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New advances in electrochemical biosensors for the detection of toxins: Nanomaterials, magnetic beads and microfluidics systems. A review

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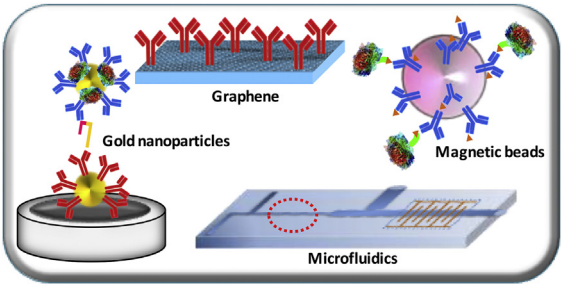
HIGHLIGHTS

- Nanomaterials improve the performance of electrochemical biosensors.
- Carbon nanomaterials can act as electrocatalysts or label supports in biosensors.
- Metal nanomaterials can act as nanostructured supports or labels in biosensors.
- Magnetic beads are exploited as immobilisation supports and/or label carriers.

GRAPHICAL ABSTRACT



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ABSTRACT

The use of nanotechnology in bioanalytical devices has special advantages in the detection of toxins of interest in food safety and environmental applications. The low levels to be detected and the small size of toxins justify the increasing number of publications dealing with electrochemical biosensors, due to their high sensitivity and design versatility. The incorporation of nanomaterials in their development has been exploited to further increase their sensitivity, providing simple and fast devices, with multiplexed capabilities. This paper gives an overview of the electrochemical biosensors that have incorporated carbon and metal nanomaterials in their configurations for the detection of toxins. Biosensing systems based on magnetic beads or integrated into microfluidics systems have also been considered because of their contribution to the development of compact analytical devices. The roles of these materials, the methods used for their incorporation in the biosensor configurations as well as the advantages they provide to the analyses are summarised.

1. Introduction

Over the past decade the number of publications describing the incorporation of nanomaterials in biosensors has exponentially increased. Several reviews on nanomaterial-based biosensors have recently been published with focus on certain types of target analytes, detection techniques and applications [1–12]. The analysis of the published literature reveals the well-established use of nanotechnology in biomedical applications, and the increasing interest of its application in the agro-food sector and the environmental field.

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This paper aims to critically review research work that exploits nanotechnology for the development of biosensors. Due to the highly productive publication records in the field, the scope of the review is limited to electrochemical biosensors for the detection of toxins of interest in agro-food and environmental applications. Biosensing systems based on the use of magnetic beads and/or microfluidics are also considered due to their key contribution towards the development of compact analytical devices. Tables 1–3 summarise the electrochemical biosensors for mycotoxins, aquatic toxins and other analytes, respectively, reviewed according to the biorecognition element, electrochemical technique, performance parameters and analysed matrix.

2. Incorporation of nanomaterials in biosensors

The International Organisation for Standardisation [13] defines the term "nanomaterial" as a material with an external dimension, or an internal or surface structure in the nanoscale (1–100 nm). Nanomaterials have unique optical and electrical characteristics that make particularly interesting their incorporation in biosensor configurations by providing advantages, such as high sensitivity, low limits of detection (LODs) and reduced matrix effects. The features of certain nanomaterials, such as their biocompatibility, conductivity, catalytic activity or stability, show them an attractive choice for certain purposes: they are widely used as label supports and signal enhancers, as they increase the electroactive surface area, and might favour the electron transfer and amplify the electrochemical signals. Carbon and metal nanomaterials have often been incorporated in electrochemical biosensor configurations. Next, these works are reviewed and classified according to the purpose of their use in the biosensor configuration as well as the method used for the nanomaterial incorporation.

2.1. Carbon nanomaterials

The unique physical and chemical properties of carbon nanomaterials, such as ultra lightweight, high mechanical strength, excellent electrical and thermal conductivity, highly ordered structure and high surface area, are responsible for the increasing interest in their incorporation in biosensor configurations. Carbon nanotubes (CNTs), carbon nanospheres (CNSs), carbon nanohorns (CNHs), graphene oxide (GO) and graphene nanoribbons (GNRs) have been immobilised onto electrodes, sometimes the carbon nanomaterial being modified with biomolecules prior or after the immobilisation step, to amplify the electrochemical signal and decrease the limit of detection in biosensing systems. Another role of carbon nanomaterials in biosensors is to act as supports for the label in competition and sandwich assay formats, also enhancing the electrochemical response.

