Recent advances in electrochemical enzymatic biosensors based on regular nanostructured materials

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A R T I C L E   I N F O

Keywords:
Enzymatic biosensors
Regular nanostructure
Nanomaterials
Control methods
Performance advances

A B S T R A C T

Due to the emergence of nanomaterials, enzymatic biosensors have achieved fast and unprecedented development in biomedicine, agriculture, food, and other fields. Recently, an increasing number of studies have verified that regular nanostructures can enable the effective promotion of enzymatic reactions and electron transfer because of their superior surface area and conductivity. Therefore, nanostructure control of biosensing materials has become a new research hotspot in the past decade. In this review, we will focus on the recent progress of electrochemical enzymatic biosensors based on regular nanostructured materials, especially emphasizing the preparation methods and advances of these regular nanostructures. In addition, we carefully discuss the advantages and disadvantages of these regular nanostructured materials during the enzymatic detection of various analytes in different fields. Finally, we conclude by identifying the main challenges and prospective research directions for regular biosensing nanomaterials, as well as providing suggestions for the development of high-performance biosensors in the future.

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1. Introduction

An enzymatic biosensor is a detection instrument that converts the concentration of the measured substance into a digital signal through a suitable physical and chemical transducer and signal amplifying device. It is based on biomolecular recognition using enzymes as sensitive components. Its main performance indicators include its sensitivity, selectivity, detection limit, and linear range. Clark and Lyons first proposed the concept of a glucose sensor using an oxygen electrode to achieve clinical detection [1]. Updike and Hicks prepared the first glucose oxidase (GOx) sensor by coating a polycrylamide gel film containing GOx on Clark’s oxygen electrode [2]. Since then, enzymatic biosensors have achieved rapid development and undergone several generations [3–6].

Due to the natural specificity of an enzyme, enzymatic biosensors often show excellent selectivity to the target in practical applications. These biosensors have gradually attracted an increasing attention of attention from people in various fields, including food safety, agricultural pollution, pharmaceutical production, fermentation and environmental monitoring, due to their excellent characteristics, namely, their high sensitivity, short response time, on-site mode and low cost [7–11]. However, when relying only on the redox ability from the active centre of the enzyme for sensing, not enough electrons are transferred to produce a strong enough response signal, which greatly affects the accuracy of the sensor. Therefore, an electrode material is often employed to magnify the enzymatic signal to enhance biosensing performance. With the rapid development of nanoscience, an increasing number of nanomaterials have been designed to obtain higher electrocatalytic activity, surface area and conductivity. In recent decades, nanostructure control of nanomaterials has gradually become a hot research direction due to its general ability to improve performance [12–14]. To date, the development of enzymatic biosensors can be divided into three generations according to the way enzyme-generated electrons transfer between the enzyme and electrode surface [15]. The first generation is the classic enzymatic biosensor, which relies on the participation of oxygen or H2O2 in solution to transfer electrons. Although its stability is poor due to the changing concentration of dissolved oxygen, it was the earliest to be reported and established the essential development direction of the enzymatic biosensor. The second generation is the mediator-based enzymatic biosensor, which often introduces an artificial electron mediator into the enzymatic electrode as a bridge between the active centre of the enzyme and the electrode surface. Employing this type of material greatly magnifies and accelerates the detection signal via its redox ability. However, artificial mediators often suffer from falling off the electrode surface because of weak interactions or affinities, thereby causing the prepared biosensors to have short lifetimes. The third generation is the mediator-free enzymatic biosensor, which transfers electrons directly between the enzyme centre and the electrode without any artificial mediator. In this case, the stability of the interface between the enzyme layer and electrode surface will be significantly improved, together with the much faster electron transfer rate. As a result of no mediator, electrocatalysis of the enzymatic reaction is not satisfactory, thus leading to low sensitivity. The development history of the enzymatic biosensor is actually accompanied by the progress made in developing electrode materials, in which a more stable and shorter transfer pathway for electrons is continuously achieved. In this way, remarkable amplification of the electrical signal derived from the enzymatic reaction is expected, producing high biosensing performance.

Nanomaterials have been successfully used in many fields, including the medicinal, pharmaceutical, chemical, and biological fields. Nanomaterials play important roles in electrochemical sensing: (1) High electron transfer rate. Low transfer resistance of nanomaterials can improve the response of the nonconductive protein on the electrode surface. (2) Amplification of electrochemical signals. Nanomaterials have excellent catalytic activity and are widely used as electrocatalysts to expand the electrochemical response signal. (3) Nanocarrier of enzymes. Nanomaterials have good biocompatibility and can immobilize protein molecules through electrostatic interactions, hydrophobic or π–π stacking interactions [16–18]. Although nanomaterials have been verified to possess various advantages, precise nanostructure control is always a challenge due to their natural aggregation behaviour when reaching the lowest enthalpy. Compared to irregular nanomaterials, materials with regular nanostructures have more active sites and showed improved catalytic capacity. Simultaneously, the uniform distribution of regular nanostructured nanomaterial makes it have a larger specific surface area, leading to an increase in enzyme loading, which in turn enhancing the signal intensity too. Furthermore, since the synthesis method of regular nanomaterials is controllable, the related biosensors have high repeatability and stability [19–22]. Therefore, an increasing number of researchers are eager for advanced nanomaterials that have a well-defined regular geometric morphology with a uniform distribution on electrodes. In the past ten years, along with a variety of regular nanostructures, such as nanocubes (NCSs), nanowires (NWs), nanotubes (NTs), and nanoflowers (NFs), various nanomaterials have been continuously developed to verify the advantages derived from their regular nanoscale appearance [23–26]. However, few reviews have evaluated the effects and progress of regular nanostructures on enzymatic biosensors developed in the last decade.

In this review, we focus on recent progress in enzymatic biosensors whose performance greatly benefits from the regular nanostructures of electrode materials (Fig. 1). According to the different elemental compositions of nanomaterials, emphasis will be given to state-of-the-art control methods of regular nanostructures and the various morphologies that are produced. Moreover, the advantages and disadvantages of these methods will be discussed to provide an overall evaluation of the effects on the enzymatic reaction in regard to conductivity and surface area. Furthermore, the performance of these regular nanostructure-based biosensors will be compared according to the detection of three types of analytes (physiological index, pollutant and food additive), showing their improved abilities in regard to sensitivity, linear range, etc. We hope that this review will provide a detailed summary of the advanced approaches of nanostructure control for producing superior enzymatic biosensors, thereby offering an essential reference for novel inspiration in biosensor design with general performance enhancements.
2. Nanostructure control of biosensing nanomaterials

The surface area and conductivity of electrode nanomaterials often determine the biosensing performance, including the selectivity, response time, and detection limit. In this case, the applied materials usually possess one or two characteristics of high catalytic activity and a fast electron transfer rate. In addition to above natural abilities, an increasing number of studies has indicated that nanostuctures enable the further promotion of performance due to their superior activity and facilitation of signal generation and magnification. Hence, many novel synthesis methods are being developed to obtain various regular nanstructured nanomaterials to continuously enhance biosensing performance. The following discusses in detail different kinds of nanomaterials (noble metals, metal oxides, carbon materials and coordination compounds) and their corresponding nanostructure control methods that are used to construct various enzymatic biosensors with regular nanostructures.

