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Review

Electrochemical Sensing for Vitamins

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Abstract: Vitamins are essential and necessary nutrients for the human body. Rapid and accurate quantification of their levels in various samples has attracted much attention. Compared with traditional analytical methods, electrochemical techniques, with the advantages of low cost, high sensitivity, flexible detection strategies, easy integration, and miniaturization, have gradually become the main tools in vitamin detection. In this paper, the advance of electrochemical sensing of vitamins in recent years is reviewed. Firstly, the basics of different vitamins are briefly introduced. Then, the commonly-used electrodes and electrochemical methods for vitamin electrochemical detection, as well as the specific implementation strategy and performance, are described in detail. The development of miniaturization devices, especially microfluidic and microsensor devices, is also presented. Finally, the challenges faced by the electrochemical detection of vitamins are discussed, and future development is prospected.

Keywords: vitamin; electrochemical sensor; biosensors; miniaturized devices; microfluidics

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1. Introduction

Vitamins are a group of complex chemical compounds with very low content in the human body but extremely important functions [1]. They play key roles in many vital biological processes, such as digestion, immunity, and metabolism, which are essential for maintaining human health. For example, vitamins can be used as cofactors to activate enzymes and assist them in completing various biochemical reactions. According to their solubility, vitamins are divided into water-soluble vitamins (B, C, P) and fat-soluble vitamins (A, D, E, K). Fat-soluble vitamins can be stored in adipose tissue, while water-soluble ones cannot be kept in the human body. Except for vitamins B3, D, and K, the human body cannot synthesize the most essential vitamins that it is necessary to obtain from diets and other sources. Vitamin deficiency caused by inadequate intake often leads to various diseases, and excessive vitamins may also be harmful.

Therefore, the determination of vitamins in the human body and the food source is of great importance and has attracted widespread attention. Due to the extremely low concentration of the vitamin in many samples, detection methods with high sensitivity are required. Various methods have been adopted for vitamin detection, including fluorescence [2], surface-enhanced Raman spectroscopy (SERS) [3], ultra-performance liquid chromatography-electrospray ionization multiple reaction monitoring/mass spectrometry (UPLC-ESIMRM/MS) [4], surface plasmon resonance [5], ultraviolet-visible spectroscopy [6], and capillary electrophoresis [7]. Although these methods have high selectivity and resolution, they have some limitations in sample consumption, analysis time, detection cost, and operational complexity. Electrochemical techniques are simple, easy to miniaturize, and portable, which have been widely used in pharmaceutical, food, and clinical analysis fields [8]. Because almost all vitamins have electrical activity, they can generate specific response signals in electrochemical detection so as to achieve their detection and discrimination. Therefore, it is promising to determine the vitamins using electrochemical sensors.

Due to the importance of vitamin analysis, there are many studies on their electrochemical detection of them. This review mainly introduces the research progress in the last three years (2020–2022), especially focusing on some new electrode materials and electrochemical detection strategies. Furthermore, microdevices integrated with electrochemical sensors for vitamin determination are also presented. Finally, the challenges and application prospects of electrochemical vitamin sensors are analyzed.

2. Vitamins and Their Properties

According to the solubility, vitamins are mainly classified into two categories. The water-soluble vitamins include vitamin C, the eight B vitamins (B1, B2, B3, B5, B6, B7, B9, B12), and vitamin P. The fat-soluble vitamins are A, D, E, and K. These organic compounds are essential for the healthy development of the human body, which is summarized in Table 1, and most of the information is from the National Institutes of Health (https://ods.od.nih.gov/factsheets/list-all/, accessed on 21 November 2022).

Table 1. Summary of different vitamins.

Vitamin	Compound	Intake	Main Roles	Source	Possible Consequence	
					Deficiency	Excessive
VB1	Thiamine	1.2 mg d^{-1}	Carbohydrate and amino acid metabolism	Rice, milk, eggs, meat	Metabolic diseases, beriberi, nervous system diseases	Excessive thiamine is excreted in the urine
VB2	Riboflavin	1.3 mg d^{-1}	Promote cell growth and regeneration	Whole-grain products, eggs, liver	Inflammation of mouth corners and oral mucosa	Oxidative damage to DNA and liver
VB3	Nicotinic acid, nicotinamide	16 mg d^{-1}	Main redox mediators in cell metabolism	Animal viscera, muscle tissues, fruits, and egg yolks	Skin disease, digestive system symptoms and dementia	Flushing, hypotension
VB5	D-pantothenic acid	5 mg d^{-1}	Amino acid catabolism, glycolysis and fatty acid metabolism	All types of animal and plant tissues	Fatigue, headache, nausea, vomiting, stomach pain	Mild diarrhea and gastrointestinal distress
VB6	Pyridoxine	1.5 mg d^{-1}	Cell growth and normal functional performance	Vegetables and protein rich foods	Dermatitis, glossitis, anemia, numbness, weakened immune	Dermatological lesions; photosensitivity
VB7	Biotin	30 μg d^{-1}	Cofactor necessary for four important carboxylases	Meats, grains and vegetables	Skin diseases, nerve problems, growth retardation	-
VB9	Folic acid	400 μg d^{-1}	Maintain and produce new cells, prevent DNA changes	Liver, dried beans, yolks, fruits, nuts, leafy vegetables	Neural tube defects, megaloblastic anemia, colon cancer	Impair the absorption of zinc and VB12
VB12	Cobalamin	2.4 μg d^{-1}	Nerve cell growth, red blood cell formation	Liver, kidney, meat, fish, clams, eggs, milk, cheese	Neuropsychiatric, cardiovascular and hematological diseases	Renal failure, liver disease and neurotoxicity
VC	L-ascorbic acid	120 mg d^{-1}	Antioxidant; synthesis of collagen, carnitine, adrenaline	Fresh fruits and vegetables	Scurvy, anemia, infections, cardiovascular disease, cancer	Headache, difficulty sleeping, skin redness

Table 1. *Cont.*

Vitamin	Compound	Intake	Main Roles	Source	Possible Consequence	
					Deficiency	Excessive
VP	Rutin	500 mg d^{-1}	Anti-radiation, analgesic, anti-inflammatory, antioxidant	Fresh plants and fruits	Increase the brittleness of capillaries	Allergies and eczema
VA	Retinoic acid	900 mg d^{-1}	Maintain normal function of human retina, bone growth	Animals (retinol); fruits, vegetables (carotenoids)	Blindness, decreased immune system efficiency, cancer	Severe headache, blurred vision, nausea, dizziness
VD	-	15 μg d^{-1}	Promote the absorption of calcium and phosphate in the intestine	Egg yolk, mushroom, cod liver oil, fresh salmon	Osteomalacia in adults and rickets in children	Nausea, vomiting, muscle weakness, pain
VE	-	15 mg d^{-1}	Prevent the formation of reactive oxygen species	Fruits and vegetables, nuts, lean meat, milk, eggs	Circulatory disorders, fertility disorder and Alzheimer's disease	Hemorrhage and interrupt blood coagulation
VK	Naphtho-quinone compounds	120 μg d^{-1}	Control calcium levels and synthesize proteins needed for blood coagulation	VK1 from Green vegetables, VK2 from eggs, cheese, liver, meat	Decrease prothrombin and the tendency of excessive bleeding	-

2.1. Water-Soluble Vitamins

Water-soluble vitamins, which are composed of various compounds and widely exist in almost all foods, are important nutrients and are of great significance to human growth and development [9]. However, they cannot be stored in the human body for a long time and are discharged quickly with urine, so they need to be regularly supplemented. Their deficiency often brings negative impacts on human health and leads to numerous diseases. Therefore, in many cases, it is necessary to detect vitamins in the human body and food samples. Except for vitamin B5, most water-soluble vitamins are electrochemically active, and they can be analyzed by examining their redox properties.

There are eight kinds of B vitamins, among which vitamin B1 (VB1), known as thiamine, can be used to synthesize pyrophosphate and thiamine diphosphate [2]. These thiamine compounds are coenzymes of many metabolic processes and are essential for the normal metabolism of the human body, especially the nervous system.

Vitamin B2 (VB2), also known as riboflavin (RF), is an important component of flavoenzyme with active forms of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). FMN and FAD are necessary cofactors for typical respiration in tissues and can help in the conversion of carbohydrates, fats, and proteins into energy [10]. RF aids in avoiding cataracts through its antioxidant property, and even high dosages can also cure migraines [11,12].

Vitamin B3 (VB3), also known as niacin, is mainly composed of nicotinic acid and nicotinamide and is the precursor of nicotinamide adenine dinucleotide (NAD). NAD, together with its phosphorylated compounds nicotinamide adenine dinucleotide phosphate (NADP) and their reduced forms NADH and NADPH, constitute the main redox mediators of various metabolic processes in cells. Nicotinic acid can increase the concentration of all major lipids and lipoproteins and also has a consistent beneficial effect on the distribution of lipoprotein subclasses [13].

Vitamin B5 (VB5) mainly refers to the D-isomer of pantothenic acid, which can be used as a precursor to form coenzyme A (CoA) and plays an important role in various life activities such as energy metabolism and fatty acid oxidation [14]. CoA is the acetyl donor of acetylation reaction, participating in the oxidation of fatty acids, pyruvate or α-ketoglutaric, the synthesis of fatty acids, cholesterol or sterols, and biological acetylation.

