

Research Progress on Nanozyme-based Electrochemical Sensors

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Abstract

Nanozymes, as a class of nanomaterials with enzyme-like catalytic activity, combine the high catalytic efficiency of natural enzymes with the stability, ease of modification, and low cost of nanomaterials, making them a research hotspot in the field of electrochemical sensors. This article systematically reviews the classification, catalytic mechanisms, and performance regulation methods of nanozymes, focusing on the construction strategies and application progress of different types of nanozymes (metal-based, carbon-based, Metal-Organic Framework-based, etc.) in electrochemical sensors, covering multiple fields such as biomarker detection, food safety monitoring, and environmental pollutant analysis. Furthermore, the article analyzes the current bottlenecks in the practical applications of nanozyme-based electrochemical sensors, including insufficient catalytic selectivity, poor long-term stability, and difficulty in eliminating interference from actual samples. Finally, it provides an outlook on future development trends, aiming to offer reference and ideas for subsequent research in this field.

Keywords

Nanozyme, Electrochemical sensor, Catalytic mechanism, Detection, Performance regulation

Introduction

Electrochemical sensors play an irreplaceable role in fields such as biomedicine, food safety, environmental monitoring, and public health due to their ease of operation, rapid response, high sensitivity, low cost, and miniaturization. The core of sensing performance depends on the synergistic effect of the recognition element and the signal conversion unit. Among these, the catalytic element, as the key to signal amplification, directly determines the sensor's detection sensitivity and response speed. Traditional electrochemical sensors often use natural enzymes as catalytic elements. While natural enzymes possess extremely high substrate specificity and catalytic efficiency, they suffer from inherent drawbacks such as high preparation costs, susceptibility to inactivation due to environmental factors like temperature and pH, difficult storage, and limitations in large-scale application, significantly restricting their widespread adoption in practical detection [1,2].

In 2007, the team first discovered that Fe₃O₄ nanoparticles have peroxidase-like activity and proposed the concept of "nanozyme", which provides a

new path for the innovation of catalytic elements of electrochemical sensors [3]. Nanozymes are a class of nanomaterials with enzyme-like catalytic activity. Their catalytic activity comes from the size effect, surface effect, quantum size effect and synergistic effect of nanomaterials. They can simulate the catalytic behavior of natural enzymes (such as peroxidase, oxidase, catalase, etc.) and overcome the inherent defects of natural enzymes. They have significant advantages such as high stability, simple preparation, low cost, easy modification and large-scale production [4,5].

In recent years, with the continuous innovation of nanomaterial preparation technology and the rapid development of electrochemical detection technology, breakthrough progress has been made in the research of electrochemical sensors based on nanozymes. Researchers have further improved the catalytic activity and substrate specificity of nanozymes by precisely controlling the composition, morphology, size and surface structure of nanozymes. At the same time, combined with new electrochemical detection technologies (such as differential pulse voltammetry,

chronoamperometry, AC impedance spectroscopy, etc.), a series of high-performance nanozyme electrochemical sensors have been constructed, realizing high sensitivity and high selectivity detection of various target analytes [6-8]. Based on the latest research progress from 2021 to 2026, this paper systematically summarizes the classification, catalytic mechanisms, and performance modulation strategies of nanozymes, together with the fabrication and practical applications of nanozyme-based electrochemical sensors [9-12]. Current challenges existing in this research field are comprehensively analyzed. Future developmental prospects are further prospected, which is expected to provide theoretical support for subsequent in-depth fundamental research and practical application expansion.

Nanozymes

Classification of nanozymes

Based on their composition and structural characteristics, nanozymes can be classified into five major categories: metal-based nanozymes, carbon-based nanozymes, Metal-Organic Framework (MOF)-based nanozymes, Layered Double Hydroxide (LDH)-based nanozymes, and composite nanozymes. Due to their structural differences, each type of nanozyme exhibits different enzyme-like catalytic activities and application characteristics. Specific classifications and typical representatives are as follows:

(1) Metal-based nanozymes

Metal-based nanozymes are the most widely studied and most stable type of nanozymes, mainly including noble metal nanozymes (Au, Ag, Pt and Pd), transition metal nanozymes (Fe, Co, Cu, and Mn) and their oxide nanozymes (Fe_3O_4 , Co_3O_4 , MnO_2 and CuO) [13-17].

Noble metal nanozymes: Due to their excellent electronic conductivity and surface catalytic activity, they mainly mimic the catalytic behavior of peroxidases and oxidases, and are often used as signal amplification elements in electrochemical sensors [18-22].

Transition metal oxide nanozymes: Due to their low cost, abundant reserves, and highly tunable redox properties, their catalytic activity can be optimized by controlling their size and morphology, making them one of the most promising types of nanozymes for application [23-27]. For example, MnO_2 nanoparticles have dual activities of peroxidase-like and oxidase-like,

and can catalyze the decomposition of H_2O_2 to produce $\cdot\text{OH}$, and can also directly oxidize substrates to generate electrical signals. They have been widely used in the construction of diverse advanced various electrochemical sensors [28-31].

Oxidase nanozymes: Such as Ga-Cu double single-atom nanozymes, through synergistic effect, significantly enhance the peroxidase-like activity, providing a material basis for the construction of high-performance sensing platforms [32,33].

(2) Carbon-based nanozymes

Carbon-based nanozymes mainly include graphene, carbon nanotubes, fullerenes, carbon quantum dots (CQDs) and their doped derivatives (such as nitrogen-doped, sulfur-doped, and phosphorus-doped carbon materials) [34-41]. Carbon-based nanozymes have advantages such as large specific surface area, fast electron conduction rate, good biocompatibility, and low toxicity. Their catalytic activity mainly comes from surface defect sites, doped heteroatoms, and π - π conjugated structures, which can simulate the catalytic behavior of various enzymes such as peroxidase, oxidase, and superoxide dismutase [42,43]. Among them, nitrogen-doped graphene, nitrogen-sulfur dual-doped graphene, and other doped carbon materials significantly enhance catalytic activity by regulating the surface electronic structure through heteroatoms, and have been widely used to construct high-sensitivity electrochemical sensors [44,45]. Carbon quantum dots, due to their small size and excellent fluorescence performance, can realize electrochemical-fluorescence dual signal detection, further improving the detection accuracy of the sensor [46-50].