2.1. Noble metals

Noble metals, including gold, silver, palladium, and platinum, normally exhibit the excellent conductivity. The catalytic properties of metal nanoparticles (NPs) facilitate the electrical contact between the redox centre of the protein and the electrode surface [27]. Moreover, noble metal NP can well control the microenvironment of biomolecules that retain their activity because it has a similar size to some biomolecules together with a large surface area. Meantime, it can also promote electron transfer between the redox centres of biomolecules and support electrode [28]. Therefore, this type of material was very widely applied early on in the preparation of enzymatic biosensors. Its nanostructure control has become a hot research direction since the 1990 s due to the rapid development of nanoscience.

Over the years, there have been various approaches to synthesis metal NPs, including physical methods such as evaporation condensation and laser ablation, biological methods using natural reducing agents, chemical methods such as chemical reduction, microwave-assisted synthesis, etc [29,30]. The most common method to synthesize metal NPs is the chemical reduction [31]. By adjusting the physical and chemical conditions during synthesis, such as temperature, pH, stirring speed, precursors, reduction agents and stabilizing agents, metal NPs of different sizes and shapes can be obtained. Many reported results have demonstrated that the successful construction of regular morphologies, such as nanorods (NRs), NCS, and nanopolyhedrons, generally enhances catalytic performance. However, it is not possible to apply the same control method to all noble metals for achieving regular nanostructures. Derived from the crystallization behaviour of various noble metals, specific preparation strategies are required when using them in designs. Below, we focus on synthesis methods with regular Au, Ag, Pd and Pt NPs in enzyme sensing research.

2.1.1. AuNPs

Gold is a material with a long history and has always served as a valuable currency. Regarding biosensor research, gold electrodes are the most commonly applied substrate for material modification due to their high surface area and conductivity. It has been reported that the presence of AuNPs on the electrode surface contributes to the enhancement of charge transfer [32]. In the last century, scientists have revealed its excellent catalytic activity when its size is reduced to the nanoscale. Since then, AuNPs have been widely adopted to fabricate various biosensors.

The first regular nanostructure of AuNPs was synthesized sometime in 1994 and showed a NR shape [33]. Lang et al. used NR-shaped AuNPs to prepare an acetylcholinesterase (AChE) biosensor [34]. This structure was obtained via a seed-mediated growth method. A seed solution was prepared by mixing cetyltrimethylammonium bromide (CTAB), HAuCl4 and NaBH4. NaBH4 could reduce AuCl4 to produce small AuNPs without regular features. Then, they utilized a growth solution that contained AgNO3, CTAB, HAuCl4, H2SO4 and ascorbic acid (AA) to further culture Au seeds at 30 °C. Finally, uniform NPs with an average length of approximately 60 nm were harvested to show excellent electrocatalysis of the AChE reaction to detect acetylcholine chloride at 0.54 V.

In addition to NRs, an increasing number of regular nanostructures have been continuously created for electrode fabrication in the last ten years. NFs with an average diameter of 30 nm were obtained in the presence of cytosine by a simple chemical reduction of tetrachlorauric acid; in this case AA solution was added to a mixture of HAuCl4 and cytosine at 60 °C [35]. During this process, cytosine was more likely to adsorb on the Au(1 1 0) and (1 0 0) planes, causing the preferential growth of Au nanocrystals along the (1 1 1) direction. They also proved the influence of the reductant on the Au morphology. If NaBH4, which possesses a high reducibility, was applied, the prepared Au...
showed a network-like nanochain structure at 0 °C. In contrast, the use of AA, which is a weaker reductant, slowed the reduction rate and promoted the uniform aggregation of AuNPs to form a flower-like shape. After the immobilization of microperoxidase-11, both Au nanochains and NFs exhibited much better H₂O₂ sensing performance than irregular AuNPs due to the Au nanochains and NFs exhibiting largely improved electrocatalysis. Kitikul et al. synthesized a NW structure of AuNPs by sputtering an aluminium oxide film with gold at 15 mA for 150 s [36]. The obtained AuNWs were uniform with an average diameter and length of 120 nm and 1 mm, respectively. After cross-linking with carbon nanotubes (CNTs) and glutamate oxidase (GMOx) on the AuNW film, the constructed glutamate biosensor showed high stability, an ultralong lifetime, and good detection performance.

These regular features of AuNPs can generally increase the specific surface area of the prepared electrode, resulting in obvious electrocatalysis improvements during enzymatic reactions. More importantly, after the realization of regular nanostructures, the aggregation problem of AuNPs remarkably decreases due to the uniform surface energy of each particle. In this case, the stability of these biosensors can be evidently improved when practically applied.

2.1.2. AgNPs

Due to the high price of gold, silver is often applied as a substitute to construct enzymatic biosensors. AgNPs normally have higher activity than AuNPs because of their elemental characteristics. Moreover, the conductivity of silver is 63.01 mS/m, which is higher than that of gold (45.20 mS/m). Therefore, AgNP-based biosensors often show much shorter response times. Additionally, AgNPs can allow for the possibility of increasing the activity of immobilized enzymes [37], the electrodes modified by AgNPs also show higher sensitivity and stability [38]. The most prepared regular nanostructures of AgNPs are NCs and NWs. In most cases, the NW is derived from the further growth of NCs in a particular direction.

Lin’s group designed an AgNW-based glucose biosensor via a thermal reduction method [39]. In this preparation, AgNO₃ and ethylene glycol (EG) served as the silver source and reducing agent, respectively, together with the assistance of poly(vinyl pyrrolidone) (PVP) as a stabilizer. Notably, MnCl₂ was also introduced during the synthesis of these AgNWs. This operation decreased the amount of reacted Ag⁺ during the initial 10 min of nucleation. In addition, Mn²⁺ could remove the adsorbed oxygen on the nucleation surface to avoid oxidation of the obtained AgNWs. The preparation of a nanocubic morphology requires more precise control of the reduction process, and Lin’s group confirmed that HCl played an important role in building a cubic shape [40], while AgNO₃, EG and PVP were still used as reactants. In this case, HCl was first added to EG and dissolved at 140 °C. As claimed by the authors, HCl and oxygen could selectively etch the seed structure to produce a perfect NC of each single Ag crystal [41]. Very uniform AgNCs were synthesized with a side length of 100 nm. These NCs were then modified on an electrode together with horseradish peroxidase (HRP) and GOx, thereby fabricating a glucose biosensor. At a very low potential of −0.15 V, compared with the biosensor based on Ag NWs, the as-prepared biosensor presented a lower detection limit of 0.6977 µM with a wider linear range of 10 µM to 1.5 mM.