2.1.1. Carbon nanomaterials-modified electrodes

The modification of electrodes with carbon nanomaterials provides advantages such as larger surface areas that can lead to higher number of immobilised bioreceptors and enhanced electrochemical signals, which as a consequence result in higher sensitivities and lower limits of detection. Several techniques, alone or in combination, have been used for the immobilisation of carbon nanomaterials: adsorption, entrapment into polymers, covalent binding, electrochemical techniques and chemical vapour deposition. Below, examples of carbon nanomaterials-modified electrodes are described and organised according to the immobilisation method.

2.1.1.1. Adsorption. Due to its simplicity, adsorption has been widely used as immobilisation technique in biosensor development. Adsorption of multi-walled CNTs (MWCNTs) onto a gold electrode was used by Yao et al. [14] to develop an electrochemical biosensor for the detection of the mycotoxin sterigmatocystin (ST), a precursor of aflatoxin B_1 (AFB_1). The enzyme sensor prepared by covalent binding of aflatoxin–detoxifizyme (ADTZ), the enzyme responsible for the detoxification of AFB_1 and ST, to activated MWCNTs showed wider working range (0.04–1.33 μg mL^{-1}) and lower LOD (42 ng mL^{-1}) than those obtained by an equivalent sensor based on the adsorption of ADTZ on adsorbed MWCNTs [15]. The improved performance of the former relies on the active centre protection undertaken during the immobilisation procedure: ADTZ was saturated with its substrate ST to protect the active centre from being chemically modified and thus to maintain its activity. Similarly, Li and coworkers [16] developed an enzyme sensor for AFB_1 detection that incorporates MWCNTs adsorbed onto the electrode surface. The enzyme aflatoxin oxidase (AFO), embedded in a silica sol–gel, was covalently bound to the activated MWCNTs previously adsorbed. The sol–gel matrix provides the biocompatibility required to retain enzyme activity and prevents enzyme leakage. The biosensor exhibited a linear range from 1 to 225 ng mL^{-1} of AFB_1 with an LOD of 0.5 ng mL^{-1}. Upon CNTs adsorption, different approaches have been suggested to incorporate both bioreceptors and electron transfer mediators onto the already modified surface, aiming to improve stability and electron transfer. As an example, Fang et al. [17] reported the immobilisation of MWCNTs on glassy carbon electrodes, followed by the subsequent incorporation of Prussian Blue (PB), chitosan and glutaraldehyde. Chitosan provides the proper environment for the immobilisation of anti-*Clostridium difficile* toxin B antibody and avoids leakage of MWCNTs and PB, thus increasing the sensor stability. After toxin binding, graphene oxide (GO) conjugated with horse-radish peroxidase (HRP) and secondary HRP-labelled antibody was subsequently incubated as part of a multienzyme amplification step. The system showed a linear working range from 3 pg mL^{-1} to 320 ng mL^{-1} with an LOD of 0.7 pg mL^{-1}. This low LOD was attributed to the high amount of immobilised antibody, the excellent electrical conductivity of MWCNTs and improved electron transfer mediated by PB, and the signal amplification provided by the use of GO as enzyme/antibody carrier.

A particular study is that reported by Wang et al. [18] on a CNT-modified paper electrode for the detection of microcystin-LR (MC-LR). Paper impregnated with single-walled CNTs (SWCNTs) and anti-MC-LR antibody was shown as a simple and low-cost method of biosensor design. The change in conductivity of the paper electrode in the presence of MC-LR allowed the detection of MC-LR at an LOD of 0.6 ng mL^{-1} and a linear range up to 10 ng mL^{-1}.

Apart from CNTs, other carbon nanomaterials have also been adsorbed on electrodes. Srivastava et al. [19] developed an impedimetric immunosensor for the detection of AFB_1 incorporating GO. Anti-AFB_1 antibodies were conjugated to GO previously adsorbed on gold electrodes. The large available surface area and numerous carboxyl groups on the GO sheets provided a high loading of anti-AFB_1 antibodies, resulting in a wide linear detection range (0.5–5 ng mL^{-1}) and a low LOD (0.23 ng mL^{-1}). Zhang et al. [20] described the development of an immunosensor for the detection of MC-LR based on a competitive immunoassay, where MC-LR was covalently conjugated to carboxyl groups on the single-walled carbon nanohorns (SWCNHs) previously adsorbed on a glassy carbon electrode. The biosensor exhibited a wide linear response, 0.05–20 ng mL^{-1}, an LOD of 30 pg mL^{-1} and, in the analysis of polluted water, good agreement with the values obtained using high performance liquid chromatography (HPLC) as reference analytical method.

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