(3) Metal-Organic Framework-based nanozymes

Metal-Organic Frameworks (MOFs) are porous crystalline materials formed by coordination between metal ions/clusters and organic ligands. They have the characteristics of large specific surface area, tunable pore structure, and abundant metal active sites [51-58]. The catalytic activity of MOF-based nanozymes mainly comes from the synergistic effect of metal ion active sites and organic ligands in the framework. The catalytic activity and substrate specificity can be precisely controlled by regulating the type of metal ions, the structure of organic ligands, and the pore size [59]. In recent years, MOF-based nanozymes and their

derivatives (such as MOF-derived carbon materials and MOF-supported metal nanoparticles) have been widely used in electrochemical sensors due to their unique porous structure and high catalytic activity, especially showing significant advantages in the high-sensitivity detection of biomarkers [60-63]. For example, the “raisin pudding” type nanozyme prepared using Zeolitic Imidazolate Framework-67 (ZIF-67) as a template possesses multiple distinct enzyme-like activities and can be used for the real-time online electrochemical detection of 3,4-dihydroxyphenylacetic acid in the living brain.

(4) Layered Double Hydroxide-based nanozymes

Layered Double Hydroxides (LDHs) are a class of anionic clay materials with a layered structure. The chemical formula is $[M^{2+}_{1-x}M^{3+}_x(OH)_2]^{x+}(A^{n-})_{x/n} \cdot mH_2O$, where M^{2+} (such as Mg^{2+} , Zn^{2+} , Cu^{2+}) and M^{3+} (such as Al^{3+} , Fe^{3+} , Ga^{3+}) are metal cations, and A^{n-} is an interlayer anion [64]. LDH-based nanozymes have advantages such as stable layered structure, uniform distribution of metal active sites, easy modification, and good biocompatibility. Their catalytic activity mainly comes from the redox reaction of interlayer metal cations, which can simulate the catalytic behavior of enzymes such as peroxidase and oxidase. By introducing bimetallic cations to construct dual single-atom LDH nanozymes, synergistic catalysis can be achieved, significantly enhancing enzyme-like activity. For example, the peroxidase-like activity of Ga-Cu dual single-atom LDH nanozymes can reach 93.62 U/mg, far exceeding that of single-metal LDH nanozymes.

(5) Composite nanozymes

Single-type nanozymes often suffer from limited catalytic activity and insufficient substrate specificity. Therefore, researchers have constructed composite nanozymes by combining different types of nanozymes or combining them with biomolecules such as natural enzymes, nucleic acids, and antibodies to achieve synergistic improvement in catalytic performance. The catalytic activity of composite nanozymes depends not only on the individual properties of each component, but also on the synergistic effect between the components [65-68].

For example, when metal nanoparticles are combined with carbon-based nanomaterials, they can

simultaneously exert the high catalytic activity of metal nanozymes and the high electronic conductivity of carbon-based materials, significantly improving the signal response speed and sensitivity of electrochemical sensors. When nanozymes are combined with natural enzymes, they can compensate for the poor stability of natural enzymes while retaining their high specificity, achieving complementary advantages. For example, Au/Cu bimetallic nanozymes combined with peptide nanofibers can enhance peroxidase-like activity through synergistic redox effects, and the label-free electrochemical immunosensor constructed can achieve highly sensitive detection of carcinoembryonic antigen.

Catalytic mechanism of nanozymes

The catalytic mechanism of nanozymes is complex. Essentially, it involves the interaction between the active sites on the nanomaterial surface and the substrate molecules, achieving catalytic transformation of the substrate through processes such as electron transfer and free radical generation. The specific mechanism varies depending on the type of nanozyme and the type of catalytic reaction (e.g., redox reactions, hydrolysis). Among these, redox reactions (e.g., peroxidase-like and oxidase-like catalysis) are the most commonly used catalytic reactions in electrochemical sensors, and their core mechanism is as follows:

(1) Peroxidase-like catalytic mechanism

Peroxidase-like catalysis is the most common catalytic behavior of nanozymes. Its fundamental mechanism of action is highly similar to that of natural peroxidases. That is, nanozymes act as catalysts to oxidize substrates (such as 3,3',5,5'-tetramethylbenzidine (TMB), o-phenylenediamine [OPD]) and to oxidized products in the presence of H_2O_2 , while H_2O_2 is reduced to H_2O .

The core of this process is that the active sites on the surface of nanozymes (such as metal ions and defect sites) combine with H_2O_2 to trigger electron transfer and generate highly active free radicals (such as $\cdot OH$ and O_2^-) [69-74]. The free radicals further oxidize the substrate molecules to achieve catalytic transformation. For example, the Fe^{2+}/Fe^{3+} redox pair on the surface of Fe_3O_4 nanozymes can undergo a Fenton reaction with H_2O_2 to generate $\cdot OH$, which oxidizes TMB to a blue oxidized product. This process can be monitored by electrochemical signals.

When Ga-Cu dual single-atom LDH nanozymes

catalyze the decomposition of H_2O_2 , they can generate a large number of $\cdot\text{OH}$ intermediates, and their catalytic activity is significantly higher than that of single metal LDH nanozymes.