In addition to the above two nanoshapes, Cathcart and Kitaev realized a controllable approach to easily adjust the morphology of AgNPs to obtain prismatic polyhedrons (Fig. 2a-f) [42]. First, they obtained a precursor solution of thiolate-protected silver clusters in the presence of captopril during the reduction reaction between AgNO₃ and NaBH₄. Flower-shaped multifaceted prismatic silver nanoparticles (AgNFs) with diameters of 130–2250 nm, thicknesses of 30–150 nm and asperities of 50–100 nm were observed. In this process, because insoluble AgCl was gradually synthesized from the combination of Ag⁺ and Cl⁻, the further formation of Ag crystals was prevented. Therefore, only the most active sites of the crystal nucleus allowed the continuous deposition of AgNPs, resulting in heterogeneous growth. By controlling the pH value and concentration of Cl⁻, other regular morphologies can also be obtained. These special architectures demonstrate that if a high surface area of AgNPs is obtained, the electron generation and transfer during enzymatic reactions will be promoted. Recently, Huang et al. proposed a glucose biosensor that was constructed by a nanocomposite with dewdrop-like PtNP-decorated AgNFs. These AgNFs were prepared by the addition of AA into a mixture of bovine serum albumin (BSA) and AgNO₃ at 55 °C (Fig. 2g) [43]. This AgNF@BSA material had a high surface area with an open nanoporous structure, together with excellent conductivity, biocompatibility and stability. After the introduction of H₂PtCl₆·6H₂O in the AgNFs@BSA water dispersion, AgNFs-Pt@BSA NPs were obtained with a diameter of ~5 µm, which further improved the catalytic activity. The corresponding glucose biosensor exhibited good performance at −0.35 V, including a wide linear range of 1 to 14 mM and a low detection limit of 0.3 mM.

2.1.3. Pd and Pt NPs

As mentioned above, regular AgNPs usually possess superior conductivity, which are beneficial to biosensing performance. However, due to its high activity and low oxidation potential, its poor stability always limits its practical application in electrochemical detection. Therefore, PdNPs and PtNPs have been newly developed to address this issue.

Vertically oriented PdNWs with a diameter of 30 nm were obtained by an electrodeposition route using an anodized aluminium oxide (AAO) template [44]. The electrolyte for deposition consisted of Pd

![Fig. 2. Schematics of the formation of AgNFls formation (a) and SEM images of the AgNFl morphologies: (b-c) triangular and (d-f) hexagonal. Schematic illustration of the preparation of (g) AgNFls@BSA and (h) MPdPnP. Reproduced from refs. [42,43,46] with permission from the Royal Society of Chemistry, IOP Publishing, and Wiley, respectively.](image-url)
(NH₄)₆Cl₂, NH₄Cl and NH₄H₂O. After being modified through loading AuNPs and immobilizing GOx, a biosensor based on PdNWs could be applied to detect glucose.

To overcome the stability and aggregation problems without a template, Krishnan et al. synthesized a nanocubic Pd@Pt core–shell structure via a seed-mediated solution-phase method [45]. First, PdNC seeds with an average length of 13 nm were obtained by heating a mixture containing PVP, AA, KBr, and Na₂PdCl₄ at 80 °C for 3 h. Subsequently, the aqueous suspension of the Pd nanocubic seeds was reacted with an aqueous solution of AA and PVP. Then, NaOH was added to adjust the pH of this mixture to 11. Finally, K₂PtCl₄ was added to finish the formation of Pd@Pt core–shell NCs. The thickness of the Pt shell layer could be well controlled by varying the concentration of K₂PtCl₄. The pH also played an important role on the reduction kinetics of the core–shell structure. The reduction ability of AA increased with increasing pH to generate more PtNPs that rapidly diffused over the whole PdNC surface. This special nanocubic composite enabled high electrocatalysis of the reaction of GOx during glucose recognition, presenting a sensitivity of 6.82 μA mM⁻¹ cm⁻² with a linear range of 0–500 μM, which could be maintained even under a large strain of 200%. This result proves the broad prospects of sensors based on gold nanomaterials in wearable biochemical diagnosis.

In 2016, Abrar and coworkers obtained AgNPs-based electrodes with a mechanically bendable cross serpentine shape on a flexible substrate using direct stamping technique with simple spray coating. On this basis, a biosensor is prepared by modifying the electrode with BSA-immobilized lactate oxidase (LOx) (Fig. 3b-c) [48]. As the biosensor has an excellent resistance to mechanical deformation and temperature fluctuations, it has the potential to be used for continuous measurement of lactic acid in sweat on the human epidermis. In the in vitro evaluation, the average sensitivity of the lactate biosensor is 256 nA mM⁻¹ cm⁻² and the linear response range is from 0 mM to 25 mM at a working potential of 0.65 V. Abellán-Llobregat et al. successfully prepared a stretchable electrochemical biosensor based on PtNPs for the determination of glucose in sweat (Fig. 3d) [49]. When preparing the working electrode, 75% Pt-graphite/25% binder (w/w) ink was used. For cost considerations, the content of PtNPs is less, but they still have high electrical activity. The GOx immobilized biosensor achieved (105 ± 3) μA cm⁻² mM⁻¹ sensitivity with 0–0.9 mM linear range, moreover, the glucose in actual human sweat samples was measured, the recovery rate is between 71% and 84%.

Generally, noble metals with regular nanostructures are widely used in the application of enzyme biosensors due to their good electrical conductivity and catalytic activity. However, noble metals are expensive. Furthermore, metal NPs easily agglomerate, which affects the storage and detection stability of enzyme biosensors.

2.2. Metal oxides

Compared with noble metals, metal oxides usually have special optoelectronic properties and are inexpensive. Since the metal elements in metal oxides often have multiple valence states and are prone to valence changes, they can quickly transfer electrons through their own redox reactions. Through appropriate chemical reaction engineering, usable functional groups can be prepared, so biomolecules and nano metal oxides can be covalently combined to reduce the cost of biosensors and extend their shelf life [50]. In the process of enzyme electrochemical sensing, metal oxides can enhance the stability of the enzyme by enhancing immobilization [51]. Because of this charac-
teristic, metal oxide materials have wide application prospects in promoting enzymatic reactions.

