

Mini-Review



Electrochemistry of Vitamin D and Biosensors for its Determination: A Review

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Abstract | Vitamin D play a vital role in health, therefore, it is necessary to find a sensitive, selective as well as quick and easy technique for its determination. This review focuses on the Vitamin D investigations of electrochemical biosensors that have been conducted in recent years. According to the research, the practical use of electrochemical biosensors is attributed to the existence of UV radiation and transactivation of pharmaceutical items, food, or even human blood plasma in the detection of Vitamin D from diverse samples, including Vitamin D production in nature. Among the most commonly used electrochemical biosensors for vitamin D detection are Ab-25OHD/SPE/ FM-TAD, CYP27B1/GCE, SiO₂/GO/Ni(OH)₂/GCE, BSA/Ab-VD₂/CD-CH/ITO, BSA/Anti VD/Fe₃O₄ PANnFs/ITO, BSA/Ab-VD/Asp-Gd₂O₃NRs/ITO, 25OHD Antibody, 25OHD, 25OHD Antibody, IoT Enabled Enzyme Embossed Biosensor, Au-Pt NPs/APTES/FTO and GCN-β-CD/Au nanocomposite. The proposed electrochemical biosensors utilized in the previous publications studied were based on glassy carbon, carbon dots, or carbon paste, functionalized with the various electrochemical biosensors. Further research should be conducted on existing problems and future opportunities of the present electrochemical sensors for the determination of vitamin D.

Keywords: Vitamin D, Biosensor, Radiation, Carbon dots, Glassy carbon, Carbon paste

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INTRODUCTION

Vitamin D (also known as “calciferol”) is a class of fat-soluble secosteroids that promotes calcium, magnesium, and phosphate absorption in the intestine, and enhances several other biological effects. Vitamin D is associated with the guideline of calcium homeostasis and bone metabolism by playing out its capacities in target tissues, including the digestion tracts, kidneys, and bones. Developing proof proposes that Vitamin D assumes a significant part in numerous tissues, including skeletal muscle. Early clinical translations of a myopathy related with extreme Vitamin D insufficiency perceived the expected

connection between nutrient D and muscle. Toxicity to Vitamin D is much rare (Holick, 2007). It is caused due to the administration of excessive doses of Vitamin D rather than the exposure to sunlight. Toxicity threshold has not yet being established for Vitamin D.; however, according to certain research, for the ages of 9 – 71 the acceptable upper intake level (UL) is 4,000 IU per day. (Ross, 2011), Other study indicates that a continuous consumption of more than 1250 g per day (50,000 IU) in healthy persons might cause overt toxicity after a few months and raise blood 25-hydroxyVitamin D levels to 150 ng/mm or higher. (Holick, 2007). Patients suffering from primary hyperparathyroidism are substantially more susceptible to Vita

Table 1: Important Chemical structure of Vitamin D

Name of Vitamin D	Chemical Name	Structure	Chemical Composition
Vitamin D ₁	(3S,9R,10R,14S)-17-((2R,5R,Z)-5,6-dimethylhept-3-en-2-yl)-10,13-dimethyl hexadeca hydro-1H-cyclopenta [a] phenanthren-3-ol		Mixture of molecular compounds of ergocalciferol with lumisterol, 1 :1
Vitamin D ₂	(Z)-3-((E)-2-(1-((Z)-5,6-dimethylhept-3-en-2-yl)-7a-methyl hexahydro-1H-inden-4(2H)-ylidene)ethylidene)-4-methylene cyclohexanol		Ergocalciferol (made from ergosterol)
Vitamin D ₃	(Z)-3-((E)-2-(7a-methyl-1-(6-methyl heptan-2-yl)hexahydro-1H-inden-4(2H)-yli dene) ethylidene)-4-methylenecyclohexanol		Cholecalciferol (made from 7-dehydrocholesterol in the skin)
Vitamin D ₄	(Z)-3-((E)-2-((3aS)-1-((Z)-5,6-dimethylhept-3-en-2-yl)-7a-methyl hexahydro-1H-inden-4(2H)-ylidene)ethylidene)-4-methylenecyclohexanol		22-dihydroergocalciferol
Vitamin D ₅	(Z)-3-((E)-2-((3aS)-7a-methyl-1-(6-methyl heptan-2-yl)hexahydro-1H-inden-4(2H)-yli dene) ethylidene)-4-methylenecyclohexanol		Sitocalciferol (made from 7-dehydrositosterol)