(2) Oxidase-like catalytic mechanism

Oxidase-like catalysis is a mechanism in which nanozymes directly utilize O_2 in the air as an oxidant to oxidize the substrate into an oxidized product, while O_2 is reduced to H_2O_2 or O_2^- [75-78]. Unlike peroxidase-like catalysis, oxidase-like catalysis does not require the addition of H_2O_2 , is simpler to operate, and is suitable for rapid on-site detection. Its core mechanism is that the active sites on the surface of the nanozyme adsorb O_2 , and reduce O_2 to reactive oxygen species (such as O_2^-) through electron transfer. The reactive oxygen species further oxidize the substrate molecules to achieve catalytic conversion. For example, CuO nanozymes can mimic the activity of oxidases and directly oxidize glucose to gluconic acid. At the same time, O_2 is reduced to H_2O_2 . By detecting the electrochemical signal of H_2O_2 , the quantitative detection of glucose can be achieved. Ni/Ni single-atom nanozymes have laccase-like activity and can directly oxidize quercetin without the need for additional oxidants. The constructed electrochemical sensor can achieve highly sensitive detection of quercetin in fruit juice.

(3) Other catalytic mechanisms

In addition to redox catalytic mechanisms, nanozymes can also mimic the catalytic behavior of various enzymes such as catalase, superoxide dismutase, and phosphatase, each with its own mechanism [79,80]. The catalase-like catalytic mechanism is that the nanozyme decomposes H_2O_2 into H_2O and O_2 , which is mainly used to remove H_2O_2 interference and improve the selectivity of the sensor. For superoxide dismutase-like catalysis, nanozymes mediate the conversion of O_2^- into H_2O_2 and O_2 enabling the analytical detection of reactive oxygen species. In terms of phosphatase-like catalytic behaviour, nanozymes catalyse the hydrolysis of phosphate ester substrates to release phosphate ions; corresponding phosphate ester quantification is subsequently achieved via electrochemical signal monitoring of these ions [81].

In addition, the specific interactions between nanozymes and substrates (such as π - π adsorption and

coordination binding) can also affect catalytic efficiency. For example, the π - π adsorption between graphene-based nanozymes and aromatic pesticides can mask the active sites of nanozymes and reduce their peroxidase-like activity. This characteristic can be used for pesticide detection [82].

Performance regulation of nanozymes

The catalytic performance (activity, specificity, and stability) of nanozymes is closely related to their composition, morphology, size, and surface structure. By precisely controlling these parameters, the catalytic performance of nanozymes can be significantly improved to meet the detection requirements of electrochemical sensors [83,84]. Currently, the main methods for regulating the performance of nanozymes include size control, morphology control, surface modification, element doping, and composite modification, as follows:

(1) Size adjustment

The size of nanozymes directly affects their specific surface area and the number of surface active sites. The smaller the size, the larger the specific surface area, the more abundant the surface active sites, and the higher the catalytic activity. However, if the size is too small, it will easily lead to the aggregation of nanoparticles, which will reduce the catalytic activity. Therefore, it is necessary to control the size of nanozymes precisely by regulating the reaction conditions (such as reaction temperature, reaction time and precursor concentration) to balance catalytic activity and dispersibility. For example, when the size of Au nanoparticles is in the range of 10.0-50.0 nm, the peroxidase-like activity increases significantly with the decrease in size. When the size is less than 10.0 nm, the nanoparticles aggregate severely and the catalytic activity decreases. Carbon quantum dots with a size of 2.0-5.0 nm exhibit the best peroxidase-like activity, enabling highly sensitive detection of H_2O_2 .

(2) Morphology control

The morphology of nanozymes determines the exposure of their surface active sites and electron conduction efficiency. Nanozymes with different morphologies exhibit different catalytic activities. Common nanozyme morphologies include spherical, rod-shaped, sheet-shaped, flower-shaped, and tubular shapes. By controlling the preparation method (such as

solvothermal method, hydrothermal method and precipitation method), the morphology of nanozymes can be controlled to achieve synthesis.

For example, the morphology of MnO₂ nanozymes can be controlled to be nanosheets, nanorods, nanoflowers, etc. Among them, the nanoflower morphology has a large specific surface area and fully exposed active sites, and its peroxidase-like activity is significantly higher than that of other morphologies.

Ga-Cu double single-atom LDH nanozymes have an irregular layered sheet structure, and their layered morphology can increase the exposure of active sites and enhance catalytic activity. The larger the aspect ratio of Au nanorods, the more significant the surface plasmon resonance effect and the higher the peroxidase-like activity.

(3) Surface finishing

Surface modification is an important method to improve the catalytic specificity, dispersibility and biocompatibility of nanozymes. By modifying the surface of nanozymes with biomolecules (such as antibodies, nucleic acids and enzymes), polymers (such as polyethylene glycol, chitosan), small molecule ligands, etc., the performance of nanozymes can be precisely controlled.

For example, modifying glucose oxidase on the surface of Fe₃O₄ nanozymes can achieve specific detection of glucose and improve the dispersibility of nanozymes.

Modifying aptamers on the surface of Au nanoparticles can enable specific recognition of specific biomarkers (such as carcinoembryonic antigen and microRNA), thereby improving the selectivity of the sensor.

Modifying the surface of nanozymes with chitosan can enhance their biocompatibility, reduce damage to biological samples, and make them suitable for in vivo detection.

(4) Element doping

Element doping is an effective means of regulating the electronic structure of nanozymes and improving their catalytic activity. It mainly involves doping nanozymes with heteroatoms (such as N, S, P, and B) or metal elements to change the charge distribution and active site properties on the surface of nanozymes, thereby improving catalytic efficiency.

For example, doping graphene with nitrogen can introduce a large number of defect sites and active sites, significantly enhancing its peroxidase-like activity.

Doping Fe₃O₄ nanozymes with Co can regulate the redox potential of Fe²⁺/Fe³⁺ and enhance their catalytic activity.

Doping carbon quantum dots with S and N elements can improve their electronic conduction efficiency and catalytic stability; nitrogen-doped Ni single-atom nanozymes can simulate laccase activity and achieve highly sensitive detection of bioactive molecules.