Vitamin B6 (VB6), also known as pyridoxine, acts as a cofactor for participating in the catalytic reaction of more than 100 enzymes in protein metabolism and hemoglobin production, can promote brain development in pregnancy and infancy, and support the nervous and immune systems [15–17].

Biotin, usually called vitamin B7 (VB7) or vitamin H, plays an important role in the main metabolic pathways in mitochondria and cytoplasm (such as amino acid catabolism, gluconeogenesis, and lipogenesis) [18].

Vitamin B9 (VB9), also known as folic acid (FA) or pteroylglutamic acid (PteGlu), plays a major role in various synthetic biological reactions such as the synthesis of purine and pyrimidine, DNA fixation and methylation [19,20].

Vitamin B12 (VB12, cobalamin) is a cobalt-containing coordination compound that plays an important role in the normal function of the nervous system and the maturation of red blood cells [21].

Vitamin C (VC) or L-ascorbic acid (AA) is one of the most common small biomolecules in human blood. It can fight against bacterial infections in our body cells to prevent damage and protect healthy cells from free radicals, harmful chemicals, cigarette smoke, and other pollutants. AA is also involved in a variety of human activities, including immune system function, wound healing, and the maintenance of cartilage, bone, and tooth. It can be used as a cofactor of several enzymes, enhance intestinal iron absorption, participate in carnitine biosynthesis and cell metabolism, and can also be used as an ideal singlet oxygen scavenger and a chelating agent [22–25].

Rutin, also known as rutoside and vitamin P (VP), is a natural flavonoid glycoside, which belongs to flavonol glycoside widely existing in plants [26,27]. In vivo, rutin shows some scavenging activities mediated by active oxygen species, such as peroxy, superoxide, and hydroxyl radicals. Rutin can maintain vascular resistance and reduce its permeability and brittleness.

2.2. Fat-Soluble Vitamins

Fat-soluble vitamins are easily soluble in organic solvents but poorly water-soluble. They are the crucial nutrients needed to maintain human health. Each fat-soluble vitamin contains some structurally related different compounds. Among them, vitamin A (VA) refers to compounds with retinol biological activity, including carotenoids, retinoids, and retinol. VA is an essential nutrient required for the synthesis of retinylidene chromophores and visual pigments [28].

Vitamin D (VD), known as the sunshine vitamin, is one of the key determinants of bone and mineral metabolism. It is an important hormone that participates in a variety of metabolic pathways [29,30]. VD helps maintain calcium homeostasis and plays an important role in strengthening bones. The two most common substances in VD are ergocalciferol (D2) and cholecalciferol (D3). Among the various VD3 metabolites, 25-hydroxy vitamin-D3 (25-OHD3) is of significance as it is the best indicator of VD deficiency and can be measured in serum samples [31].

Vitamin E (VE) represents a group of molecules with the antioxidant activity of α-tocopherol, including all tocopherols and their derivatives. The liver absorbs various forms of VE and only secretes α conformation (α-tocopherol), which has greater biological activity in the human body and can be used as an antioxidant [32]. VE maintains immune and neural health. VE also affects the proliferation and differentiation of smooth muscle cells, platelets, and monocytes and helps regulate blood cell expansion and inhibit platelet aggregation.

Vitamin K (VK) includes 2-methyl-1,4-naphthoquinone and all its derivatives, among which phylloquinone (K1) and menaquinone (K2) are the most common. VK1 is a redox

mediator in photosynthesis and the predominant form of VK. VK2 is a kind of compound with a quinone structure, including more than 12 different types from MK-4 to MK-15 according to the length of its unsaturated side chain [33,34].

3. Electrochemical Methods and Working Electrodes

3.1. Electrochemical Methods

Electrochemical sensing detection is based on the charge transfer between two phases caused by the electrochemical reaction on the electrode so as to establish the metrological relationship between the electrical parameters, such as potential, conductance, current and electric quantity, and the level of the measured substance. Based on the difference in measured electrical signals, electrochemical sensors are generally divided into conductometric, amperometric, and potentiometric sensors. Electrochemical impedance spectroscopy (EIS) measures the impedance change with the frequency of a sinusoidal potential signal [35]. During the detection process, a small amplitude alternating current potential wave with different frequencies is applied, and the change of the ratio of the alternating current potential to the frequency of the sinusoidal wave or the change of the phase angle of the impedance with the frequency is measured [16,27,36–41].

Amperometry, which depends on the amount of charge transfer in the redox process, can measure the current changes generated when the electroactive substances are oxidized or reduced [21,35,36]. Its main advantages are high sensitivity, good selectivity, wide response range, and simple structure. According to the different ways of applying a potential, it can be divided into constant potential amperometry (CPA), pulsed amperometry (PA), and integrated pulsed amperometry (IPA) [37–40]. The CPA has a simpler structure and is more suitable for miniaturization and even wearable applications [42]. The amperometry is easy to be interfered with by the electroactive compounds or biometric molecules in the sample, resulting in wrong detection signals [43].

Voltammetry is the most commonly used electrochemical sensing method because the current and voltage are kept dynamic, reflecting the most basic electrochemical reaction process. Moreover, the corresponding detection system is cheaper, more sensitive, and faster. In voltammetry, differential pulse voltammetry (DPV) [9,24,42–47] and square wave voltammetry (SWV) [32,35,48–51] can distinguish charging current with high sensitivity and gradually become the main detection methods. However, traditional techniques such as cyclic voltammetry (CV) [40,47,52–59] and linear scanning voltammetry (LSV), which are simple and easy to operate, are still used extensively.

Because different electrochemical detection methods have their own advantages, they can be applied to various aspects of vitamin sensing detection [60]. For example, CV is typically used for electrode treatment and condition exploration, EIS is often used to analyze electrode characteristics, and DPV and SWV are mostly used for the analysis of electrochemical reactions.

Some new electrochemical detection technologies are also used for vitamin analysis. For example, photoelectrochemical (PEC) methods combine photochemical and electrochemical techniques to study that the light is absorbed by vitamin-sensitive electrode materials (e.g., Dy-OSCN monocrystal) [61] or reagents, thus generating specific electrical signals. PEC sensing shows significant advantages over conventional electrochemical detection in terms of low background and potential high sensitivity. Moreover, electrochemical detection methods can be combined with other detection technologies to obtain higher resolution. Ali et al. combined UV-Vis spectrophotometric and electrochemical sensing approaches to measure the mixture of a variety of biomolecules, such as dopamine, epinephrine, and VB12. This method could overcome the problem of poor resolution that could not be solved by using any of them alone [19]. Amine-modified carbon quantum dots (NH2-CQDs) have good electron transport capability and display blue fluorescence so they can be used for AA detection by the combination of the electrochemistry method and fluorescence analysis [62].

3.2. Electrode Materials

The working electrode is the main carrier of electrochemical reaction and the basic pathway of electrochemical signal acquisition. Based on different materials and structures, various electrodes have been used for analyzing numerous vitamin samples, including carbon-based electrodes, metal and metal oxide-based electrodes, and functional material-modified electrodes.

3.2.1. Carbon-Based Electrodes

Carbon-based electrodes are widely used for electrochemical sensing applications due to their low-cost, low background current, and excellent stability [37]. Glassy carbon electrode (GCE) is adopted for electrochemical sensing applications due to their low porosity, good conductivity, and high chemical stability [45,63].

A carbon paste electrode (CPE) is made by mixing graphite powder and binder [37]. It has the advantages of low resistance, low background current, easy availability of raw materials, low cost, good surface reproducibility, excellent stability, wide operating potential ranges, etc. It is also a mainstream working electrode in the electrochemical analysis of vitamins. However, its sensitivity may be reduced due to the addition of a non-conductive binder in the preparation process. Therefore, the surface of CPE is often coated with modifiers to improve its electrochemical performance [12].

Graphite electrode (GE) has the advantages of easy processing, good stability, low toxicity, environmental protection, high repeatability, and uniform quality [55]. At present, the pencil graphite electrode (PGE) is the most widely used graphite electrode in electrochemical vitamin detection. PGE is easily available, inexpensive, and disposable without tedious cleaning procedures [51].

3.2.2. Metal and Metal Oxide Electrodes

Gold is a noble metal with high chemical inertness, but under certain conditions, its surface atoms can be activated to obtain good catalytic properties. In addition, the good conductivity, chemical inertness, and processability make it an ideal electrode material [23]. Owing to the strong affinity between gold and a variety of organic or biological molecules, gold is often used as a substrate to prepare self-organized monolayers for electrode surface modification.

ITO (indium-tin oxide) conductive glass is prepared by magnetron sputtering of indium tin oxide on a glass slide to form a layer of film. ITO is a kind of metal compound with good transparent and conductive properties, high bandgap, and low resistivity. The indium tin oxide film is only a few thousand angstroms, so the ITO electrode is of high light transmittance and strong conductivity [52]. In vitamin sensing analysis, the ITO electrode is very suitable for the simultaneous realization of electrochemical detection and optical analysis due to its unique light transmittance [19,31].

3.2.3. Functional Material-Modification Electrodes

In order to further improve the response of the electrodes, different functional components were designed to modify the surface of the electrodes for vitamin detection. The modification of functional material can provide more active sites by increasing the roughness of the electrode surface and promoting the electron transfer and the enrichment rate of the electrodes.