Based on the type of medium in which the oxidation reaction occurs, the synthetic methodology of metal oxide NPs can be roughly divided into three categories: solution-based methods (sonochemical synthesis, co-precipitation, hydrothermal/solvothermal synthesis, sol–gel technique, microwave-assisted synthesis, microemulsion synthesis, electrochemical deposition (ECD)), gas phase methods (laser ablation, chemical vapor deposition (CVD), combustion, template/surface-mediated synthesis) and biological methods [52]. The morphology of NPs can be adjusted by changing the concentration of chemical substances and reaction conditions. Compared with physical methods, for manufacturing biosensors based on nanomaterials, chemical methods or solution-based chemical methods is more conducive to obtaining metal oxides NPs with regular nanostructured NPs, such as NWs, NRs, nanofibers, NTs, and NFs [53]. In this section, we chose ZnO, TiO2 and Fe3O4 as typical examples to study the synthesis of regular nanostructured metal oxides.

ZnO is often used in the preparation of biosensors due to its biocompatibility, low toxicity, high electron mobility and easy manufacturing. Moreover, ZnO has a high isoelectric point (IEP ~ 9.5) and positive surface charge for ZnO quantum dots, which is beneficial for enzyme loading at a low IEP [54]. Through the use of an aqueous solution of Zn(NO3)2 and KCl as the electrolyte, vertically oriented ZnO NWs with diameters and lengths of approximately 80 and 300 nm are obtained through an electrodeposition process at 70 °C [55]. These synthesized ZnO NWs are positively charged and firmly combine with negatively charged GOx through electrostatic attraction. In addition, due to their biocompatibility and large surface area, ZnO NWs provide an excellent microenvironment for a high loading of GOx. The as-prepared glucose biosensor shows good electrocatalytic performance and fast electron transfer at a potential of 0.8 V. Using a seed-assisted hydrothermal method, our group recently reported a 3D aloe-like ZnO-based nanocomposite to construct a phenolic biosensor (Fig. 4a) [56]. Before the hydrothermal reaction, a ZnO seed layer was first spin-coated on an ITO electrode. The pH of the reaction system was proven to be critical to the formation of the aloe-like morphology. The aloe-like nanostructure was observed through the uniform assembly of NRs with a diameter and length of approximately 150 nm and 6 μm, respectively, when the pH was increased to 10.5. Moreover, to enhance the conductivity of zinc oxide, AuNPs were electrodeposited on the ZnO surface. After the immobilization of laccase, this biosensor could accurately detect catechol, while avoiding interference from other dihydroxybenzenes and phenol. Thus, this biosensor exhibited remarkable performance, including a sensitivity of 131 μA mM⁻¹, a wide linear range of 75 nM to 1100 μM and a low detection limit of 25 nM.

TiO2 NPs are also widely applied to fabricate enzymatic biosensors because of their satisfactory stability and photochemical activity. Among all studies of TiO2 NPs, a one-dimensional TiO2 nanostructure is special because it has a high surface-to-volume ratio, an increased number of delocalized carriers and improved charge transport [57]. Highly ordered TiO2 NT arrays with an inner pore diameter of ~ 100 nm and a tube thickness of ~ 25 nm can be directly synthe-
sized on a titanium substrate by anodic oxidation. This process is carried out at 60 V for 1 h in water and an EG solution (volume ratio, 1:99) with ammonium fluoride mixed in. Anatase TiO₂ NTs are obtained after calcination at 450 °C for 2 h [58]. After the modification of BSA-glutaraldehyde and GOx enzymes via a cross-linking technique, the unhybridized TiO₂ NT array electrode exhibits satisfactory glucose sensing performance at an operating potential of −0.5 V. Guo et al. successfully obtained free-standing TiO₂ hollow nanofibers (HNF-TiO₂) by a template-assisted sol–gel method [59]. Fig. 4b shows that an electrospun nanofiber film was encapsulated by the TiO₂ sol using a calcination process at 550 °C for 2 h. HNF-TiO₂ was observed to possess a rough, porous and hollow tubular structure with a diameter of 100–500 nm and a wall thickness of 50 nm. During the immobilization of GOx, this rough and hollow structure could capture enzyme molecules in the pores of HNF-TiO₂, resulting in a shorter electron tunnel length between the active centre of the enzyme and HNF-TiO₂. Thus, the electrochemical performance of the HNF-TiO₂-modified electrode was significantly improved under both O₂-free and O₂-containing conditions.

Fe₃O₄ magnetic NPs (MNPs) were confirmed to present intrinsic peroxidase-like activity in various biotechnological applications [60]. Distinguished from other metal oxides, magnetic properties can often induce self-aggregation to form special Fe₃O₄ architectures. Cheon et al. fabricated Fe₃O₄ MNPs-embedded GOx-copper hybrid nanoflowers (GOx-HFs) to realize ultrasensitive glucose recognition [61]. Initially, amine-functionalized MNPs were coupled with GOx in sodium phosphate buffer (pH = 5.5), followed by the addition of a buffer solution containing CuSO₄. The pH of the applied buffer solution was important to the synthesis of MNPs-GOx NFs. To maintain the electrostatic attraction between MNPs and GOx and ensure effective coordination between Cu²⁺ and the amine moieties of MNPs and GOx, the pH needed to be controlled at 5.5. They found that MNPs-GOx NFs had highly enhanced peroxidase activity and substrate channels for cascade reactions, realizing the accurate detection of serum glucose levels. Yu et al. reported a glutamate biosensor constructed of Fe₃O₄/graphene (GR)/chitosan (CS) nanospheres (NSs) [62]. Fe₃O₄ NSs were obtained by a solvothermal process on the surface of GCE modified with GR and CS. Well-defined spheroidal Fe₃O₄ NSs were observed with an average diameter of 400 nm through the aggregation of small Fe₃O₄ NPs. Due to the synergistic effect of Fe₃O₄ NPs, GR and CS, the biosensor showed good performance for the determination of NH₄⁺ in PM₂.₅.

In studying the effect of metal oxide nanomaterials on enzyme sensing, regular nanostructured metal oxide materials such as NRs, NWs, NTs and so on can provide a direct and stable way for rapid electron transmission. Moreover, Shukla and co-workers [63] found the aspect ratio of the NRs is positively related to the enzyme loading. Among the glucose sensors based on different ZnO NRs they prepared, the NR with the highest aspect ratio exhibited the lowest electron transfer resistance and the corresponding biosensor showed the best sensitivity. Additionally, Kim et al. studied the glucose detecting performance of ZnO NRs with three different surface area and found that the larger...
the surface area of the ZnO NRs, the higher the sensitivity of the glu- cose biosensor [64].