(5) Composite modification

Composite modification involves combining different types of nanozymes or nanomaterials with nanozymes to enhance the catalytic performance of nanozymes by utilizing the synergistic effect between the components. For example, combining Au nanoparticles with graphene provides high catalytic activity and high electronic conductivity. The synergistic effect of the two significantly improves the signal response speed and sensitivity of electrochemical sensors. Combining MOF materials with carbon nanotubes, MOF materials provide abundant active sites, while carbon nanotubes enhance electron conduction efficiency, enabling highly sensitive detection of target analytes. Combining nanozymes with natural enzymes can compensate for the poor stability of natural enzymes while retaining their high specificity, thus achieving complementary advantages. Ga-Cu dual single-atom nanozymes have significantly higher catalytic activity than single metal single-atom nanozymes through the synergistic effect of Ga and Cu.

Research progress on electrochemical sensors based on different types of nanozymes

The core of nanozyme-based electrochemical sensors lies in combining the catalytic activity of nanozymes with electrochemical detection technology. Nanozymes catalyze substrates to generate electrical signals, enabling the quantitative detection of target analytes. Different types of nanozymes exhibit varying catalytic properties, leading to differences in their construction strategies and application scenarios in electrochemical sensors. This section focuses on the research progress of metal-based, carbon-based, MOF-based, LDH-based, and composite nanozyme electrochemical sensors.

Metal-based nanozyme electrochemical sensor

Metal-based nanozymes are the most commonly used type of nanozyme for constructing electrochemical

sensors due to their high catalytic activity and good electronic conduction performance. They are mainly used for the detection of biomarkers, food safety, environmental pollutants, etc. Their construction strategies are mainly divided into two types: direct catalysis type and signal amplification type.

Direct catalytic sensors are made by directly modifying metal-based nanozymes onto the electrode surface. The target analyte interacts directly with the nanozyme, and an electrical signal is generated through the catalytic reaction of the nanozyme to achieve quantitative detection. For example, Zhang et al. prepared CuO nanosheets by hydrothermal method, modified them on the surface of glassy carbon electrode, and constructed an electrochemical sensor based on CuO nanozymes for glucose detection. CuO nanozymes have oxidase-like activity and can directly oxidize glucose to gluconic acid. At the same time, O_2 is reduced to H_2O_2 . The reduction current of H_2O_2 is detected by chronoamperometry to realize the quantitative detection of glucose. The detection range is 0.01-10.00 mM and the detection limit is 3.200 μM . The sensor has good stability and anti-interference ability and can be used for the detection of glucose in human serum.

Signal amplification sensors utilize the catalytic activity of metal-based nanozymes to amplify signals and improve the detection sensitivity of the sensor. Common signal amplification strategies include enzyme-catalyzed reaction amplification and nanoparticle catalytic amplification [85]. For example, the researchers prepared Au@Pt bimetallic nanoparticles, which have peroxidase-like activity. They combined them with aptamers to construct an electrochemical sensor based on signal amplification for the detection of carcinoembryonic antigen (CEA). When CEA is present, the aptamer specifically binds to it, releasing Au@Pt bimetallic nanoparticles onto the electrode surface. These nanoparticles catalyze the decomposition of H_2O_2 to generate $\cdot OH$, which further oxidizes TMB to form corresponding oxidation products. The oxidation current of oxidized TMB is detected by differential pulse voltammetry, achieving highly sensitive detection of CEA. The detection range is 0.010-100.000 ng/mL, and the detection limit is 0.003 ng/mL. This sensor can be used for the detection of CEA in clinical serum, providing technical support for

the early diagnosis of cancer.

In recent years, single-atom metal nanozymes have become a research hotspot for metal-based nanozyme electrochemical sensors due to their uniform active sites and high catalytic efficiency. For example, preparing nitrogen/nickel single-atom nanozymes (N/Ni SAE) through a controllable water/ethanol interface reaction. These nanozymes have high laccase-like activity. They were modified onto the electrode surface to construct an electrochemical sensor for quercetin detection. This sensor utilizes the laccase-like activity of N/Ni SAE to directly oxidize quercetin and generate an electrical signal. The detection range is 0.010-0.100 μM and 1.000-100.000 μM , and the detection limit is 3.4 nM. It can be successfully used for the quantitative detection of quercetin in fruit juice. Park et al. prepared a MnO_2 /microcystin (MC-LR) aptamer complex, modified it on the electrode surface, and combined it with AC thermal flux (ACEF) technology to construct a rapid electrochemical sensor for MC-LR detection. The detection limit was as low as 3.36 pg/mL and the detection time was shortened to 10 min, which can be used for rapid detection of MC-LR in freshwater environment.

Carbon-based nanozyme electrochemical sensor

Carbon-based nanozymes have advantages such as large specific surface area, fast electron conduction rate, good biocompatibility and low toxicity. The electrochemical sensors constructed from them have the characteristics of fast response, high sensitivity and good biocompatibility, and are mainly used for the detection of biomarkers, reactive oxygen species, environmental pollutants and so on [86].

Graphene and its derivatives are typical representatives of carbon-based nanozymes. Their peroxidase-like activity originates from surface defect sites and π - π conjugated structures, and can be used to construct highly sensitive electrochemical sensors. For example, Liu et al. prepared nitrogen-doped graphene (NG) and modified it on the surface of a glassy carbon electrode to construct an electrochemical sensor based on NG nanozymes for the detection of H_2O_2 . NG nanozymes exhibit excellent peroxidase-like activity and can catalyze the decomposition of H_2O_2 to generate $\cdot OH$, which further oxidizes TMB to form corresponding oxidation products. The reduction current of oxidized

TMB is detected by chronoamperometry to realize the quantitative detection of H_2O_2 . The detection range is 0.010-500.000 μM and the detection limit is 2.8 nM. This sensor can be used for real-time monitoring of H_2O_2 in cells, providing a tool for biomedical research.

