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Review

Nanoparticle-based immunosensors and immunoassays for aflatoxins

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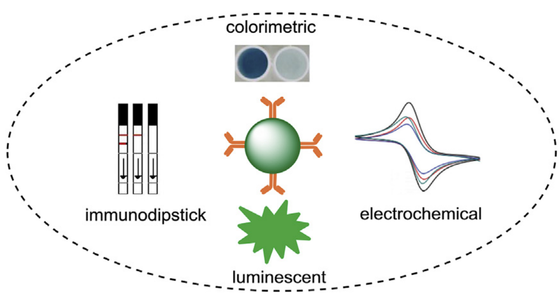
H I G H L I G H T S

- Novel concepts and promising applications of nanoparticle-based immunological methods for the determination of aflatoxins.
- Inclusion of most important nanomaterials and hybrid nanostructures.
- Inclusion of electrochemical, optical and mass-sensitive biosensors as well as optical and immunochromatographic assays.

G R A P H I C A L A B S T R A C T



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A B S T R A C T

Aflatoxins are naturally existing mycotoxins produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*, present in a wide range of food and feed products. Because of their extremely high toxicity and carcinogenicity, strict control of maximum residue levels of aflatoxins in foodstuff is set by many countries. In daily routine, different chromatographic methods are used almost exclusively. As supplement, in several companies enzyme immunoassay-based sample testing as primary screening is performed. Recently, nanomaterials such as noble metal nanoparticles, magnetic particles, carbon nanomaterials, quantum dots, and silica nanomaterials are increasingly utilized for aflatoxin determination to improve the sensitivity and simplify the detection. They are employed either as supports for the immobilization of biomolecules or as electroactive or optical labels for signal transduction and amplification. Several nanoparticle-based electrochemical, piezoelectric, optical, and immunodipstick assays for aflatoxins have been developed. In this review, we summarize these recent advances and illustrate novel concepts and promising applications in the field of food safety.

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1. Introduction

During the last decade, nanomaterials of various shapes, sizes and compositions have been synthesized, e.g., noble metal nanoparticles, metal oxide nanoparticles, carbon nanomaterials, fluorescent nanoparticles and hybrid nanostructures [1–5]. Compared with bulk materials, nanomaterials exhibit some inherent advantages such as good biocompatibility, high surface-to-volume ratio, and unique physical and chemical properties, which endow nanomaterials as excellent candidates for the fabrication of chemical and biological probes [6–11].

Immunoassays, based on the biospecific reaction between an antigen and a selective antibody, are a powerful tool in bioanalysis which use different labels like radioactive isotopes, enzymes and fluorophores for signal development [12–15]. Nowadays, these molecular tags are increasingly being replaced by nanomaterials since they have superior optical or electrochemical properties and substantially greater chemical stability [16–18]. A large number of nanoparticle-based biosensors and immunoassays have been developed for the detection of various targets such as cells, proteins, pathogens and small molecular toxins [19,20].

The aflatoxins, one of the most important mycotoxins in agricultural products, have gained much attention since their discovery in the early 1960s [21]. Approximately 18 aflatoxins (e.g., AFB1, B2, G1, G2 and M1) have been identified [22]. They may exist in food products and are extremely harmful to the health of humans. Food safety issues drive the development of highly sensitive and selective methods for the detection of aflatoxins [23,24]. Among them, several nanoparticle-based immunological methods, using electrochemical, optical, and piezoelectric detection have been developed. Nanoparticles are used as either support materials for the immobilization of biomolecules or as tracer (signal tags) to obtain amplified detection. These assays are generally fast, easy to operate, cost-effective (require fewer reagents), highly sensitive and could be combined with microfluidics. In this review we summarize the analytical performance of these methods and discuss some representative examples to illustrate novel concepts and promising applications in the field of food safety monitoring.

2. The importance of aflatoxins

2.1. Toxicity and carcinogenicity

Aflatoxins are highly toxic secondary metabolites produced mainly by the fungal species *Aspergillus flavus* and *Aspergillus parasiticus* [25]. They are present in a wide range of agricultural products such as maize, rice, pistachio, cereals and peanuts [26]. Chemically, aflatoxins are a group of difuranocoumarin derivatives, which consist of a double-furan-ring and a coumarin moiety, where the furofuran moiety is essential for toxic and carcinogenic activity [27]. Six aflatoxins are considered important, i.e., B1, B2, G1, G2, M1 and M2 (Fig. 1). The B-group aflatoxins (B1 and B1) contain a cyclopentane ring while the G-group counterparts have a lactone ring. They can emit blue or green (B = blue, G = green) fluorescence under the irradiation of ultraviolet light. Aflatoxins M1 and M2 are hydroxylated products of aflatoxins B1 and B2, which are mainly present in milk. Among the 6 compounds, AFB1 is usually predominant in amount and constitutes the most poisonous substance.

The main route that people are exposed to aflatoxins is through diet. Moldy foods like maize and peanuts can contain a high amount of aflatoxins. In addition, milk products might contain aflatoxins (AFM1 and M2) if the animals were fed on moldy feeds containing AFB1 and B2 [28]. The intake of a large amount of aflatoxins in short time can cause vomiting, abdominal pain, edema, jaundice, acute liver damage or even death [29]. Moreover, humans may be affected by liver cancer after exposure to small amounts of aflatoxins over a long period. Reactive metabolites like AFB1-8,9-*exo*-epoxide are formed, which can covalently react with nucleophilic sites of DNA or RNA, resulting in gene mutations that are associated with hepatocellular carcinoma [30,31]. In 1993, the International Agency for Research on Cancer (IARC) classified AFB1 as Group I carcinogen in humans [32].

Because of the extremely high toxicity and carcinogenicity of