min D. All patients with primary hyperparathyroidism will have low levels of Vitamin D. Maternal hypercalcemia may enhance fetal susceptibility to Vitamin D effects and lead to mental retardation syndrome and facial abnormalities. (Vieh, 1999). Idiopathic infantile hypercalcemia is caused by a CYP24A1 gene mutation, which reduces Vitamin D decomposition. Infants with this mutation are more sensitive to Vitamin D and are at risk of hypercalcemia if they consume more of it (EFSA, 2006). Dahlquist et al. (2015) found that deleterious effects were only observed at 25(OH)D serum concentrations over 200 nmol/L. It is important that women who are pregnant or breastfeeding should get doctor's advice prior to using a vitamin D sup-

plement. The Food and Drug Administration (FDA) has recommended makers of liquid Vitamin D supplements that droppers containing such goods should be clearly and precisely labelled for 400 foreign units (1 IU is the biological equivalent of 25ng cholecalciferol/ergocalciferol); FDA, 2017). The indication of hypercalcemia is increased urination and thirst. Excessive calcium accumulation in soft tissues and organs such as the liver, kidneys, and heart result in discomfort and organ damage if hypercalcemia is not treated. (Holick, 2007; Brown, 2013; Insel, 2015). Anorexia, vomiting, and diarrhoea are the most common symptoms of Vitamin D insufficiency. and they are the same as hypercalcemia. The toxicity of Vitamin D is

controlled by the discontinuation of the supplementation of this vitamin and restricting calcium intakes. Sunlight exposure over lengthy periods of time does not generally result in Vitamin D toxicity. The amounts of Vitamin D precursors produced in the skin reach equilibrium, and any more Vitamin D produced is decomposed. (Vieth, 1999). These facts show that determination of Vitamin D in human organism is important especially if one uses food supplements with this vitamin. Therefore, the fast and effective methods for analysis of this vitamin are needed. One of such methods are electrochemical sensing, this review determination of Vitamin D with electrochemical sensors or methods are overviewed. In order to understand how this vitamin could be determined electrochemically, basics of compounds and transformations within Vitamin D are explained and discussed.

BASIC INFORMATION ON VITAMIN D AND ITS ANALOGUES

Vitamin D (Vitamin D) production in nature is dependent on the presence of UV light and transactivation in the liver and kidneys. Vitamin D comes in a variety of forms in living beings (Horst et al., 1986; Table 1). Vitamin D₁ contains a 1:1 ratio of the lumisterol and D₂ molecular compounds. Vitamin D₂ or trivial name - ergocalciferol & Vitamin D₃ or trivial name - cholecalciferol are the two primary form. Without a subscription, Vitamin D is usually D₂ or D₃, or both. Collectively, these are recognized as calciferol (Karim & Hoor, 2015). In 1931, Vitamin D₂ was characterized for the first time (Vaja, 2019). In 1935, the molecular structure of Vitamin D₃ was discovered and proved to be the result of UV irradiation of 7-dehydrocholesterol. The various forms of Vitamin D are secosteroids, which are steroids formed by the breakage one of the bonds in the steroid rings. The structural distinction between Vitamin D₂ and D₃ is that the side chain of D₂ includes a double bond between carbons 22 and 23 and the carbon 24 methyl groups. The structural difference among Vitamin D₂ and D₃ is that the side chain of D₂ has a methyl groups on carbon 24 and a double bond between carbons 22 and 23 (Trashin & Pchelintsev, 2010).

Number of species synthesize the 7-dehydrocholesterol (trivial name - cholecalciferol) Vitamin D₃ and several fungi synthesize the ergosterol Vitamin D₂ (trivial name - ergocalciferol). Vitamin D₂ and Vitamin D mechanism of action (Bikle, 2014). According to the International Union of Pure and Applied Chemistry, metabolites generated by Vitamin D₂ are frequently referred to as er- or ergo prefixes to distinguish them from D₃ equivalents. (IUPAC, 1982). Vitamin D₂ metabolites tend to link the Vitamin D binding protein. Alternatively, Vitamin D₃ may be hydroxylated to calcifediol via sterol 27-hydroxylase (CYP27A1), while Vitamin D₂ doesn't. From position 24, ergocalciferol

can be hydroxylated directly. This hydroxylation also adds to a greater degree of inactivation: whereas calcitriol activity reduces to 60% of its original following 24-hydroxylation, (Holick et al., 1973), the activity of ercalcitriol on conversion to ercalcitrol decreases 10-fold.