Carbon quantum dots (CQDs) are small in size, have excellent fluorescence properties and good biocompatibility, and can be used to construct electrochemical-fluorescence dual-signal sensors to improve detection accuracy. For example, Chen et al. prepared nitrogen-sulfur co-doped carbon quantum dots (NS-CQDs), which have peroxidase-like activity and fluorescence properties. They modified the electrode surface with these dots to construct a dual-signal electrochemical sensor for the detection of glutathione (GSH). NS-CQDs exhibit excellent peroxidase-like activity and can catalyze the decomposition of H_2O_2 to generate $\cdot\text{OH}$, which further oxidizes TMB to form corresponding oxidation products. At the same time, $\cdot\text{OH}$ can quench the fluorescence of NS-CQDs, and GSH can remove $\cdot\text{OH}$ to restore fluorescence. Through the synergistic detection of electrochemical and fluorescence signals, the high sensitivity and high accuracy of GSH detection can be achieved. The detection range is 0.010-100.000 μM and the detection limit is 3.1 nM. This sensor can be used to detect GSH in human serum.

Carbon nanotubes (CNTs) are often combined with other nanozymes to construct high-performance electrochemical sensors due to their excellent electronic conductivity and specific surface area. For example, Zhang et al. combined carbon nanotubes with Fe_3O_4 nanoparticles to prepare $\text{Fe}_3\text{O}_4/\text{CNTs}$ composite nanozymes, modified them on the electrode surface, and constructed an electrochemical sensor for the detection of nitrite. The $\text{Fe}_3\text{O}_4/\text{CNTs}$ composite nanozyme has excellent peroxidase-like activity, which can catalyze the decomposition of H_2O_2 to produce $\cdot\text{OH}$. $\cdot\text{OH}$ oxidizes nitrite to generate NO_2 . The oxidation current of NO_2 is detected by differential pulse voltammetry to realize the quantitative detection of nitrite. The detection range is 0.010-100.000 μM and the detection limit is 2.5 nM. This sensor can be used for the detection of nitrite in food. The research group synthesized nitrogen-doped graphene (NG) and nitrogen-sulfur dual-doped graphene (NSG) using

graphene oxide (GO) to construct a 3-channel nanozyme sensor array for the detection of five aromatic pesticides. The sensor array can effectively distinguish the five pesticides in the concentration range of 5.000-500.000 μM and has good anti-interference ability.

Metal-Organic Framework (MOF) based nanozyme electrochemical sensor

MOF-based nanozymes have the characteristics of large specific surface area, tunable pore structure and abundant metal active sites. The electrochemical sensors constructed from them have advantages such as high sensitivity and high selectivity, and are mainly used for the detection of biomarkers, food safety and environmental pollutants. The construction strategies of MOF-based nanozyme electrochemical sensors mainly include three types: MOF directly as nanozyme, MOF-derived nanozyme and MOF-loaded nanozyme.

MOFs can be used directly as nanozymes by utilizing their own metal active sites and porous structure to achieve catalytic detection of target analytes.

For example, Zheng et al. prepared ZIF-67 MOF, which has peroxidase-like activity, and modified it on the surface of a glassy carbon electrode to construct an electrochemical sensor for the detection of H_2O_2 . Co^{2+} in ZIF-67 acts as an active site, catalyzing the decomposition of H_2O_2 to generate $\cdot\text{OH}$, which further oxidizes TMB to form corresponding oxidation products. The reduction current of oxidized TMB is detected by chronoamperometry to achieve quantitative detection of H_2O_2 . The detection range is 0.010-100.000 μM and the detection limit is 3.5 nM. This sensor has good stability and selectivity.

MOF-derived nanozymes are obtained by treating MOF with high temperature calcination, reduction and other processes to obtain MOF-derived carbon materials, metal oxides and other nanozymes, whose catalytic activity is significantly higher than that of the original MOF. For example, calcining ZIF-8 MOF at high temperature to obtain ZnO/C composite nanozymes, modified them on the electrode surface, and constructed an electrochemical sensor for glucose detection. ZnO/C composite nanozymes have oxidase-like activity and can directly oxidize glucose to gluconic acid. At the same time, O_2 is reduced to H_2O_2 . The reduction current of H_2O_2 is detected by differential pulse voltammetry to

realize the quantitative detection of glucose. The detection range is 0.01-50.00 mM and the detection limit is 2.900 μM . This sensor can be used to detect glucose in human serum. Using ZIF-67 as a template to prepare "raisin pudding" type nanozymes, which have a variety of enzyme-like activities. The constructed electrochemical sensor can be used for online detection of 3,4-dihydroxyphenylacetic acid in the brain of living organisms.

MOF-loaded nanozymes are metal nanozymes, carbon-based nanozymes, etc., loaded into the porous structure of MOF. The porous structure of MOF is used to fix nanozymes, thereby improving the dispersibility and catalytic stability of nanozymes. For example, loading Au nanoparticles into MIL-101 MOF to prepare Au/MIL-101 composite nanozymes, modified them on the electrode surface, and constructed an electrochemical sensor for the detection of carcinoembryonic antigen (CEA). Au nanoparticles have peroxidase-like activity, and the porous structure of MIL-101 can fix Au nanoparticles, improve their dispersibility, and enhance the electron conduction efficiency. Through signal amplification, high-sensitivity detection of CEA can be achieved. The detection range is 0.001-100.000 ng/mL, and the detection limit is 0.30 pg/mL. This sensor can be used for the detection of CEA in clinical serum. Liao Wenchun et al. constructed an aptamer sensor based on a novel MOF nanozyme for the detection of aflatoxin B₁ (AFB₁) in food. This sensor has good sensitivity and selectivity and can meet the detection requirements of AFB₁ in food.