Carbon-based materials are being used extensively because of their biocompatibility. There are many types of carbon materials that have been developed for vitamin detection due to their prominent electrical and chemical properties, including carbon nanotubes (CNT) [64,65], graphene oxides [58,66], graphene [17], and graphene quantum dots. For example, copper-poly(1,8-diaminonaphthalene)/graphene was used as a modifier, in which graphene can promote electron transfer, while copper has a significant electrocatalytic ability for the oxidation of pyridoxine [17]. Pushpanjali et al. chose graphene nanoplatelets to paste electrodes (GNpPE) with better porosity and greater surface activity than ordinary

CPE [58]. The presence of N,S-GQD promotes charge transfer in the electrical sensing system, providing a higher current intensity and lower charge transfer resistance [63].

Metal materials are often used to form a modified layer of nanoparticles [46] on the electrode surface to increase the electrochemical reaction area and sensitivity [67]. Metals, metal oxide, metal nitrides, metal phosphates, and alloys are the main forms of metal materials for vitamin detection due to their good electrical and catalytic properties. Transition metals and their metal oxides have been widely used due to their exceptional catalytic activity, precise selectivity between various functional groups, green synthesis methods, and unique ability to accelerate electron transfer [60]. For example, the modification of non-metal nanosized catalysts, such as cobalt phosphide (R-CoP) [53], can provide a high active surface area and low charge transfer resistance, which can enhance the contact between electrodes and the working electrolyte and also promotes the participation of more active sites in the electrochemical reaction.

3.2.4. Other Functional Materials

Aptamers are single-stranded oligonucleotide fragments selected in vitro by systematic evolution of ligands (SELEX) technology [68]. They can recognize various targets, including proteins, peptides, cells, and small molecules, and have some advantages, such as low cost, high purity, and stability, as well as easy to prepare, label, or immobilize [43]. Like antibodies, they have strict recognition ability and a high affinity for binding ligands, so they become an important means to specifically recognize specific vitamins [40].

Molecular imprinting technology uses molecularly imprinted polymers (MIPs) to simulate the interaction between enzyme and substrate or antibody and antigen for the special recognition of imprinted molecules [69–72]. Because of its predictability, reusability, recognition, remarkable stability, and super sensitivity, this technology has great potential in the specific recognition of vitamins [30,73]. The combination of electrochemical methods with MIPs can be a competitive alternative for the detection of vitamins, and good surface adhesion of various electrodes also paves the way for this application [16].

In conclusion, in the selection of electrode materials, in order to obtain stronger electrochemical signals, it is necessary to select those materials with high electrical activity and catalytic ability, such as some new metal oxides. The formation of nanostructures of modified materials can increase the electrochemically active surface area and sites. At the same time, some materials with molecular recognition ability, such as enzymes, antibodies, aptamers, and molecular imprinting materials, can be selected to improve the selectivity of electrodes for different vitamins. In commercial products and portable designs, screen-printed electrodes have become the first choice for cost and miniaturization.

In the electrochemical detection of vitamins, there are numerous interference factors from samples, buffers, electrodes, circuits, etc. Among them, the major ones are the interference of non-target substances in the sample and the stability of the electrode itself. Therefore, electrode materials with high physicochemical stability as well as high sensitivity and selectivity to target vitamins should be selected. Meanwhile, the prepared electrodes should have high consistency and structural stability. It is worth noting that the shape of electrode, especially the surface area, which has a great influence on the electrochemical response of the sensor. And different electrode space distribution also has a certain degree of influence on the electric field and current. In practical applications, simulation analysis and experimental testing methods can be used to obtain optimized electrode morphology design.

4. Electrochemical Sensing of Vitamins

4.1. Electrochemical Sensors for Water-Soluble Vitamins

4.1.1. Vitamin B1 Sensors

Adsorptive chronopotentiometric stripping analysis (AdCSA) and non-specific adsorption of VB1 onto a mercury film electrode were used for VB1 determination [74]. Under an optimal experimental condition, the linear detection range of VB1 was 5–50 mg L^{-1}

with a LOD (limit of detection) of 1.64 mg L^{-1}. This method was applied for the analysis of VB1 in pharmaceutical products.

4.1.2. Vitamin B2 Sensors

Various sensitive and accurate electrochemical detection methods were developed for VB2 detection [58,64,75–91]. For example, Wu et al. grew a covalent-organic framework nanobelt (COFTFPB-Thi) vertically on three-dimensional porous carbon (3DKSC) to achieve double signal ratiometric electrochemical detection of RF (Figure 1a) [10]. Due to the reduction of COFTFPB-Thi and its conjugate structure, when two different response signals were used, the linear ranges were 0.13 μM–0.23 mM and 0.30 μM–0.23 mM, respectively, and the LODs were 44 nM and 90 nM respectively. Double signal-based detection can correct the measurement results and improve their reliability.

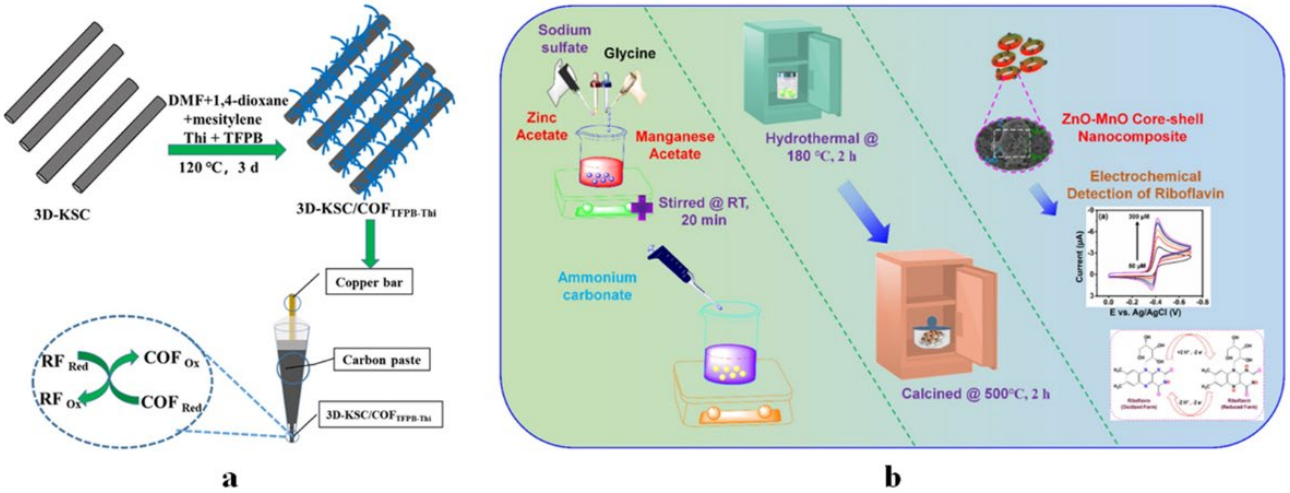


Figure 1. (**a**) The preparation process of 3D-KSC/COFTFPB-Thi and the structure of 3D-KSC/COFTFPB-Thi integrated electrode. Reprinted with permission from Ref. [10]. Copyright 2022, Elsevier. (**b**) Schematic representation of the preparation of ZnO-MnO core-shell nanocomposites for the electrochemical evaluation of RF. Reprinted with permission from Ref. [60]. Copyright 2022, the Electrochemical Society.

The combination of nanocomposites and the screen-printed electrode (SPE) was conducive to the construction of highly sensitive miniaturized RF sensing devices. The screen-printed carbon electrode (SPCE) was modified with Ni/NiO nanocomposites, and the sensor could achieve high detection performance after optimizing the parameters, such as the proportion of nanocomposites. In Britton Robinson (BR) buffer, the linear range was 0.5–75 μM, and the LOD was 0.15 μM [38].

Using binary transition metal oxides (ZnO-MnO) to construct core-shell nanocomposites, its unique structural morphology could improve the specific surface area and increase the number of active sites (Figure 1b) [60], thus showing a superior electron transfer rate. Based on this electrode, a wider linear range (0.05–1102 μM) could be achieved in RF detection, and the LOD also decreased to 13 nM.

4.1.3. Vitamin B3 Sensors

A graphite paste microelectrode (GPE) modified with 5,10,15,20-tetrakis(4-methoxyphenyl)21H,23H-porphine cobalt (II) (CoTMPP) was used to detect NA, and a wide linear detection range (1.00×10^{-7}–1.00×10^{-4} M) with a LOD of 3.03×10^{-8} M could be achieved [92].

4.1.4. Vitamin B6 Sensors

Many methods have been used to quantify VB6 accurately. [36,37,93–97]. Based on the high recognition ability of MIP (3-amino benzoic acid), good selectivity and sensitivity could be obtained when VB6 is detected on MIP modified carbon fiber paper electrode (CFPE) (Figure 2a). Its linear detection range was 0.6 μM to 700 μM with a LOD of 0.010 μM [16].