In recent years, there are many researches about enzymatic electrochemical biosensor devices based on metal oxides. Fung and cowork- ers applied roll-to-roll printing technology to the preparation of ZnO NWs glucose biosensor, demonstrating the possibility of industrial pro- duction of sensors in high-yield and low-cost (Fig. 5a-c). Use printing technology to pattern the precursor to achieve the selective growth of ZnO NWs on the porous carbon working electrode, the obtained glu- cose biosensing device presented a typical sensitivity of 1.2 ± 0.2 μA mM⁻¹ cm⁻² with a linear range from 0.1 mM to 3.6 mM [65]. In 2018, Kiranjana an et al. first prepared a flexible and independent sen- sor on the basis of Fe₃O₄/reduced graphene oxide (rGO) paper for the detecting of H₂O₂ after the immobilization of catalse (Cat) [66] (Fig. 5d). Through electrodeposition on rGO paper by constant potent- ial electrolysis (-1000 mV), they obtained Fe₃O₄ octahedral crystals which could be used as the active binding centre for Cat immobilization (Fig. 5e-f). The analysis of the electrochemical performance of the electrode shows that the dense Fe₃O₄ crystals will increase the electroactive area of the electrode, and then increase the actual surface area of the electrode. Moreover, the electron transfer resistance of Fe₃O₄/rGO paper (24.67 Ω) is smaller than that of rGO paper (48.35 Ω), that is to say, the existence of Fe₃O₄ crystals is conducive to the increase of electron transfer.

Although metal oxide NPs promote enzymatic oxidation activity to present high biosensing sensitivity, their poor conductivity and high oxidation potential (normally higher than 0.4 V) will easily cause interference from the overoxidation of other coexisting substances when used in practical applications, such as the analysis of blood and wastewater. The decrease in operating potential is always a main chal- lenge for this type of nanomaterial and affects detection accuracy and selectivity.

2.3. CNTs and GR

Carbon materials often have a large specific surface area, excellent photoelectric thermal conducting properties and a high electron trans- fer rate. Moreover, based on their good biocompatibility, biologically active substances can be loaded on the surface of carbon materials for functional modification through physical adsorption or chemical crosslinking. Among them, CNTs and GR are attracting increasing attention from biosensor researchers [67], especially since Geim and Novoselov were awarded the Nobel Prize in 2004.

2.3.1. CNTs

CNTs are unique one-dimensional nanochannels that provide ultra- fast molecular transport and excellent mechanical strength. The hol- low structure of CNTs is favourable for the adsorption of enzymes, enzymes could be immobilized on the open end of CNTs to provide a scaffold structure [68]. Because CNTs are small in size, have excel- lent electrochemical and electrical properties, meanwhile, their con- ductivity is about 100 times that of copper wires, thus they are very suitable for the conduction of electrical signals generated after identi- fying the target [69].

CNTs are generally divided into single-walled CNTs (SWCNTs) and multwalled CNTs (MWCNTs). The earliest method of producing car- bon nanotubes was high-temperature preparation techniques, such as arc discharge or laser ablation. Now the most practical method is low-temperature CVD (≤ 900 °C), which can precisely control the ori- entation, alignment, diameter, purity, and density of CNTs [70]. In biological systems, the main problem of using CNTs is their poor dis- persibility in solvents. This result is because there are strong van der Waals interactions between each tube. Therefore, surface functional- ization is a widely used method to improve solubility and provide other functional properties in biological applications [71], it has been reported that the solubility and stability of CNTs can be improved through chemical adsorption functionalization.

Zhu et al. synthesized bamboo-shaped CNTs (BCNTs) with a regular morphology and doped nitrogen via a CVD method using acetonitrile as the nitrogen precursor [72]. The bamboo structure of the nanotubes was observed as segmented compartments, and the curvature of the compartment layers was directed to the tube tip. The diameter and wall thickness of these BCNTs were 20–25 nm and 7–10 nm, respec- tively, and the bamboo segment distance was 15–20 nm. After the introduction of CS for immobilizing HRP, the obtained HRP/BCNT/ CS nanocomposite film showed a high catalytic activity for H₂O₂. The adsorption of CS promoted the dispersion of CNTs, while the BCNTs increased the electron transport performance and porosity of the nanocomposite.

Functionalyzed multwalled CNTs (fMWCNTs) with carboxyl groups on the sidewall can be obtained by acid functionalization of MWCNTs [73]. Subsequently, Nafton (NF) is used to decrease the aggregation of fMWCNTs in the electrolyte solution. Thus, a highly electroactive film of polypryrolyl (PPy)-NF-fMWCNTs can be uniformly formed on a GCE surface through simple one-step electrochemical polymerization. The carbonyl groups of fMWCNTs can form hydrogen bonds with PPY generated by the anodic polymerization of pyrrole, which promotes mechanical stability and the proper conjugation of the nanohybrid composite (Fig. 6a). After loading GOx, the encapsu- lated enzyme inside the bio-nanocomposite decreases the surface porosity of the film, producing a modified electrode with a smooth sur- face. This biosensor shows an ultrahigh sensitivity of 2860.3 μA M⁻¹ cm⁻², with a linear range up to 4.7 mM and a detection limit of 5 μM at a potential of 0.25 V.

During manufacturing CNT-based biosensors, the main challenges are the precise control of tube size and structural orientation for real- izing the fastest electron transfer. Since GR is verified to possess even higher conductivity than CNTs, most researchers have turned their attention to GR to construct an increasing number of enzymatic biosensors.

2.3.2. GR and its derivatives

GR, due to its unique single atom-thick two-dimensional structure, has high mechanical strength and extraordinary flexibility, together with excellent chemical stability [74]. Excellent electric conductivity and a small band gap of GR would facilitate the conduction of elec- trons from biomolecules [75]. The surface of GR reaches 2630 m²/g, and the conductivity of GR is approximately 64 mS cm⁻¹, which is 60 times better than that of SWCNTs [76].

The synthesis of GR can be carried out in two main ways: one is the top-down method such as mechanical exfoliation, arc discharge, oxida- tive exfoliation-reduction, liquid-phase exfoliation, and unzipping of CNT, the other is the bottom-up method such as CVD, epitaxial growth, substrate-free gas-phase synthesis, template route and total organic synthesis [77]. For the preparation of monolayer GR, mechanical exfoliation and CVD are commonly used methods [78]. However, GR and its derivatives are usually hydrophobic due to their inherent conjugated structure, which hinders the diffusion of detection targets and the bonding of water-soluble enzymes. Therefore, GR is often modified with other components to increase its affinity to enzymes during biosensor construction.