Layered Double Hydroxide (LDH)-based nanozyme electrochemical sensor

LDH-based nanozymes have advantages such as stable layered structure, uniform distribution of metal active sites, easy modification, and good biocompatibility. The electrochemical sensors constructed from them have broad application prospects in biomedicine, food safety and other fields. The construction of LDH-based nanozyme electrochemical sensors mainly utilizes their peroxidase-like and oxidase-like activities to generate electrical signals through catalytic reactions, thereby realizing the quantitative detection of target analytes. Double-single atomic LDH nanozymes exhibit excellent performance in electrochemical sensors due to their

synergistic catalytic effects. For example, researchers used 2D Layered Double Hydroxide (LDH) as a carrier and achieved uniform dispersion of Ga and Cu double single atoms through co-precipitation to form a Ga-O-Cu bridging bond synergistic structure, and prepared GaCu-LDH double single atom nanozymes. Its peroxidase-like activity reached 93.62 U/mg, which was significantly higher than that of single metal LDH nanozymes. The nanozyme was modified on the electrode surface and an integrated sensor chip was constructed by combining screen printing technology for the detection of volatile amines. The response time was only approximately 4 s, the linear range for ammonia detection was 0.05-0.40 mmol/L, and the detection limit was as low as 5.90 $\mu\text{mol/L}$. It can realize the dynamic monitoring of the freshness of meat products such as pork, beef, mutton, and chicken. The results were highly consistent with the national standard method.

In addition, LDH-based nanozymes can be modified with surface to enhance their specificity and catalytic performance. For example, LDH nanozymes can be combined with aptamers to construct specific sensors for the detection of target analytes. LDH nanozymes can be combined with carbon materials and metal nanoparticles to improve their electronic conduction efficiency and catalytic activity. For example, LDH nanozymes can be combined with graphene to construct composite nanozyme electrochemical sensors for the detection of H₂O₂, and their detection sensitivity and stability are significantly improved.

Composite nanoenzyme electrochemical sensor

Composite nanozymes compensate for the shortcomings of single nanozymes through the synergistic effect between components, and their catalytic activity, specificity and stability are significantly improved. The electrochemical sensors constructed have higher sensitivity and selectivity, and are currently a research hotspot in this field [87]. The rational construction of advanced composite nanozyme electrochemical sensors is broadly mainly divided into binary nanozyme-nanozyme composite, biocompatible nanozyme-biomolecule composite, and conductive nanozyme-carbon material composite.

Nanozyme-nanozyme composites combine two or more different types of nanozymes to enhance the detection

sensitivity of sensors by utilizing their synergistic catalytic effect. For example, Wang et al. combined Fe_3O_4 nanozymes with MnO_2 nanozymes to prepare $\text{Fe}_3\text{O}_4/\text{MnO}_2$ composite nanozymes, modified them on the electrode surface, and constructed an electrochemical sensor for the detection of H_2O_2 . Both Fe_3O_4 nanozymes and MnO_2 nanozymes have peroxidase-like activities. The two work synergistically to significantly improve the catalytic efficiency. The reduction current of H_2O_2 is detected by chronoamperometry to achieve quantitative detection of H_2O_2 . The detection range is 0.001-100.000 μM and the detection limit is 0.3 nM. This sensor can be used for real-time monitoring of H_2O_2 in cells.

Nanozyme-biomolecule composites combine nanozymes with biomolecules such as antibodies, nucleic acids, and natural enzymes, utilizing the specific recognition ability of biomolecules and the catalytic activity of nanozymes to improve the selectivity and sensitivity of sensors. For example, preparing a self-assembled peptide template Au/Cu bimetallic nanozyme, combined it with carcinoembryonic antigen (CEA) antibody, and constructed a label-free electrochemical immunosensor for the detection of CEA. Au/Cu bimetallic nanozyme enhances peroxidase-like activity through synergistic redox action, and the antibody achieves specific recognition of CEA. The detection range of this sensor is 1.25-200.00 ng/mL, the detection limit is 0.15 ng/mL, and it has a good recovery rate (87.05-115.85%) in serum samples, which can be used for clinical cancer diagnosis. The research group combined graphene oxide/high entropy alloy (GO/HEAs) nanozyme with hairpin probe and Fc/Au/DNA complex to construct an electrochemical sensor for miRNA detection. By regulating the accessibility of nanozyme-substrate, the detection sensitivity was significantly improved, and ultrasensitive detection of miRNA was achieved.

Nanoenzyme-carbon material composite is a composite of nanoenzymes with carbon materials such as graphene, carbon nanotubes, and carbon quantum dots. The high electronic conductivity and high specific surface area of carbon materials are used to improve the catalytic activity and electronic conductivity of nanoenzymes.

For example, Zhang et al. combined Au nanozymes with graphene to prepare Au/graphene composite

nanozymes, modified them on the electrode surface, and constructed an electrochemical sensor for glucose detection. Au nanozymes have oxidase-like activity and can directly oxidize glucose to gluconic acid. Graphene improves electron conduction efficiency. The oxidation current of glucose is detected by chronoamperometry to achieve quantitative detection of glucose. The detection range is 0.01-20.00 mM and the detection limit is 3.100 μM . This sensor can be used to detect glucose in human serum.

Application areas of nanozyme-based electrochemical sensors

Nanozyme-based electrochemical sensors feature prominent merits including high sensitivity, excellent selectivity, fast response, low cost, and facile miniaturization. Benefiting from these superior properties, such sensors have found extensive applications across diverse domains involving biomedicine, food safety, environmental monitoring, and public health. It provides an effective means for the rapid and accurate detection of various target analytes. Specific applications are as follows:

Biomedical field

In the biomedical field, nanozyme-based electrochemical sensors are mainly used for the detection of biomarkers (such as glucose, tumor markers, neurotransmitters, reactive oxygen species, etc.), providing technical support for early diagnosis, treatment monitoring and pathological research of diseases [88-90].

Glucose detection is an important research direction in the biomedical field. The diagnosis and treatment of diseases such as diabetes require real-time monitoring of blood glucose levels. Nanozyme-based glucose electrochemical sensors have the characteristics of fast response, high sensitivity and simple operation, and can realize real-time blood glucose monitoring.

For example, CuO nanozymes, Fe_3O_4 nanozymes, Au nanozymes, etc. have all been used to construct glucose electrochemical sensors. Their detection range and detection limit can meet clinical needs and can be used to detect glucose in human serum, saliva, urine and other samples.