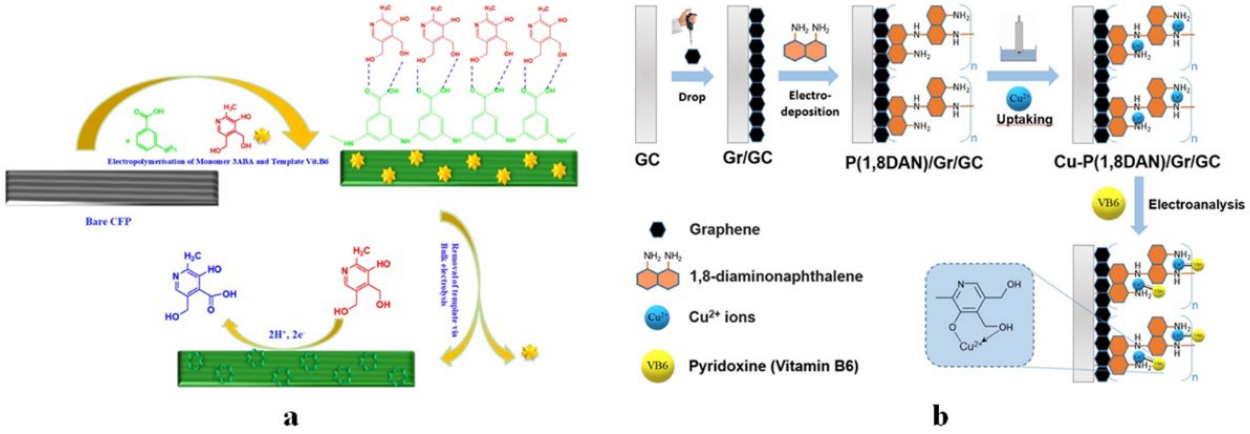


Figure 2. (**a**) The mechanism of oxidation reaction of VB6 at MIP P-(3ABA)/CFP electrode. Reprinted with permission from Ref. [16]. Copyright 2021, the Electrochemical Society. (**b**) Schematic of fabrication procedure of Cu-P(1,8DAN)/Gr/GC electrode for detection of pyridoxine. Reprinted with permission from Ref. [17]. Copyright 2022, Wiley-VCH GmbH.

The glassy carbon electrode was modified with nickel zeolite/carbon black to form a special durable layer [15]. The synergistic effect produced by the unique characteristics of zeolite material and carbon black provides excellent repeatability and short-term stability, as well as good reproducibility and advanced electrical performance. When determining VB6 with DPV, the linear range was 0.050–1.0 mg L^{-1} with a LOD of 15 μg L^{-1}.

Using the unique properties of different materials will help to improve the sensitivity and specificity of VB6 detection. Vu et al. electrodeposited poly(1,8-diaminonaphthalene) onto graphene/GCE using potential cycling technology (Figure 2b) [17]. Then the electrode was immersed in copper (II) aqueous solution to adsorb copper ions onto poly(1,8-diaminonaphthalene)/graphene-modified GCE. The synthetic materials not only obtained the ability of graphene to promote electron transfer but also had the electrocatalytic capacity of copper for the oxidation of pyridoxine. In SWV detection within 0.1 M phosphate buffer solution (pH 7.4), the linear detection range of pyridoxine was 0.58–24.51 μM, and the LOD was 0.3 μM.

4.1.5. Vitamin B9 Sensors

In order to achieve highly sensitive and accurate electrochemical sensing detection of FA, special recognition elements are often required [98–101]. Ali et al. developed a FA sensor based on UV-Vis spectroelectrochemical technology using Ni-tipped carbon nanofibers (Ni-CNFs) anchored over a transparent ITO electrode, which could simultaneously detect the electrochemical response of FA oxidized by Ni-CNF electrocatalyst and the optical sensing signal of FA oxidation products [19]. In the presence of interfering molecules, the linear detection range of the FA sensor was 1–100 μg mL^{-1} with LOD 0.14 μg mL^{-1}.

Because UV-Vis spectroelectrochemistry can simultaneously obtain complementary information from both electrochemistry and spectroscopy, as well as the unique electrical activity and optical characteristics of FA, this method has significant application value in FA quantitative analysis. Olmo et al. [20] used this method to quantitatively analyze FA concentration under various conditions and obtained ideal results (the linear detection range of the FA sensor was 5–100 μM with LOD ~1 μM). Especially, spectral analysis can

more accurately capture the dynamic electrochemical response signals of FA, providing conditions for real-time analysis.

4.1.6. Vitamin B12 Sensors

The determination of VB12 in human plasma, urine, food and drugs is of great significance in the diagnosis and treatment of metabolic diseases [102–105]. Guo [21] et al. modified the GCE with a nanocomposite of Au and polypyrrole nanoparticles and functionalized carbon nanotubes (Au-PPy NPs@f-CNTs), and conducted electrochemical detection using DPV and amperometric techniques. Due to the high conductivity of Au NPs, PPy NPs, and f-CNTs, as well as the uniform distribution of electrochemically active sites on CNTs, the stable and strong electrochemical signals could be obtained, and the linear range, LOD, and sensitivity of electrochemical detection of VB12 were respectively 0–85 μM, 0.9 nM and 4.3597 μA μM^{-1}.

4.1.7. Vitamin C Sensors

The electrochemical AA sensor is mainly divided into enzyme sensors and non-enzyme sensors [42,44,106–129]. The enzyme sensor uses the super ability of biological enzymes in recognition and catalysis, with high sensitivity and selectivity, but it still has limitations in cost and preservation. The non-enzymatic method is the focus of current research [50]. Common bare electrodes are gradually replaced by various modified electrodes due to their low electrical activity and easy passivation by electrochemical reactants, and nanomaterials have become the most commonly used modifiers [35,52,130–132].

Transition metal oxides have high electrochemical activity and are common modifiers for electrochemical electrodes. Sampathkumar et al. used a molybdenum disulfide (MoS_2) modified GCE to determine AA electrochemically [25]. The linear range of detection was 90 to 590 nM, the sensitivity was 5.83×10^{-2} μA μM^{-1}, and the LOD was 41 nM. It could achieve excellent detection effects in artificial urine samples with complex components.

Tortolini et al. studied the effect of different nanostructured modified gold electrodes on the electrochemical detection of AA [23]. Using CV and EIS, the developed sensor had strong electrocatalytic activity against the oxidation of AA. Compared with the bare gold electrode (1.0×10^{-2} μA μM^{-1} cm^{-2}), the detection sensitivity of gold single-walled carbon nanotubes (Au/SWCNT) and gold modified electrode with high nanoporous gold (h-nPG) film was improved to 1.2×10^{-2} μA μM^{-1} cm^{-2} and 2.5×10^{-2} μA μM^{-1} cm^{-2}, respectively. The h-nPG electrode was also successfully used to determine AA in human urine.

Compared with ordinary nanomaterials, nanocomposites have more advantages in porosity and synergistic effect of various electrochemical active substances. Ahmed et al. [133] developed an AA electrochemical sensor based on porous silicon-mesoporous carbon nanocomposites (PSi-MC NCs) modified GCE (Figure 3a), which could measure AA in a very wide range (0.5–2473 μM) in PBS with a sensitivity of 0.1982 μA μM^{-1} cm^{-2} and a LOD of 30.0 ± 0.1 nM. The detection of AA in human blood showed good anti-interference ability and high selectivity.

Gold nanoparticles (Au NPs), chicken egg white (CEW), copper phosphate ($Cu_3(PO_4)_2$), and graphene oxide (GO) were assembled together to form organic/inorganic hybrid nanoflowers (Au-CEW-$Cu_3(PO4)_2$-GO HNFs) (Figure 3b) [57]. When HNFs-modified GCE was used for electrochemical detection of AA, a linear range of 8–300 μM, the LOD of 2.67 μM, and a high sensitivity of 6.01×10^{-3} μA μM^{-1} cm^{-2} could be achieved due to many reasons, such as the high conductivity of Au NPs, the large specific surface area of GO, the strong electrocatalytic activity of HNFs, and so on.

Combined with the outstanding electrical activity of transition element oxides and the superior performance of nanostructures on the surface, Atacan et al. studied the electrochemical sensing of AA with Au-MoS_2/NiO modified GCE (Figure 3c) [24]. The detection linear range and LOD were 2–50 μM and 0.13 μM, respectively, realizing the detection of AA in vitamin C tablets.