Through the use of a thiol GR-modified Au electrode as a substrate, uniform [TBA][Ni(mnt)₂] (mnt = maleonitriledithiolate) NCs with a diameter of 500 nm can be crystallized in situ on a gold surface [79]. Only disordered and ununiform [TBA][Ni(mnt)₂] will be obtained in the absence of thiol GR. This case illustrates that the introduction of GR will induce the regular growth of [TBA][Ni(mnt)₂] crystals to form a nanocubic morphology. In addition, the presence of GR can greatly increase the electron transfer rate due to its excellent conduc- tivity. After immobilizing GMOx, this biosensor can be used for detect-
ing both glutamate and alanine amino-transferase (ALT) with excellent
results.
Recently, Wang et al. described the synthesis of an integrated com-
posite of mesocellular silicate foam (MCF)-modified rGO (MCF@rGO)
[80]. The use of polyethylene oxide–polypropylene oxide–polyethylene oxide (P123) could prevent aggregation between each GR layer, which was essential to obtain uniform MCF@rGO. More importantly, as a template for the preparation of mesoporous silicates,

Fig. 6. Schematic illustration of the preparation of fMWCNTs (a) and MCF@rGO (b). Low-resolution Cs-corrected FETEM image of fMWCNTs with PPy (c). TEM images (d, e) and enlarged TEM image (inset of d) of MCFs@rGO. Reproduced from refs. [73] and [80] with permission from Elsevier and the American Chemical Society, respectively.

Fig. 7. Flow diagram of the preparation route of (a) flexible glucose biosensor with the incorporation of VACNTs and CP; (b) 3D GR-PLA biosensor. Reproduced from refs. [81] and [82] with permission from Elsevier.
the modified P123 could significantly enhance the affinity between the nanosheets and formed MCF. Thus, the as-prepared MCF@rGO exhibited a unique sandwich structure with an inner skeleton of rGO and two outer layers of MCF with large mesopores (Fig. 6b, d-e). The external modification of MCFs with the existence of large mesopores made the surface property of rGO change from hydrophobic to hydrophilic, providing a favourable microenvironment for enzyme loading. In addition, although the conductivity of MCFs is weak, the inner skeleton of rGO with high conductivity enables the direct electron transfer of various redox enzymes. On the basis of this novel composite, the biosensor immobilized with GOx showed outstanding H2O2 and glucose sensing performance.

For the application of carbon materials-based biosensor, Gökgölan et al. first prepared a new type of biosensor platform that combines vertically aligned CNTs (VACNTs) and conjugated polymer (CP) to detect glucose [81]. The aluminum foil with grown VACNTs was first assembled on a polyethylene terephthalate (PET) substrate, after decorating poly (9,9-di-(2-ethylhexyl))-fluorenyl-2,7-diyl)-end capped with 2,5-diphenyl-1,2,4-oxadiazole (PFLO) on VACNT, using glutaraldehyde as a cross-linking agent to immobilize GOx, the resulting flexible glucose biosensor could successfully detect the glucose content in the beverage (Fig. 7a). During the manufacturing process, the conductive network of the VACNT array provides a high surface matrix for biomolecules, and provides communication mediation between bio-molecules and electrochemical transducers. Meanwhile, using appropriate CP to coat the surface of VACNT could avoid the adverse effect of the hydrophobicity of VACNT on the immobilization of the enzyme.

In 2020, Marzo and coworkers used 3D printed GR/polylactic acid (PLA) electrodes to construct a third-generation electrochemical biosensor. In the process, HRP was directly immobilized on the electrodes for hydrogen peroxide detection [82]. Importantly, the 3D GR/PLA electrode should be activated through chemical (ultrasound in DMP) and electrochemical treatments. After the activation, the exposed functional groups of GR can immobilize enzymes without any other binders (Fig. 7b). In addition, GR itself is an excellent electron carrier, it is possible to successfully fabricate a mediator-free enzymatic biosensor using 3D printed electrodes as a direct electron transfer platform. Comparing the calibration curves of the activated 3D-printed GR/PLA electrode with the non-activated one at different H2O2 concentration, the sensitivity of the biosensor based on the activated electrode has been significantly improved, indicating that the activated 3D printed electrode is beneficial to the effective direct electron transfer from the enzyme to the electrode.

Both CNTs and GR can provide a shorter response time and higher conductivity than traditional electrode materials at low working potentials when applied in biosensor applications; however, their extra functionalization modification and weak catalysis limit their application in trace detection because of their low sensitivity.

2.4. Coordination compounds

Coordination compounds are a type of complex molecule formed by the combination of a central atom or ion (usually metallic) and several ligand molecules or ions through coordination bonds. Because they contain metals, they usually possess good catalytic activities. In this review, we focus on PB and metal organic frameworks (MOFs), as they are typical inorganic and organic coordination compounds.

PB consists of metals and cyanide groups, and its unit cell shows a face-centred cubic structure with two different metal valences. Therefore, PB is a large family holding many analogues by utilizing various metal elements. Due to the mixed-valence metal centres in the unit cell of PB and its analogues (PBA), they can promote the amplification of electrochemical signals through redox reactions. Additionally, the working potential of PB is usually lower than 0 V (vs. Ag/AgCl), which leads to the excellent anti-interference ability of PB-based biosensors against common chemicals. Among them, PB, which is composed of Fe(II) and Fe(III) in its cell, is the most famous enzyme-mimicking material to be called “artificial peroxidase”. Because most oxidases can produce H2O2 during their oxidation reactions, PB can be used as a versatile electron mediator to construct different kinds of oxidase-based biosensors for the detection of various analytes.

The approaches to obtain PB is relatively simple, including microemulsion, solvothermal, hydrothermal, sonochemical, co-precipitation and ECD method [83]. The traditional methods for the preparation of PB contain ECD and co-precipitation. PB was obtained for the first time through the chemical reaction between potassium salt and animal blood [23]. Then a PB film-modified electrode was first prepared by Neff in the 1980 s through ECD method [84]. Using mechanical technology and abrasive transfer technology, Zakharchuk et al. immobilized a PB slurry on a carbon paste electrode [85]. Although these two methods are the most commonly used, the morphology of the resulting PB is difficult to control. Because the crystallization speed of PB is too fast; thus, the formation of regular nanostructured PB is always a challenge and limits the improvement in biosensing performance.

Zhou et al. first obtained a highly ordered PBNW array (50 nm diameter) by electrodeposition PB crystals into the cavities of AAO, which was formed via a two-step anodizing method [86]. This was the first time that a regular nanostructure of PB was created; however, the template was found to be difficult to remove. Furthermore, the high electric resistance of AAO greatly affected the sensing performance.

In recent years, our group tried to obtain a regular Prussian blue structure without a template. In early 2010, to avoid template influence, we developed an in situ aerosol deposition approach to obtain a nanocubic PB structure on a Pt electrode without any template (Fig. 8a) [87]. We revealed that if the deposition temperature was effectively controlled, well-defined PBNCs would grow on the Pt surface due to van der Waals forces. This method could convert a traditional solution reaction into an aerosol reaction, remarkably decreasing the molar amount involved in the reaction per unit time. Therefore, the crystallization rate of PB could be highly slowed to promote the regular growth of PB. This PBNC-based biosensor promoted a nearly 2-fold sensitivity increase compared with irregular PB crystals. Through the use of this method, PBNCs could be obtained in situ on an AgNW network with an average diameter of 200 nm, and the corresponding prepared GOx biosensor exhibited high selectivity and sensitivity along with excellent stability and repeatability [88].