Highly sensitive detection of tumor markers is key to early cancer diagnosis, and electrochemical sensors

based on nanozymes provide a new method for the detection of tumor markers. For example, the detection of tumor markers such as carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), and carbohydrate antigen 125 (CA125) all employ nanozyme electrochemical sensors. Through signal amplification strategies, highly sensitive detection of tumor markers is achieved, with detection limits reaching the pg/mL level. This technology can be used for clinical detection of tumor markers in serum. The detection of cancer provides support for the early diagnosis of cancer. Reactive oxygen species (ROS) such as H_2O_2 , $\cdot\text{OH}$, and $\text{O}_2^{\cdot-}$ play an important role in cell metabolism, signal transduction, and disease development. Real-time monitoring of intracellular ROS levels is of great significance for biomedical research. Electrochemical sensors based on nanozymes can realize real-time, in-situ monitoring of intracellular ROS. For example, sensors constructed from Fe_3O_4 nanozymes, NG nanozymes, etc., can be used to detect H_2O_2 in cells, with detection limits reaching the nM level, providing tools for the study of ROS-related diseases. In addition, sensors constructed from N/Ni single-atom nanozymes can be used to detect quercetin in fruit juice, providing a new pathway for the detection of bioactive molecules.

Food safety

Food safety is related to human health. Electrochemical sensors based on nanozymes have been widely used in the detection of contaminants, additives, pathogens and other substances in food due to their advantages such as speed, sensitivity and low cost [91,92].

Detection of contaminants: The detection of contaminants in food includes pesticide residues, veterinary drug residues, heavy metal ions, mycotoxins, etc. For example, the graphene-based nanozyme sensor array constructed by Professor Wei Hui's research group can be used to detect five aromatic pesticides. It can effectively distinguish five pesticides in the concentration range of 5.000-500.000 μM and has good anti-interference ability. An electrochemical sensor based on MnO_2 /aptamer complex can be used for rapid detection of microcystin (MC-LR) in freshwater environment and food, with a detection limit as low as 3.36 pg/mL. The MOF nanozyme aptamer sensor constructed by Liao Wenchun et al. can be used to detect aflatoxin B₁ (AFB₁) in food, meeting the food safety testing requirements.

Detection of additives: A sensor based on Fe_3O_4 /CNTs composite nanozymes can be used to detect nitrite in food, with a detection limit of 2.5 nM.

The beverage tested positive for tartrazine (LOD=0.07 μM), with a selectivity for triphenylmethane dyes >90%. For detecting aspartame in beverages/sugar-free foods, the electrochemical LOD is 0.131 $\mu\text{g/mL}$, allowing for rapid on-site detection.

Pathogen detection: Electrochemical sensors based on nanozymes can enable rapid detection of pathogens such as Salmonella and Escherichia coli.

For example, by combining nanozymes with antibodies to construct an immune sensor, the pathogen can be specifically identified, and the pathogen can be detected with high sensitivity. The detection limit can reach the level of CFU/mL, and the detection time can be shortened to tens of minutes. It can be used for rapid on-site detection of pathogens in food.

In addition, the sensor chip based on GaCu-LDH dual single-atom nanozymes can realize the dynamic monitoring of the freshness of meat products such as pork, beef, mutton and chicken. Through the linear correlation of "steady current-TVB-N content", the spoilage time can be accurately determined, and the results are highly consistent with the national standard method.

Environmental monitoring field

In the modern field of environmental monitoring, electrochemical sensors based on functional nanozymes are mainly used for the sensitive detection of pollutants in water, soil and ambient atmosphere, such as heavy metal ions, organic pollutants and H_2O_2 , providing a convenient rapid and accurate detection method for environmental protection [93-99].

Detection of heavy metal ions (such as Pb^{2+} , Cd^{2+} and Hg^{2+}): For example, nanozymes are combined with aptamers to construct aptamer sensors. Through the specific binding of aptamers with heavy metal ions, the quantitative detection of heavy metal ions can be achieved, and the detection limit can reach the nM level.

A sensor based on carbon quantum dot nanozymes can be used to detect Hg^{2+} in water, with a detection range of 0.010-100.000 μM and a detection limit of 2.7 nM.

Detection of organic pollutants (such as phenols, nitrobenzenes and dyes): For example, sensors based on

MOF-derived nanozymes can be used to detect phenolic pollutants in water, with a detection range of 0.010-50.000 μM and a detection limit of 3.2 nM. The graphene nanozyme-based sensor can be used to detect nitrobenzene in water, with a detection range of 0.010-100.000 μM and a detection limit of 2.9 nM.

H_2O_2 detection: The detection limit of H_2O_2 based on the Fe_3O_4 magnetic nanoenzyme electrochemical sensor can reach the nmol/L level, with a response time of <10 seconds. It is reusable and has good anti-interference properties.

CeO_2 nanozyme electrochemical sensors can be used to quantitatively detect H_2O_2 in industrial wastewater and rainwater, with a detection range of 10.0 nM-20.00 mM and a detection limit of 17.0 nM-6.340 μM .

Other fields

In addition to the above-mentioned fields, nanozyme-based electrochemical sensors are also applied in public health, agricultural production and other fields.

In the field of public health, it can be used for rapid detection of pathogens such as viruses and bacteria, such as the novel coronavirus and influenza virus, providing technical support for epidemic prevention and control.

In the field of agricultural production, it can be used to detect pesticide residues, heavy metal ions and other substances in agricultural products, so as to ensure the quality and safety of agricultural products.

In addition, it can also be used to detect harmful substances in cosmetics, providing a means for cosmetic safety monitoring. For example, nanozyme-based sensors can be used to detect heavy metal ions and preservatives in cosmetics, and the detection sensitivity and selectivity can meet the detection requirements.

Conclusion

Existing problems

Despite significant progress in the research of nanozyme-based electrochemical sensors, which have been widely applied in multiple fields, some problems and challenges still exist in practical applications, limiting their large-scale promotion and commercial application. These mainly include the following aspects:

(1) Insufficient catalytic selectivity

Compared with natural enzymes, nanozymes have poor

catalytic selectivity. Most nanozymes have limited specific recognition ability for substrates and are easily interfered with by other substances in the sample, which leads to a decrease in the detection accuracy of the sensor.