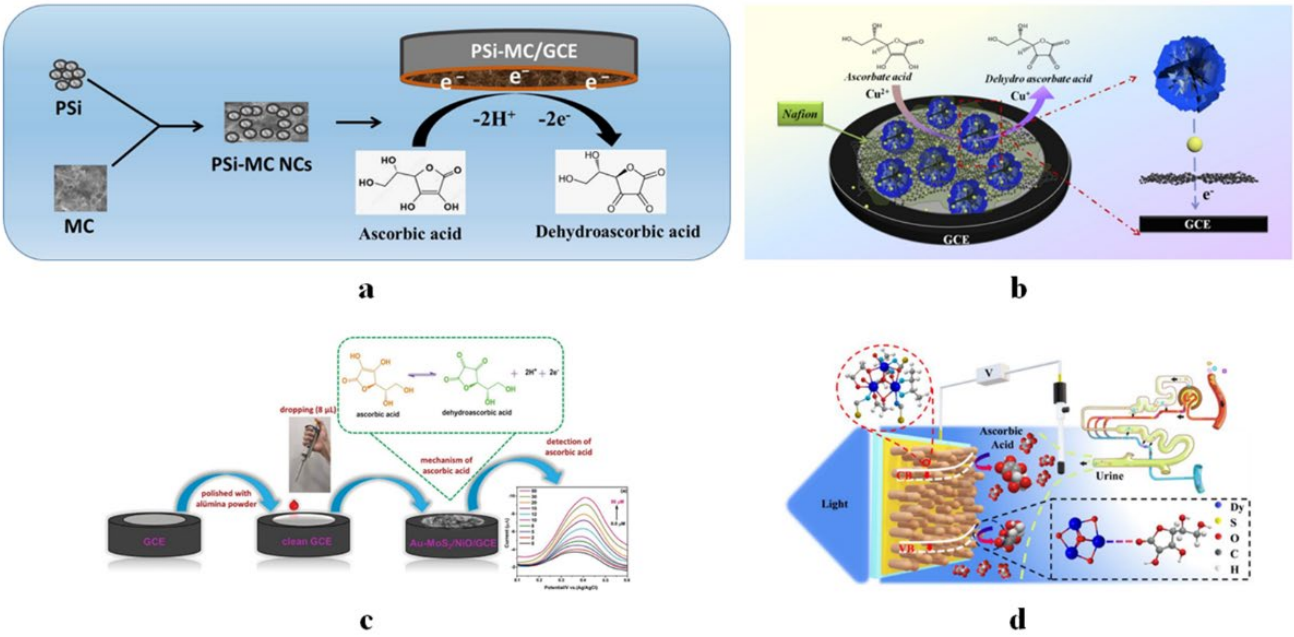


Figure 3. (**a**) Schematic representation of PSi-MC/GCE-based ascorbic acid electrochemical sensor. Reprinted with permission from Ref. [133]. Copyright 2022, Wiley-VCH GmbH. (**b**) Au-CEW-$Cu_3(PO_4)_2$-GO HNFs-based electrochemical sensor for AA detection. Reprinted with permission from Ref. [57]. Copyright 2021, IOP Publishing Ltd. (**c**) The fabrication of Au-MoS_2/NiO composite on GCE for AA detection. Reprinted with permission from Ref. [24]. Copyright 2022, the Korean Institute of Chemical Engineers. (**d**) Schematic diagram of Dy-SCN/FTO PEC sensor detecting AA. Reprinted with permission from Ref. [61]. Copyright 2022, MDPI.

Zhao et al. designed a photoelectrochemical (PEC) sensor based on Dy-OSCN for the detection of AA (Figure 3d) [61]. Dy-OSCN single crystal has a regular microstructure, which accelerates the separation and transmission of internal carriers under light excitation, and can effectively improve the sensing performance. Under the best conditions, the linear range of AA sensing detection was from 7.94 μM to 1.113 × 10^4 μM, and LOD was 3.35 μM. The analysis of human urine samples was realized.

4.1.8. Vitamin P Sensors

Rutin is an effective antioxidant polyphenol with an important protective effect [134,135]. In BR electrolyte, it was determined by differential pulse stripping voltammetry (DPSV) using GCE modified with graphene oxide nanocomposite reduced (rGO) by ferric oxide containing cerium (Ce) and chromium (Cr) (Figure 4a) [26]. The integration of Ce^{4+}, Cr^{3+}, and rGO has a synergistic effect on the sensor performance and excellent activity. Under the optimized construction conditions of the nanocomposite, the linear range of rutin detection was 0.075–12.00 μM, and the LOD was 52 nM.

Elancheziyan et al. developed a highly porous graphitic-activated carbon-coated iron oxide nanocomposite (Fe_2O_3/GAC) and then deposited it on an SPE to construct Fe_2O_3-GAC/SPE for electrochemical detection of rutin (Figure 4b) [27]. The synergistic effect of Fe_2O_3 and GAC can enhance the electrocatalytic reaction ability. A wide linear detection range (0.1–130 μM) could be obtained by DPV, and the LOD was 0.027 μM.

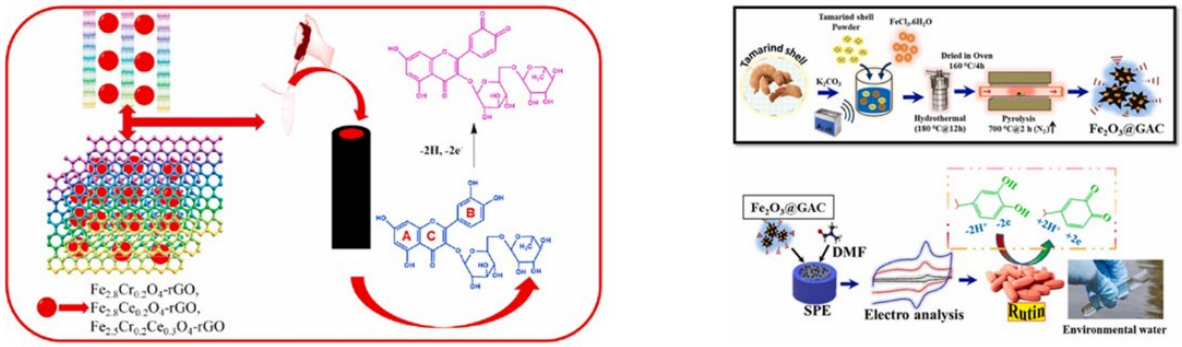


Figure 4. (**a**) The proposed structure of the magnetite-rGO composite materials and proposed electrochemical oxidation mechanism of rutin. Reprinted with permission from Ref. [26]. Copyright 2022, Elsevier. (**b**) Synthetic route of Fe_2O_3/GAC nanocomposite. Reprinted with permission from Ref. [27]. Copyright 2022, Elsevier.

4.1.9. Simultaneous Analysis of Multiple Water-Soluble Vitamins

Since various vitamins in the body have their own unique and important functions, it is of great significance to detect and analyze them [136–142]. Simultaneous detection of multiple vitamins can greatly improve detection efficiency, which is also a research hotspot in recent years. Based on the requirements of the electrochemical detection environment, vitamin samples that can be detected at the same time basically have similar solubility. There are two common detection and discrimination methods for different water-soluble vitamins. One is to rely on the difference of electrochemical response signals of a specific electrode material to different vitamins, such as different detection peak positions. The other is to select or design electrode materials with selectivity for each of several target vitamins and then combine them in a certain means to detect and discriminate their mixtures.

In B vitamins, VB2 and VB6 play an essential role in the normal function of the body and are interrelated, so Buleandra tried to detect and analyze them simultaneously by electrochemical method [51]. Preliminary cyclic voltammetry analysis showed that VB2 underwent a quasi-reversible electron transfer reaction in BR buffer with a pH of 5.0, the oxidation of VB6 was irreversible, and their electrochemical properties were of obvious differences. Therefore, PGE and SWV could be used to determine both of them simultaneously. For VB2, the two linear concentration ranges of 1.00×10^{-7}–5.00×10^{-5} M and 5.00×10^{-5}–7.50×10^{-4} M were achieved, and for VB6, the linear range was 2.50×10^{-5}–2.50×10^{-3} M. The detection limits of VB2 and VB6 were 7.38×10^{-8} M and 1.10×10^{-5} M, respectively. The method was further realized in the simultaneous determination of VB2 and VB6 in tablets. Using magnetite nanoparticles to improve the detection sensitivity of disposable electrochemical paper-based analysis equipment, Pereira et al. also realized simultaneous quantitative analysis of VB2 and VB6 (Figure 5a) [49]. The calibration plot for VB2 was in the range of 2.0 to 20.0 μM with a detection limit of 0.25 μM, and the calibration plot for VB6 was in the range of 0.2 to 2.0 mM with a detection limit of 29.5 μM.

Porada et al. used a nickel zeolite/carbon black modified GCE (NiZCBGCE) to determine VB2, VB9, VB12, and VB3 simultaneously (Figure 5b) [9]. Based on the enhancement of zeolite and carbon black on the vitamin reduction signal, the linear response range of vitamin detection was 0.008–0.24 mg L^{-1}, 0.004–0.22 mg L^{-1}, 0.003–0.1 mg L^{-1}, and 0.15–10 mg L^{-1}, and LOD was 2.3 μg L^{-1}, 1.3 μg L^{-1}, 1.092 μg L^{-1} and 45 μg L^{-1} for VB2, VB9, VB12, and VB3 respectively. Chitosan, in which nitrogen and sulfur co-doped graphene quantum dots are immobilized, was used to modify GCE for monitoring a variety of B vitamins [63]. Using the optimized parameters, vitamins B2, B6 and B12 were detected by SWV with detection limits of 0.30, 30.1, and 0.32 nM, respectively, and vitamins in energy drinks could be quantified.