The transition of 7-dehydrocholesterol to Vitamin D₃ occurs in two steps. (Fig.1; Holick 1987; Deluca, 2014). First, 7-dehydrocholesterol is photolyzed by UV light in a 6-electron conrotatory ring-opening electro cyclic process, yielding previtamin D₃. Second, previtamin D₃ spontaneously isomerizes to Vitamin D₃ in an antarafacial-sigmatropic hydride transfer. In an organic solvent, the transition of previtamin D₃ into vitamin D₃ takes around 12 days at room temperature. The conversion of previtamin D₃ into vitamin D₃ in skin is approximately ten times quicker than in an organic solvent. (Holick, 2004). The conversion of ergosterol into Vitamin D₂ bears some resemblance, with photolysis yielding previtamin D₂, which isomerizes to Vitamin D₂ (Eyley, 1975). The rate of previtamin D₂ transformation to methanol from Vitamin D₂ is similar with that of previtamin D₃. (Banerjee & Bhunia, 2009). Photochemically, vitamin D₃ is generated from 7-dehydrocholesterol with in skin of majority vertebrate species, which includes humans. (Keegan, 2013).

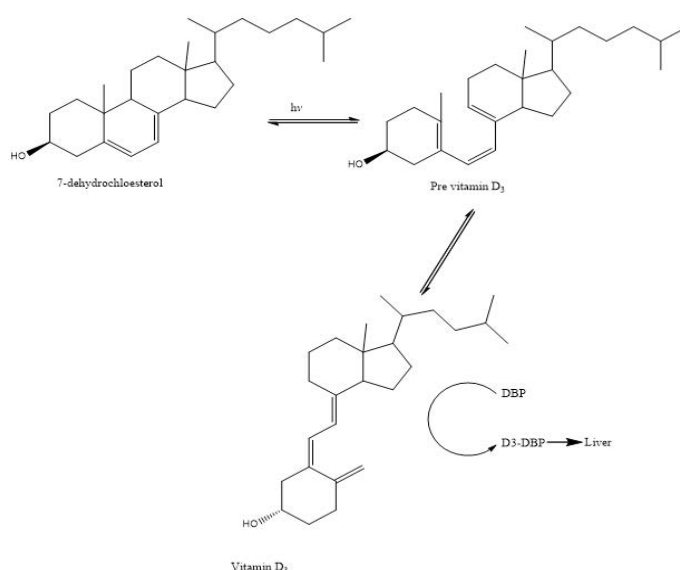


Figure 1: Transformation form 7-dehydrocholesterol to Vitamin D₃ (Source: Carlucci et al., 2013)

7-dehydrocholesterol, a precursor of vitamin D₃, is produced in quite substantial quantities. (Crissey et al., 2003). At wavelengths of 290–315 nm, 7-dehydrocholesterol interacts with UVB light (Holick, 2018). These wavelengths are found in sunshine and also the light generated by UV lamps in sunbeds (which emit ultraviolet mostly in UVA spectrum but often generate 4 -10% of overall UV emissions as UVB). Because glass nearly totally filters UVB rays, there is insufficient light availability from windows.

(Ray, 2005; Bolton, 2013). Adequate quantities of Vitamin D can be obtained by exposing the arms, legs, and face to the sun for 15 to 30 minutes twice per week, or around 25% of the typical sunburn duration. The lower level of sunshine and more exposure time is required for the darker skin.

The toxicity of Vitamin D from UV radiation is impossible since the skin reaches a point where the vitamin is degraded at the same rate as it is produced. (Holick, 2007; Holick, 2009; Holick, 2002). UV radiation is absorbed or reflected by sunscreen, preventing it from reaching the skin (Holick et al., 1987). Sunscreen with an SPF of 8 on the UVB spectrum diminishes Vitamin D's synthetic potential by 95%, while SPF 15 reduces it by 98 percent. (Ross et al., 2011). The skin consists of two main layers: the inner layer called the dermis while outer layer is known as thinner epidermis, mainly composed of connective tissue (Yousef et al., 2021). The thick epidermis consists of five strata in the soles and palms; from outside to inside they are stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale. In keratinocytes, the basal stratum and the spinosum stratum are the two innermost strata that synthesize vitamin D (Holick et al., 1987; Blumberg et al., 2016). Table 2 summarizes various biosensors that are used to determine vitamin D levels.