The preparation of PB can also be carried out by a self-assembly method that relies on electrostatic adsorption and the reaction between two oppositely charged reactive ions, in this case, Fe3+ and [Fe(CN)6]4-. In 2009, through the careful study of self-assembly parameters, we proved that the decrease in pH and the increase in the K+ concentration in the electrolyte were both beneficial to the formation of smaller PB particles with a more uniform distribution [89,90]. In 2011, we further investigated the influence of the modification conditions of polyelectrolytes on PB nanostructures during the self-assembly process. We used poly(diallyldimethylammonium chloride) (PDDA) to pretreat a bare electrode to provide a positively charged layer for the further adsorption of [Fe(CN)6]4- ions. The results showed that if the deposition temperatures of PDDA and PB were controlled exactly at 30 and 35 °C, well-defined PBNCs could be obtained (Fig. 8b) [91]. Later, it was shown that regular PBNCs could also be grown on the surface of graphite electrodes without poly-electrolyte modification at 35 °C [92]. This electrode was then prepared for three kinds of biosensors by the immobilization of GOx, GMOx and LOx, and showed high sensitivities of 127, 238 and 12.5 µA M−1 cm−2 for the detection of glucose, glutamate and lactate, respectively. We also successfully obtained a single-layer PB grid with uniform nanopores on a Pt substrate using polystyrene (PS) beads as the template through a self-assembly method with NS lithography.
PBNC-based ultrasonic deposition and self-assembly methods, we could obtain a film with two heterogeneous nanocrystal layers that could be applied to glucose detection [94]. Recently, we proposed a confined growth strategy of PB in membrane pores to construct a heterogeneous-nanostructured architecture, wherein a nanoporous surface layer continuously extracted serum, while the biosensing nanochannels underneath dynamically recognized biotargets (Fig. 8) [95]. After the immobilization of different oxidases in the biosensing channels, the as-prepared separation-sensing membrane achieved continuous testing of vital clinical indices (including glucose, lactate, and glutamate) as blood was drawn.

By etching solid PB mesocrystals from the inside with HCl, hollow mesoporous-structured PB mesocrystals (HMPB) can be formed with a diameter of 90 nm [96]. HMPB has a mesoporous nanostructure of approximately 20 nm in the shell and an inner hollow cavity of approximately 60 nm. As a result, in the process of glucose biosensing, the mesoporous shell provides more catalytic reaction sites, and the large hollow space is conducive to an increase in GOx loading. At a working potential of −0.1 V, the GOx-based biosensor shows high sensitivity and a low detection limit.

In contrast to PB, MOFs are composed of metals as coordinate centres and organics as ligands. They are considered as a new type of highly porous material, most of them can be prepared under mild conditions via different routes containing diffusion method, hydrothermal-solvothermal synthesis, electrochemical synthesis, mechanochemical method, microwave assisted method, heating, ultrasound, etc [97]. Due to the organic having longer chains than the cyanide group, MOFs are a larger unit cell with weak coordination interactions and longer pathways for electron transfer, often exhibiting higher resistance and weak stability in water. However, MOFs also possess special characteristics, including their superior affinity for enzymes. In addition to providing a high surface area for enzyme loading, MOF can also form a strong interaction between the organic pairing of the organic framework and the enzyme, thereby preventing them from leaching out of MOFs. As the enzyme is protected by a highly ordered framework, the MOF-enzyme complex shows extraordinary catalytic performance in terms of stability, selectivity, storage stability and recyclability [98]. According to different binding mechanisms, the preparation of MOF-enzyme complexes mainly occurs through the following three ways: surface bioconjugation (physical adsorption or covalent grafting), infiltration into MOFs, and encapsulation [99].

In physical adsorption, enzymes are immobilized on the surface of an MOF through weak forces, such as hydrogen bonds, van der Waals forces, or electrostatic interactions. This method is simple to conduct and requires fewer chemical reagents. For example, Patra et al. anchored GOx on the surface of the nanocomposite of MIL-100 (Fe) and PtNPs by simple deposition [100]. MIL-100(Fe) NPs were pre-prepared through a microwave-assisted hydrothermal approach, showing a well-faceted octahedral morphology with a diameter of 130 ± 30 nm. As a result, the MOF material provided an extremely high specific surface area and pore volume, and the GOx–MIL-100 (Fe)–P2NP bioelectrode exhibited remarkable electrocatalytic efficiency.

In the presence of the organic ligands in MOFs, the functional groups on MOFs can be covalently coupled to the corresponding active groups of enzymes. Glucose oxidase can be grafted to a spindle-shaped MOF (Fe-MIL-88B-NH₂) via an EDC-/NHS- induced amidation coupling process, obtaining biomimetic cascade nanozymes Fe-MOF-GOx [101]. Due to “nanoscale proximity effects”, H₂O₂ produced by the GOx-catalysed oxidation of glucose will be immediately oxidized by Fe-MOF in situ, thereby decreasing the influence of the diffusion resistance and minimizing the decomposition of H₂O₂. Therefore, Fe-MOF-GOx has excellent reusability and strong resistance to various temperature, acid and alkali conditions.

As is well known, MOFs have an abundance of micropores or channels. When the size of the enzyme is compatible with that of the channel, the enzyme can be inserted into the channel of the MOF. There are two kinds of channels in zirconium-based MOF NU-1000: hexagonal channels with a diameter of 3.1 nm and triangular channels with an edge length of 1.5 nm. Fusarium solani pisi cutinase has a small-axis length of ~3.0 nm and can penetrate into the large hexagonal channels of NU-1000 [102]. When using this MOF as an enzyme support, the large pores can be used for enzyme immobilization, and the small pores can be used for reactant/product diffusion. Therefore, compared with the free enzyme, cutinase@NU-1000 has similar activity and shows high stability in the presence of THF or urea.

For some enzymes larger than the MOF, the encapsulation method can be used to synthesize MOF biocomposite materials. GOx/ZIF-8 powder can be obtained from a mixed solution of GOx, 2-methylimidazole and Zn(NO₃)₂. It shows a rhombic dodecahedral structure with an average size of 400 nm. GOx is confirmed to embed in the ZIF-8 framework because the enzymes could not be washed away but could only be released by dissolving ZIF-8 using acetic acid. After incubating the powder in a freshly prepared polydopamine (PDA) solution in Tris...
buffer, highly stable PDA@GOx/ZIF-8 can be simply prepared; furthermore, this material still retains its activity after 10 cycles [103].