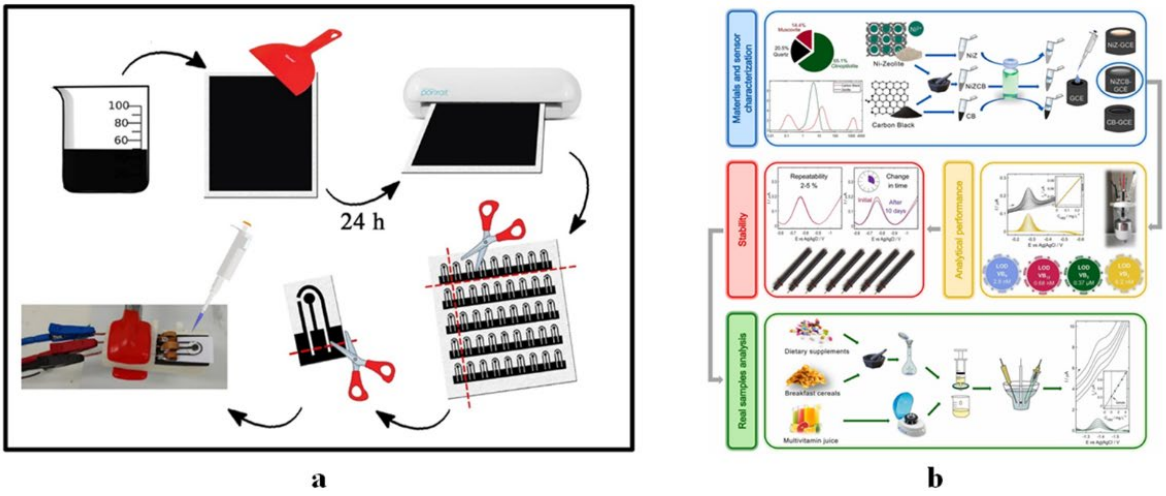


Figure 5. (**a**). Schematic representation of the production of Fe_3O_4 NPs-ePADs. Reprinted with permission from Ref. [49]. Copyright 2022, Elsevier. (**b**) The preparation of NiZCB-GCE and following experiments. Reprinted with permission from Ref. [9]. Copyright 2022, Elsevier.

4.2. Electrochemical Sensors for Fat-Soluble Vitamins

Like water-soluble vitamins, fat-soluble vitamins are essential nutrients in the human body and play an important role in human growth and development. It is also important to effectively detect their concentrations in the human body and supplementary nutrients. However, compared with water-soluble vitamins, there are relatively few electrochemical studies on fat-soluble vitamins. The use of various organic solvents requires higher chemical inertness of various devices, and the selection of organic materials is often limited by the physical and chemical properties of vitamins to be tested. Due to the non-polar characteristics of organic solutions, the charge movement is far inferior to the polar water environment. Therefore, the detection and exploration of various fat-soluble vitamins are still of great concern.

4.2.1. Vitamin D Sensors

Serum 25 hydroxyvitamin D (25-OHD) has been considered a new biomarker of vitamin D deficiency, and its concentration analysis is the focus of vitamin D-related electrochemical detection [143–145]. Immunological methods are often used for this purpose. Anusha et al. proposed a label-free impedimetric immunosensor, using a carbodiimide chemical method to covalently immobilize Ab-25-OHD antibody on GCN-b-CD@Au/GCE for specific recognition of 25-OHD in the serum (Figure 6a) [41]. The combination of antigen and antibody was detected by EIS, and the linear concentration range was 0.1–500 ng mL^{-1} with a LOD of 0.01 ng mL^{-1}. Zirconia nanoparticles modified with L-cysteine functionalized gold (Cys-Au@ZrO_2 NPs) were electrodeposited onto ITO substrate to enhance the electrochemical behavior, stability, and availability of covalent binding of functional groups and biomolecules (Figure 6b) [31]. Immunosensing analysis of 25-OHD3 could be realized by further modifying the electrode with its antibody (Ab-25-OHD3). Its sensitivity was increased to 2.01 μA ng^{-1} mL cm^{-2}, and the linear detection range and LOD were 1–50 ng mL^{-1} and 3.54 ng mL^{-1}, respectively. Polli et al. modified the graphite SPE with cysteamine-functionalized core-shell magnetic nanoparticles and then fixed the 25-OHD3 antibody through glutaraldehyde cross-linking [29]. Utilizing DPV, the linear detection range was between 7.4 and 70 ng mL^{-1}, and the LOD was 2.4 ng mL^{-1}.

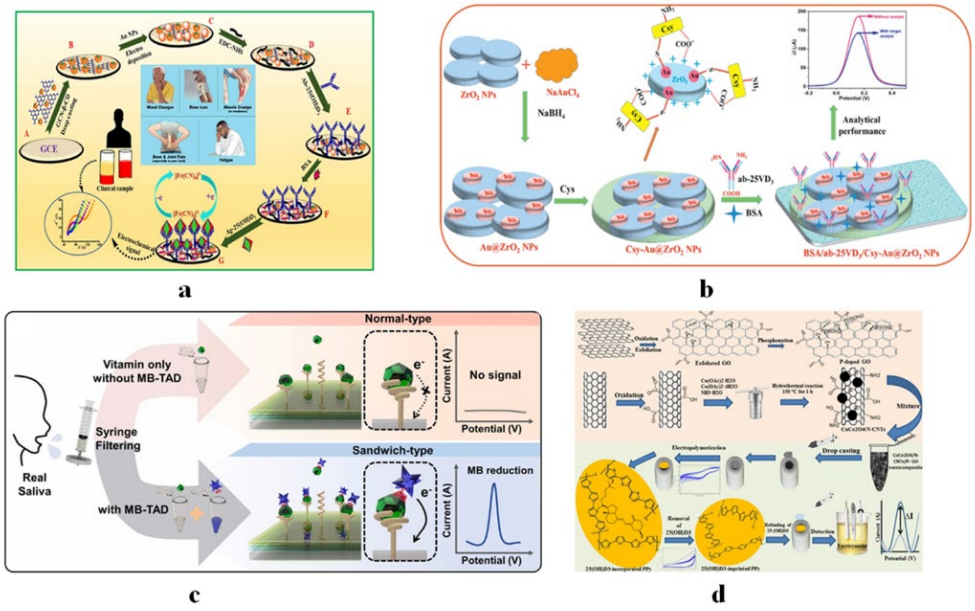


Figure 6. (**a**) Synthesis of GCN-b-CD nanocomposite. Reprinted with permission from Ref. [41]. Copyright 2022, Elsevier. (**b**) Schematic of BSA/Ab-25-OHD3/Cys-Au@ZrO_2/ITO immunoelectrode fabrication. Reprinted with permission from Ref. [31]. Copyright 2021, the Electrochemical Society. (**c**) Detection principle of 25-OHD3 in real saliva using MTES aptasensor. Reprinted with permission from Ref. [43]. Copyright 2022, Elsevier. (**d**) Preparation process of $CuCo_2O_4$/N-CNTs/P-GO nanocomposite and 25-OHD3-imprinted PPy and their application for designing an electrochemical sensor for detection of 25-OHD3. Reprinted with permission from Ref. [30]. Copyright 2022, International Union of Biochemistry and Molecular Biology.

Aptamers are also widely used in the highly sensitive and selective electrochemical analysis. Yin et al. fixed the DNA tetrahedron on the gold surface and then assembled the hairpin DNA probe-25-OHD3 complex [40]. Its electrochemical detection showed a wide linear range of 0.1–1000 nM, and the LOD was 0.026 nM. Park et al. prepared heterogeneous nanostructures composed of molybdenum disulfide and an electrochemically reduced graphene oxide composite to modify the electrode for amplifying the electrochemical signal (Figure 6c) [43]. Then, 1,2,4-triazol-3,5-dione (MB-TAD) labeled with methylene blue was modified on the electrode, where MB was used as the redox mediator to generate electrons, and TAD could combine with 25-OHD3 to form the aptamer/25-OHD3/MB-TAD complex. The binding of the aptamer to 25-OHD3 was detected by DPV. At the same time, 1,6-hexanedithiol was modified to avoid nonspecific adsorption and reduce the steric hindrance between aptamers. The linear range of 0.1–150 ng mL^{-1} could be obtained by electrochemical detection, and the LOD was 0.02 ng mL^{-1}.

Similarly, as an important specificity analysis technology, molecular imprinting is also used in vitamin D determination. GCE was modified with $CuCo_2O_4$/nitrogen-doped carbon nanotubes and phosphorus-doped oxygraphene nanocomposites and then coated by electropolymerization of 25-OHD3 imprinted polypyrrole to construct a specific recognition electrochemical sensor (Figure 6d) [30]. Using ferricyanide as the signal mediator, the signal was significantly reduced after recombining 25-OHD3 on the electrode, and the linear sensing detection range and LOD were 0.002–10 μM and 0.38 nM, respectively.

Vitamin D (such as VD3) can be directly detected by electrochemical analysis in biological samples [146–148]. For example, Bora et al. used nitrogen doped carbon nanotubes to improve the hydrophilicity and specific surface area of the working electrode, thereby improving the electrochemical detection performance [55]. The VD3 sensor based on it could provide high performance in the concentration range of 0–10 nM, with a LOD of 16 pM, and the sensitivity value 0.000495 mA cm^{-2} nM^{-1} was achieved.

4.2.2. Vitamin E Sensors

Jashari et al. used the SWV method to simultaneously detect three natural isomers of tocopherol (α, γ, and δ) [32]. Under optimized conditions, there were similar linear detection ranges (3.0×10^{-6}–1.0×10^{-5} M) for these isomers. For α, γ, and δ-tocopherol, quantification limits were 11.28, 2.70, and 3.67×10^{-6} M, and LODs were 3.72, 0.89, and 1.21×10^{-6} M, respectively. This method achieved the same detection effect as the popular chromatographic methods.