Biosensor/Electrode/Recognition element	Technique	References
Ab-25OHD/SPE/FMTAD	SPR	Carlucci et al., 2013
	DPV	Chauhan et al., 2019
CYP27B1/GCE	CV	Ozbakir et al., 2015
SiO ₂ /GO/Ni(OH) ₂ /GCE	DPV	Canevar et al., 2014
BSA/Ab-VD ₂ /CD-CH/ITO	DPV	Sarkar et al., 2018
BSA/AntiVD/Fe ₃ O ₄ -PANnFs/ITO	DPV	Chauhan et al., 2018
BSA/Ab-VD/Asp-Gd ₂ O ₃ NRs/ITO	DPV	Chauhan et al., 2019
25OHD Antibody	SPR	Carlucci et al., 2013
25OHD	SPR	
25OHD Antibody	DPV	
IoT Enabled Enzyme Embossed Biosensor	DPV	Ghosh et al., 2021
Au-Pt NPs/APTES/FTO	FE-SEM, FT-IR, XRD, XPS, CAM, CV, DPV, EIS.	Kaur et al., 2020
GCN-β-CD/Au nanocomposite	EIS, CLIA	Anusha et al., 2022

*DPV: Detection Using Differential Pulse Voltammetry, SPR: Surface Plasmon Resonance, CV: Cyclic Voltammetry, Anti-VD3: anti-VD3

1 A hydroxylase enzyme antibody, Au-Pt NPs/APTES/FTO: gold-platinum nanoparticles supported on 3-(aminopropyl)triethoxysilane modified fluorine tin oxide glass electrode, FE-SEM: field emission scanning electron microscope, FT-IR: Fourier Transform Infrared Spectroscopy/Analysis, XRD: X-ray Powder Diffraction, XPS: X-ray photoelectron spectroscopy, CAM: contact angle measurement, EIS: electrochemical impedance spectroscopy, GCN-β-CD@Au/GCE: graphitic carbon nitride hybridized with β-cyclodextrin, CLIA: Chemiluminescent immunoassays

CONCLUSIONS

Considering the significance of Vitamin D in health, it is vital to develop a sensitive, selective, quick, and simple technique for determining it. This review outlines some of the novel electrochemical biosensors used to determine Vitamin D in the presence of UV radiation and subsequent activation of pharmaceutical products, food, or human plasma, since there is a global need to be able to employ a quick approach to evaluate Vitamin D levels from a range of samples. The biosensors used for the electrochemical determination of Vitamin D were functionalized via various electrochemical materials based on carbon dots, glassy carbon, or carbon paste. Also, the option of electrochemical biosensor for Vitamin D determination should be affected by the form of specific samples used in the applications. For practical applications, highly selective and efficient analytical techniques that can span vast concentration ranges and fulfill low concentration limits for Vitamin D are required. The advantages of the electrochemical biosensors presented in this review, such as high sensitivity, selectivity, stability, and repeatability, allow them to be employed as prospective instruments for evaluating Vitamin D in medical, therapeutic, food, or other domains. The carbon-based electrochemical discussed in this study demonstrates a rapid electrochemical biosensor determination of Vitamin D on the functionalized electrochemical surface, as well as a fast kinetic process and high electrochemical biosensor activity. Furthermore, the design and production of the material utilized to detect Vitamin D is focused on lowering the fouling impact of the electrochemical surface, which might lead to biological application.

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CONFLICT OF INTEREST

The authors have declared no conflict of interests.

NOVELTY STATEMENT

In this study, some efficient electrochemical biosensors for Vitamin D detection has been reviewed.

AUTHORS CONTRIBUTION

Nargis Sardar designed the work and drafted the manuscript; AKM Rezwan Sardar and Fahamida Zaman collected the data; Arsalan Rasheed performed data analyses and co-write the manuscript; Sufian Rasheed and Maria Binte Sarfraz critically revised the manuscript for necessary actions. All the authors approved the final manuscript for publication.

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