Recently, our group developed a screen-printed biosensing microchip (SPBM) on the basis of AuNPs/nickel hexacyanoferrate (NFPBA) NC, which can realize accurate and rapid monitoring of the ethanol concentration in the real fermentation reaction after immobilizing alcohol dehydrogenase [104]. We used an excess of NiCl₂ solution in the synthesis of NFPBA NCs to create a positively charged surface that can attract [AuCl₄]⁻ ions. Through electrostatic adsorption, AuNPs grow uniformly in situ on the surface of the NFPBA crystal (Fig. 9a). By comparing the electrochemical oxidation–reduction capabilities of bare chips, NFPBA and AuNPs/NFPBA SPBMs, we found that AuNPs can effectively enhance the conductivity of NFPBA, which is conducive to the generation and amplification of enzymatic reaction response signals. Moreover, because AuNPs cover the surface of NFPBA, AuNPs/NFPBA SPBM has a larger effective electrode area and more enzyme binding sites.

In 2020, for on-site monitoring of the enzymatic activity, Guo et al. designed a MOFs-in-nanochannels architecture [105]. They used ZIF-8 grown in TiO₂ nanochannels as a platform, and cytochrome C (CytC) was a model enzyme encapsulated in ZIF-8 (Fig. 9b). It was found that the protection of ZIF-8 is beneficial to the long-term stability of CytC at room temperature. Furthermore, the ZIF-8 encapsulating CytC showed a significantly enhanced enzymatic activity in a wide temperature region (37–80 °C), indicating that ZIF-8 provides a favourable environment for activating enzymes and can also reduce the denaturation caused by high temperature heating.

Although the application of MOF biocomposites in the field of biosensing is being advanced, the interfacial interaction between the enzyme and the MOF support still needs to be promoted. In addition, as most MOF materials have poor water stability with low conductivity, the organic segments contained in MOFs are difficult to dissolve in water. Thus, attaining long lifetime stability and satisfactory sensitivity in practical applications of blood or fermentation detection is still a challenge.

3. Applications of regular nanostructure-based enzymatic biosensors

During the enzyme reaction, analytes are catalysed by enzymes to induce electron transfer. However, if only relying on the redox ability of enzymes, the transfer rate is low, and the signal is weak, which cannot meet actual detection requirements. Therefore, nanomaterials are usually used to supply extra catalysis for biosensing, thereby achieving the magnification of the signal to improve the sensitivity and accuracy. According to the materials described above, the targets most commonly used for enzyme detection can be roughly divided into three categories: physiological indexes, pollutants, and food additives (Table 1).

Abnormalities in certain metabolites in the human body may indicate the occurrence of some diseases. The value of glucose in human blood is an important indicator of diabetes. For this reason, studies on glucose sensing have been more commonly studied and have the longest history of enzymatic biosensors. Many nanomaterials have been used as electrode modifiers to immobilize glucose oxidase for measuring glucose. Among them, different nanomaterials enable the detection of different functions. For example, metal NPs exhibit excellent catalytic effects and often present high sensitivity. Carbon nanomaterials possess superior conductivity that generally decreases the work potential of the prepared biosensor. Although metal oxides only exhibit their redox ability at a high potential, they can clearly promote the oxidation reaction of enzymes. In addition to glucose, uric acid is one of the important indicators of gout [136]. Elevated total cholesterol is an important risk factor for cardiovascular disease [137]. Glutamate is the main excitatory neurotransmitter, and its level is closely related to the activity of certain aminotransferases in the human body [138]. The amount of lactate produced during the process of human metabolism will increase in the case of hypoxia, and the continuous accumulation of lactic acid can lead to lactic acidosis [139]. The monitoring of these compounds is of great significance in clinical diagnosis. Therefore, biosensors based on uricase, GMOx, ChOx and LOx have been developed for the detection of uric acid, glutamate, cholesterol, and lactate, respectively. Since γ-glutamic acid is a common product formed by the reaction of α-ketoglutarate and α-alanine in the presence of ALT, the [TBA]₂[Ni(mnt)₂]/GR-based GMOx biosensor can also achieve ALT testing. Using the TiO₂/Au/EVIMC/PANI nanocomposite, after the immobilization of LDH and NAD⁺, the biosensor shows excellent electrocatalytic performance for the detection of lactate.

Unlike many enzymatic biosensing methods based on enzyme oxidation, AChE-based sensing is normally achieved through the inhibition principle of enzymes. Common detectable substances (always pesticides) such as organophosphates or carbamates can covalently bind to the hydroxyl group on the active site of the enzyme, thereby inactivating the enzyme [140]. During the sensing process, AChE can catalyse the hydrolysis of acetylthiocholine (ACh) to generate thiocholine (TCh), but the natural catalytic activity of AChE will be inhibited in the presence of inhibitors, resulting in a decreased production of TCh [141]. In sensing applications, precious metals are often supplemented with other materials. In the process of pesticide detection, the working potential is always approximately 0.6 V, and most biosensors can only detect a single pesticide. Doping AuNPs@MS core–shell NPs into TiO₂ can significantly enhance the conductivity and bioelectrocatalytic activity of the biosensor; moreover, this biosensor can be used for the detection of dichlorvos and fenthion.

As shown in Table 1, when MAC-ZIF-8 is applied as a carrier for the immobilization of lipase, the biosensor can be applied for detecting nitroen. The lipase biosensor, on the basis of CNP-L/CuO/MWCNT/pectin, shows good performance for the determination of TGs. In addi-
As substrates can achieve the highly selective detection of different phenolic compounds. The use of different regular nanomaterials relies on the various enzymes immobilized on these nanomaterials. To various nanomaterials, the recognition of different analytes achieves.

The Abbreviations: 2,6-DMP: 2,6-dimethoxyphenol; CMC: carboxymethylcellulose; CNP-L: chitosan coated magnetic nanoparticles; EVIMC: 1-ethyl-3-vinylimidazolium chloride; GAA: α-glucosidase; GDH: glutamate dehydrogenase; HQ: hydroquinone; LDH: lactate dehydrogenase; MAC: macro-microporous; MS: mesoporous SiO2; NAD+/-: dehydrogenase; PANI: polyaniline; TGs: totals triglycerides.

<table>
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<th>Detection type</th>
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<th>Enzyme</th>
<th>Material</th>
<th>Nanostructure</th>
<th>Work potential (V)</th>
<th>Sensitivity (µAM^-1cm^-2)</th>
<th>Linear range (mM)</th>
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Declarations of Competing 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.

Acknowledgement

This work was financially supported by the National Natural Science Foundation of China (No. 21727818, 22078148, and 21921006) and the Top-notch Academic Programs Project of Jiangsu Higher Education Institutions (TAPP).

References