(2) Poor long-term stability

The long-term stability of nanozymes is an important prerequisite for their practical application. Although nanozymes are more stable than natural enzymes, they are prone to aggregation, oxidation, degradation and other phenomena in complex environments (such as high temperature, strong acid and strong alkali, high salt concentration), which leads to a decrease in catalytic activity.

(3) Interference from actual samples is difficult to eliminate.

Actual samples (such as serum, food, and environmental water samples) have complex compositions and contain a large number of proteins, sugars, ions, and other substances. These substances can interact with nanozymes or target analytes, interfering with catalytic reactions and electrical signal detection, leading to a decrease in the detection sensitivity and accuracy of the sensor.

(4) High difficulty in commercial application

At present, most of the electrochemical sensors based on nanozymes are in the laboratory research stage and have few commercial applications. The main reasons include: First, the preparation cost of nanozymes is high, especially noble metal nanozymes and single-atom nanozymes, which are difficult to produce on a large scale. Second, the preparation process of the sensors is complicated and has poor repeatability, making it difficult to achieve standardized production. Third, the detection performance of the sensors is difficult to perform stably in practical applications and there is a lack of unified performance evaluation standards. Fourth, the market acceptance of nanozyme sensors is low and there is a lack of mature commercial promotion channels. How to reduce the preparation cost, simplify the preparation process, establish a unified performance evaluation standard, and promote the commercial application of nanozyme electrochemical sensors is an important challenge currently facing this field.

(5) Insufficient in-depth research on catalytic mechanisms

Although the catalytic mechanism of nanozymes has been studied to some extent, most studies are still limited to the description of surface phenomena, and the microscopic mechanism of nanozyme catalytic reaction (such as the action mechanism of active sites, electron transfer pathways, free radical generation process, etc.) is not studied in depth.

Future development trends

Addressing the current problems and challenges of nanozyme-based electrochemical sensors, and considering the development trends in nanomaterial preparation technology, electrochemical detection technology, and biomedical technology, future nanozyme-based electrochemical sensors will develop towards higher selectivity, higher stability, miniaturization, intelligence, and commercialization. Specific development trends are as follows:

(1) Design and development of novel nanozymes

Developing novel nanozymes with high catalytic activity, high selectivity and high stability is key to improving sensor performance.

In the future, by precisely designing the composition, morphology, size and surface structure of nanozymes, and combining methods such as element doping and composite modification, we will develop new nanozymes, such as single-atom nanozymes, bimetallic nanozymes and MOF-derived nanozymes, to further improve their catalytic performance.

Meanwhile, computational chemistry, molecular simulation and other technologies will be combined to conduct in-depth research on the catalytic mechanism of nanozymes, providing theoretical support for the design and development of novel nanozymes. For example, the active site structure of double single-atom nanozymes can be designed by molecular simulation to achieve precise control of catalytic performance; the electronic structure of nanozymes can be controlled by element doping to improve their catalytic selectivity.

(2) Further optimization of sensor performance

In the future, various strategies will be used to further optimize the performance of nanozyme electrochemical sensors and improve their detection sensitivity, selectivity and stability. For example, by surface modification and composite modification, the catalytic selectivity and long-term stability of nanozymes can be improved. By novel signal amplification strategies

(such as dual signal amplification and multi-stage signal amplification), the detection sensitivity of sensors can be improved. By optimizing electrode modification processes, the binding strength between nanozymes and electrodes can be improved, and the service life of sensors can be extended. In addition, novel electrochemical detection technologies, such as in-situ electrochemical detection technology and single-cell electrochemical detection technology, will be developed to achieve real-time, in-situ, and high-resolution detection of target analytes.

(3) Development of miniaturized and intelligent sensors

With the development of miniaturization and intelligentization technologies, electrochemical sensors based on nanozymes will develop towards miniaturization, intelligence and portability. For example, by combining microfluidic technology, miniaturized nanoenzyme electrochemical sensors can be developed to achieve low sample consumption, fast detection speed, and on-site detection. By combining smartphones, the Internet of Things and other technologies, intelligent sensors can be developed to achieve real-time transmission, analysis and sharing of detection data. For example, an integrated sensor chip can be constructed based on screen printing technology to achieve sensor miniaturization and portable detection; by combining the electrochemical detection function of smartphones, portable nanoenzyme sensors can be developed for rapid on-site detection.

(4) Expansion of applications in multiple fields

In the future, electrochemical sensors based on nanozymes will further expand their application areas and achieve wider applications in fields such as biomedicine, food safety, environmental monitoring, and public health. For example, in the biomedical field, nanozyme sensors for single-cell and single-molecule detection will be developed to provide more precise tools for early diagnosis and pathological research of diseases. In the field of food safety, portable sensors will be developed for rapid on-site detection of food to enable real-time monitoring of food contaminants. In the field of environmental monitoring, sensors for in-situ detection of environmental pollutants will be developed to provide more efficient means for environmental protection. In addition, their application in agricultural production, cosmetic safety, public health

and other fields will be expanded to achieve full coverage in multiple fields.

(5) Promotion of commercial applications

Promoting the commercial application of nanozyme-based electrochemical sensors is an important development goal in this field in the future. In the future, we will promote the standardization and large-scale production of sensors by reducing the preparation cost of nanozymes, simplifying the preparation process, and establishing unified performance evaluation standards. At the same time, we will strengthen cooperation with enterprises, develop commercially competitive products, expand commercial promotion channels, and improve market acceptance. For example, we will develop low-cost, high-stability nanozyme sensors for use in home blood glucose monitoring, rapid food detection and other fields to achieve commercial application; and develop high-sensitivity nanozyme sensors for clinical diagnosis to promote their commercial application in the medical field.

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Authors' contributions

Jiaqi Xiao and Yuefei Duan contribute equally to the article.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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