4.2.3. Vitamin K Sensors

Rostami-Javanroudi et al. modified PGE by electrodepositing silver nanoparticles and 2-amino-5chloro benzophenone [149]. Utilizing their electro-catalytic performance for the reduction of VK1, the sensor could achieve a linear detection range of 50–700 nM with an LOD of 16.58 nM, and it was applied in the quantifying of VK1 in human blood serums.

4.2.4. Simultaneous Analysis of Multiple Fat-Soluble Vitamins

Similar to water-soluble vitamins, it has outstanding application value in detecting multiple fat-soluble vitamins simultaneously [150,151]. Avan et al. modified GCE by using a β-cyclodextrin/multi-wall carbon nanotube and constructed an electrochemical sensor for the detection of various fat-soluble vitamins (VA, VD3, VE, and VK1) in an aqueous media of micellar solutions [65]. In the BR buffer at pH 5.0, the linear calibration curves of VA, VD3, VE, and VK1 could be obtained as 8–100, 0.8–60, 0.5–60, and 0.1–20 μM, respectively. The detection results have high reproducibility and selectivity.

4.3. Simultaneous Detection of Vitamins and Non-Vitamin Substances

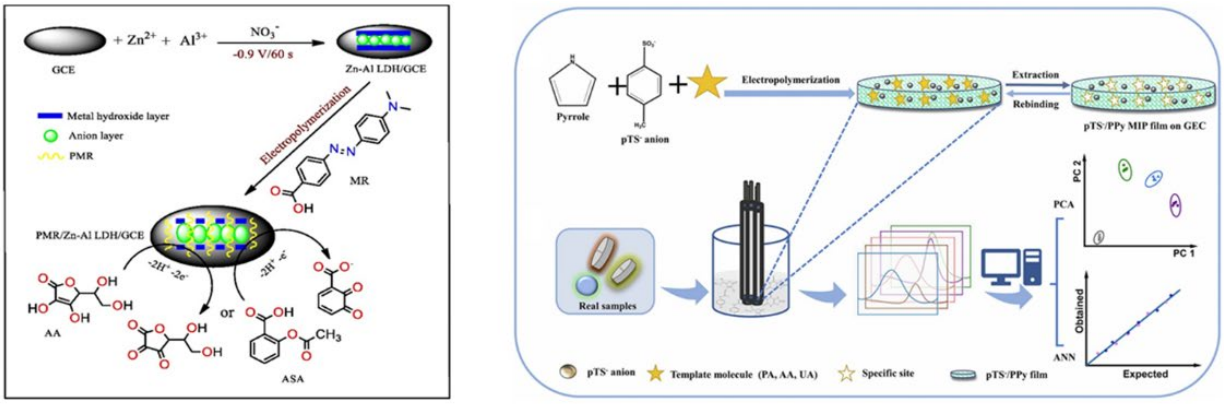
In actual biomedical samples (such as serum), besides vitamins, there are often many other substances related to health. Simultaneous quantitative analysis of biological components concerned is an important goal of inspection science, and it is also the goal of many new electrochemical sensors [152–160].

Based on Pt-Pd nanoparticles/chitosan/nitrogen-doped graphene (N-Gra) nanocomposite, Luo et al. established an electroanalytical method for the simultaneous detection of AA, sulfite, and oxalic acid (OA) [46]. The nanocomposites have remarkable electrochemical activity for the electrooxidation of sulfite and OA. At the same time, the full separation of multiple oxidation peaks ensured high electrochemical resolution, allowing simultaneous quantification of three substances of interest. The linear ranges of AA, sulfite, and OA measured by DPV were 2–400 μM, 8–600 μM, and 1.5–500 μM, respectively. Accordingly, the LODs were 0.97 μM, 5.5 μM, and 0.84 μM, respectively.

GCE modified by Zn-Al layered double hydroxide (Zn-Al LDH) and methyl red (PMR) polymer film (Figure 7a) [47] has a high electrocatalytic activity for the oxidation of AA and aspirin (ASA). There were well-spaced anodic peaks in the CV sensing detection spectrum, which could simultaneously identify trace AA and ASA. The anode peak current and the concentration of AA and ASA were of good linear range in 0.10–53.17 μM, and LODs were 1.26 and 1.27 μM, respectively. Meanwhile, the quantification limits of AA and ASA were 4.21 and 4.25 μM, respectively. On the other hand, according to the DPV method, the LODs of AA and ASA oxidation were 0.47 and 0.21 μM, respectively.

The GCE modified with sunset yellow (SY) food dye could be used to simultaneously detect AA, dopamine (DA), and uric acid (UA) [39]. Under the optimal conditions, the linear ranges of AA, DA, and UA electrochemical detection were 7–320, 0.2–45, and 0.2–50 μM, respectively, the LODs were low (4.78, 0.12, 0.12 μM, respectively), and the stability was high.

The electrochemical sensor constructed by molecular imprinted polymer (MIP) could realize the simultaneous determination of paracetamol, AA, and uric acid mixture in pharmaceutical samples (Figure 7b) [73]. Good linearity at μM level, LOD (1–24 μM), and repeatability could be obtained.



a **b**

Figure 7. (**a**) Schematic fabrication of the PMR/Zn-Al LDH/GCE for the determination of AA or ASA. Reprinted with permission from Ref. [47]. Copyright 2022, Wiley-VCH GmbH. (**b**) Preparation of the different pTS-/PPy MIPs by electropolymerization of Py in the presence of the template molecules and incorporation of the fabricated MIP-based sensors into the ET sensor array and its application to the analysis of APIs. Reprinted with permission from Ref. [73]. Copyright 2022, Elsevier.

A summary of various types of electrochemical vitamin sensors is shown in Table 2.

Table 2. A summary of electrochemical vitamin sensors.

Vitamin	Electrode	Technique	Medium	Linear Range	LOD	Application	Ref.
VB2	ZnO NPS-CPE	CV, SWV	PBS (pH 6)	0.005–10 μM	0.7 ± 0.01 nM	Beverage, milk	[12]
	Bi_2WO_6 (PVP + NaOH)/GCE	DPV	0.05 M PBS (pH 7.0)	0.03–457 μM	3.65 nM	Almond milk, soymilk	[11]
	Ru/S-GCN-SPCE	CV, DPV	0.1 M PBS (pH 7.0)	0.003–75 μM 95–260 μM	54.3 pM	Oral solution, syrup, tablets	[81]
VB6	IONCPE	DPV	0.1 M PBS (pH 6.0)	8.88–1000.0 μM	9.06 μM	Urine, pharmaceuticals	[37]
	Pt/β-CD-GR/PGE	DPV	0.1 M PBS (pH 7.0)	5–205 nM	1.2 nM	Juice	[36]
	P-doped/PGE	DPV	0.1 M PBS (pH 3.0)	0.5–300 μM	0.219 μM	Beverage	[90]
VB9	$ZnFe_2O_4$MNPs/SPE	DPV	0.1 M PBS (pH 7.0)	1.0–100.0 μM	0.3 μM	Tablets, urine	[99]
	GCE/f-MWCNT-$Ni(OH)_2$-Si4Pic$^+$Cl$^-$	DPV	PBS (pH 7.0)	0.5–26 μM	0.095 μM	Dietary supplement, fortifier compound, wheat flour	[100]
VB12	Copper oxide-GUITAR	LSV	Neutral (pH = 7) solutions	0.15–7378 nM	0.59 nM	Bacterial strains	[103]
	Mn-CPE	SWV	Acetate buffer (pH 4.6)	13.86–1500 ng L^{-1}	4.34 ng L^{-1}	Tablets, dietary supplements	[48]
VC	Pyrolytic graphite sheet	CV, SWV	KNO_3 (pH 7.0)	1.0–400 μM	0.4 μM	Extract of cultivated arugula	[50]
	Gr/NiHCF	CV, Amp *	0.01 M PBS (pH 7.4)	1–16,280 μM	0.25 μM	Supplements, fruit juices	[130]
	PBNPs/GCE	CV, Amp	PBS (pH 5)	1–1100 μM	0.47 μM	Domestic water, medicine samples	[131]

Table 2. *Cont.*

Vitamin	Electrode	Technique	Medium	Linear Range	LOD	Application	Ref.
VP	Co-BPDC-MOF/GCE	DPV, Amp	0.1 M PBS (pH 7)	0.5–1000 μM	0.03 μM	BROMEZER tablet	[134]
	Co/ZIF-C/GCE	DPV	BR buffer (pH 2.0)	0.1–30 μM	22 nM	Tablets	[135]
VD (25-OHD3)	Ab-25(OH)D3-Cys/Au/MoS_2/FTO	DPV	5 mM $[Fe(CN)_6]^{3-/4-}$ + 0.9% KCl (pH 7.4)	1 pg mL^{-1}–100 ng mL^{-1}	0.38 pg mL^{-1}	-	[143]
	Glut/Au-Pt/APTES/FTO	DPV	50 mM PBS (pH 7.4, 0.9% NaCl) + $[Fe(CN)_6]^{3-/4-}$	0.1 pg mL^{-1}–1 μg mL^{-1}	0.49 pg mL^{-1}	Serum	[145]
VD3	Co-Ag/PANI-PPY/IL/GCE	CV, SWV, Amp	PBS (pH 7)	0.0125–22.5 μM	0.0073 μM	Serum, urine	[146]
	Paper-sensor	CV, SWV, Amp	PBS (pH 7)	0.025–0.125 μM	0.015 μM	Serum, urine	[146]
	GQD-Au	EIS	0.1 M PBS (pH 7.0)	1–500 nM	0.70 nM	Serum	[147]
	CuNPs-NiNPs@reduced-fullerene-C60/GCE	CV, SWV	PBS (pH 7.0)	1.25–475 μM	0.0025 μM	Serum, urine	[148]

* Amp: Amperometry.

