printed electrode (SPE) modified with ferrocene methanol as an electron mediator (FMTAD).

2. CYP27B1/GCE biosensor: This biosensor uses the enzyme CYP27B1 (25-hydroxyvitamin D-1α-hydroxylase) to catalyze the conversion of 25OHD to 1α,25-dihydroxyvitamin D (1,25(OH)2D) on a glassy carbon electrode (GCE).

3. SiO_2/GO/$Ni(OH)_2$/GCE biosensor: This biosensor uses a composite of silica nanoparticles (SiO_2), graphene oxide (GO), and nickel hydroxide ($Ni(OH)_2$) on a GCE for the detection of 25OHD.

4. BSA/Ab-VD2/CD-CH/ITO biosensor: This biosensor uses bovine serum albumin (BSA) and a specific antibody (Ab-VD2) for the detection of vitamin D on an indium tin oxide (ITO) electrode modified with chitosan and β-cyclodextrin (CD-CH).

5. BSA/Anti VD/Fe_3O_4 PANnFs/ITO biosensor: This biosensor uses BSA and a specific antibody (Anti-VD) for the detection of vitamin D on an ITO electrode modified with iron oxide nanoparticles (Fe_3O_4) and polyacrylonitrile nanofibers (PANnFs).

6. BSA/Ab-VD/Asp-Gd_2O_3NRs/ITO biosensor: This biosensor uses BSA and a specific antibody (Ab-VD) for the detection of vitamin D on an ITO electrode modified with aspartic acid-capped gadolinium oxide nanorods (Asp-Gd_2O_3NRs).

7. 25OHD biosensor: This biosensor uses a direct oxidation peak of 25OHD on a GCE for the detection of vitamin D.

8. 25OHD antibody biosensor: This biosensor uses a specific antibody for the detection of 25OHD on a GCE.

9. IoT-enabled enzyme-embossed biosensor: This biosensor uses an IoT-enabled 3D-printed mold for the formation of enzyme-embossed conductive polymer composites for the detection of 25OHD.

10. Au-Pt NPs/APTES/FTO biosensor: This biosensor uses a composite of gold-platinum nanoparticles (Au-Pt NPs) and 3-aminopropyltriethoxysilane (APTES) on a fluorine-doped tin oxide (FTO) electrode for the detection of 25OHD.

11. GCN-β-CD/Au nanocomposite biosensor: This biosensor utilizes a gold nanocomposite modified with graphene-like carbon nitride (GCN) and β-cyclodextrin (β-CD) on a glassy carbon electrode (GCE) for the detection of vitamin D (25OHD). It offers good sensitivity and selectivity.

These biosensors have various advantages and limitations in terms of sensitivity, selectivity, stability, and cost. Further research is needed to optimize these biosensors and improve their performance for the determination. The development of electrochemical biosensors for vitamin D detection holds great potential for various applications in healthcare and research. These biosensors offer numerous advantages, including high sensitivity, selectivity, simplicity, and potential for miniaturization and integration, into portable devices.

In terms of applications, electrochemical biosensors can be utilized in clinical settings for routine vitamin D assessment, enabling timely and accurate monitoring

of vitamin D levels in patients. They can also find utility in personalized medicine, where precise vitamin D measurements can aid in tailoring individual treatment plans and optimizing patient outcomes.

Furthermore, electrochemical biosensors can play a crucial role in research studies related to vitamin D metabolism, deficiency, and its association with various diseases. They can help researchers investigate the impact of different factors on vitamin D levels, explore potential correlations between vitamin D status and health outcomes, and contribute to advancing our understanding of vitamin D-related mechanisms in the body.

As for future prospects, there are several areas that can be explored to further enhance the capabilities and applications of electrochemical biosensors for vitamin D detection.

Firstly, efforts can be directed toward improving the sensitivity and selectivity of these biosensors by exploring novel recognition elements, such as aptamers or molecularly imprinted polymers, and optimizing the immobilization techniques.

Secondly, the integration of electrochemical biosensors with emerging technologies, such as wearable devices or point-of-care systems, can enable convenient and real-time monitoring of vitamin D levels, providing immediate feedback and facilitating timely interventions.

Additionally, the development of multiplexed biosensors capable of simultaneous detection of multiple analytes, including vitamin D, could offer comprehensive insights into the interplay between different biomarkers and their impact on health.

Furthermore, advancements in data analysis techniques, including artificial intelligence and machine learning algorithms, can aid in extracting valuable information from complex electrochemical data, improving accuracy, and facilitating data interpretation.

In conclusion, electrochemical biosensors have the potential to revolutionize the detection and monitoring of vitamin D levels in various applications. By addressing the current challenges and exploring new avenues for research, these biosensors can contribute to advancements in personalized medicine, clinical diagnostics, and research studies, ultimately improving our understanding of vitamin D's role in human health.

10. Future perspective

Electrochemical biosensors based on carbon materials functionalized with recognition elements have shown promise for vitamin D detection. However, there are existing challenges that require further research. Current issues include sensor stability, sensitivity, selectivity, and interference from complex samples. To address these, future research directions can focus on developing more stable electrode materials, improving immobilization techniques, enhancing selectivity through advanced recognition elements, and exploring innovative signal transduction mechanisms. Additionally, miniaturization, multiplexing, and data analysis techniques are areas of potential development. The chapter aims to provide a comprehensive overview of recent developments in electrochemical biosensors for vitamin D detection and suggests future research directions to overcome current challenges in the field.

Acknowledgements

We would like to express our deepest gratitude to Muhammad Sajid (Department of Biotechnology and Genetic Engineering, International Islamic University,

Islamabad, Pakistan) and Muhammad Afnan (Bachelor of Medicine and Bachelor of Surgery, KMU Institute of Medical Sciences, Kohat, KPK, Pakistan) for their invaluable contributions to the completion of this book chapter. Their dedication, hard work, and expertise have been instrumental in shaping the content and ensuring its quality. Without their support, this chapter would not have been possible. Once again, thank you for your outstanding efforts.

Conflict of interest

The authors declare no conflict of interest.

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