5. Microfabrication-Based Electrochemical Sensors for Vitamin Detection

Due to the application requirements of portable, wearable, and implantable vitamin detection devices, the research and development of small, high-precision electrochemical sensors based on microfabrication technology have attracted more and more attention [44]. Especially the integration of sample processing methods (such as microfluidics) and microsensors has greatly improved the analytical throughput, automation, accuracy, and practicability of vitamin detection.

5.1. Implantable Devices

Compared with in vitro electrochemical detection, in vivo analysis can reflect the dynamic change of vitamin levels in the human body in real-time, which is of great significance for clarifying the biological function of vitamins and understanding the occurrence and development of related diseases [161]. In order to explore the role of AA in Parkinson's disease (PD), Qu et al. used a carbon fiber electrode (CFE) as the matric electrode, coated graphene oxide by potentiostatic deposition, and then electrochemical reduction was performed to remove epoxy groups to accelerate the electron transfer of AA oxidation (Figure 8a) [66]. Meanwhile, an inner-reference signal was generated by increasing the carbonyl group through electrochemical oxidation. The biocompatible polyethylenedioxythiophene (PEDOT) was further rationally assembled to improve the antifouling performance of graphene so as to establish an AA electrochemical measurement platform with high sensitivity, good selectivity, and reproducibility, which could analyze AA levels in different regions of the brains of a living mouse.

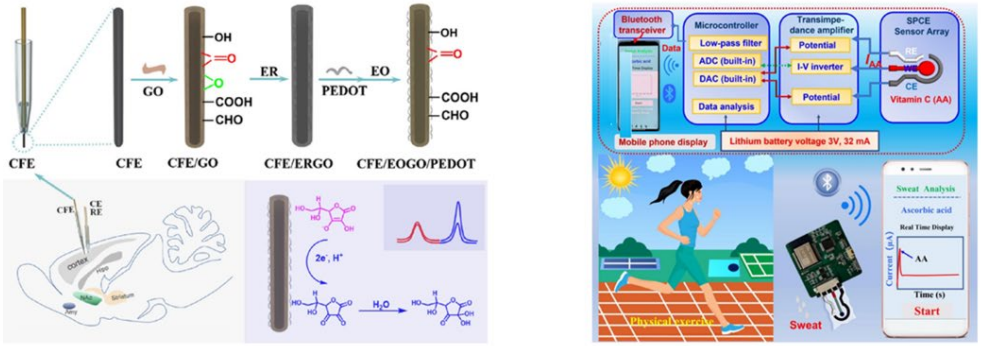


Figure 8. (**a**) Schematic illustration of the designed electrochemical platform for the ratiometric measurement of AA in a living mouse brain. Reprinted with permission from Ref. [66]. Copyright 2021, the American Chemical Society. (**b**) Block diagram of part of the circuitry in the portable enzyme-free AA detection system and the illustration showing the concept of non-invasive human health monitoring using this system with an integrated ASIC, $Ni_3(HITP)_2$/SPCE sensing electrode, and smartphone with App. Reprinted with permission from Ref. [162]. Copyright 2022, Elsevier.

5.2. Microfluidic Devices

The microfluidic technology, combining the microchannel network with sophisticated fluid control, can greatly improve the flexibility of liquid manipulation, automation, and analysis throughput and reduce sample consumption, cost, and environmental pollution. Combined with electrochemical sensing technology, it can effectively process vitamin samples, improve the sensitivity and selectivity of vitamin detection, and become an indispensable means for portable vitamin sensing applications. Stojanovic [22] et al. developed a small microfluidic platform for the selective detection of AA. A microfluidic channel was formed between the silver electrodes by xurography. In order to improve the conductivity of the device and enhance the electron transfer process, graphene sheets were deposited in the gap between the electrodes. With the increase of AA concentration, the total conductivity increased, resulting in the reduction of resistance parameters and the increase of capacitance parameters of the proposed equivalent circuit; thus, an obvious electrochemical signal could be captured.

Due to its low cost and high design flexibility, the paper-based microfluidic device has a good application prospect in electrochemical sensing. Pereira et al. used graphite powder, automotive varnish, and magnetite nanoparticles to make a conductive ink, which was used to develop a disposable electrochemical paper-based analysis equipment [49]. The magnetite nanoparticles in the conductive ink improved the sensitivity of the analytical equipment. In SWV detection, the linear range of VB2 in the BR buffer (pH 2.0) was 2.0–20.0 μM with a LOD of 0.25 μM. For VB6 in McIlvaine buffer solution (pH 4.0), the linear range was 0.2–2.0 mM with a LOD of 29.5 μM.

5.3. Portable Devices

Combined with the integrated circuit and microelectrode technology, miniature electrochemical sensors can be obtained for the development of portable devices. Wang et al. synthesized nanorods of the conductive compound Ni_3(2,3,6,7,10,11-hexaiminotriphenylene)$_2$ ($Ni_3(HITP)_2$) with a high degree of crystallinity from HITP ligands and Ni^{2+} ions and used them to modify SPE (Figure 8b) [162]. The electrode combines the two-dimensional superimposed honeycomb lattice of $Ni_3(HITP)_2$, the highly active Ni-N_4 catalytic site in the nanorods, and morphological characteristics of a large specific surface area. Their synergistic effect greatly improves the catalytic activity, and the electron transfer ability of the sensor could be greatly improved. A portable electrochemical AA detection system was developed by combining this microsensor with integrated circuits and smartphones. The system had good sensing and detection performance, including a wide linear range

(2–200 μM), high sensitivity (0.814 $\mu A \cdot \mu M^{-1} \cdot cm^{-2}$), and a low detection limit (1 μM). Pradhan et al. reported a fully organic 3-electrode sensor (O3ES) based on photolithographically micropatterned PEDOT:PSS and silk protein, which could simultaneously detect dopamine, AA, and uric acid using voltammetry [163]. This disposable point-of-care sensor could detect blood metabolites with ultralow sample volumes.

5.4. Wearable Devices

Wearable electrochemical vitamin detection equipment is promising in personal health fields. Ma et al. directly loaded the bifunctional polyaniline/reduced graphene oxide (PANI/RGO) film on the textile substrate so that the substrate has good capacitive and biosensing performance. Using this new textile substrate, they developed a wearable self-powered textile smart sensor that can be used for remote real-time detection of vitamin C [164]. Sempionatto et al. presented a skin-worn noninvasive electrochemical biosensor to detect sweat VC by immobilizing ascorbate oxidase on flexible, printable tattoo electrodes and monitoring its reaction [165].

6. Conclusions and Future Perspectives

Vitamins are indispensable nutrients in human life activities. Since most vitamins cannot be synthesized and stored in the body, a daily supplement is necessary. However, excessive intake of certain vitamins often brings new problems. Therefore, it is important to detect vitamin levels in the body and food sources. In order to detect vitamins in different types of samples quickly, accurately, and efficiently, electrochemical sensors with simple structure, low cost, high sensitivity, easy miniaturization, outstanding reliability, and reproducibility have attracted extensive attention. In this paper, the basic properties of various vitamins are reviewed, and the frequently-used electrodes, as well as the common electrochemical detection methods, are discussed in detail. For water-soluble and fat-soluble vitamins, the detection strategy and performance of corresponding electrochemical sensors are also introduced in detail. At the same time, for the great demand for vitamin detection in portability and real-time, the application of microfabrication and integrated electrochemical detection devices is discussed. In particular, the latest development of electrochemical sensors based on microfluidic technology, as well as portable, wearable, and implantable design, is described. Due to the increasing requirement for fast, efficient, and on-site detection, such sensors have significant application potential.

Although considerable progress has been made in the research of electrochemical detection of vitamins in recent years, there is still abundant room for improvement. Due to the lack of sufficient understanding of the sensing mechanism, the selection of appropriate electrodes and electrochemical detection methods still mainly depends on tedious experimental exploration, and the detection strategy of specific vitamins needs more explicit theoretical guidance. The implantable vitamin detection and POCT (point-of-care testing) devices are important development directions in the future. How to effectively combine microfluidic and other microanalysis technologies to improve the detection sensitivity of trace samples is an important demand for the development of such devices. Simultaneous analysis of multiple vitamins and other important biomarkers and effective quantification of vitamins in complex samples has significant potential in clinical disease screening, but the research and development of efficient, specific, and multi-purpose electroanalytical methods still face significant challenges. With the development of microfabrication and microfluidic technology, artificial intelligence, nanomaterials, and bio-recognition methods, these problems will eventually be solved, and convenient, efficient, and accurate vitamin detection will also play an increasingly important role in the future.

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