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Disposable amperometric immunosensor for simple and sensitive determination of aflatoxin B_1 in wheat

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ABSTRACT

A disposable and label-free electrochemical immunosensor was developed for aflatoxin B_1 (AFB_1) determination in wheat based on immobilization of anti-AFB_1 antibodies on the chitosan-gold nanoparticles (chitosan-AuNPs) modified gold microelectrode. The chitosan-AuNPs nanocomposite formed by one-step electrodeposition using chronoamperometry not only exhibited improved conductivity but also offered porous three-dimensional morphology, plentiful amine groups and good biocompatibility for antibody immobilization via covalent binding. To evaluate the effect of electrodeposition conditions on the surface structure and amperometric response of the chitosan-AuNPs modified microelectrode, both scanning electron microscope (SEM), cyclic voltammetry (CV) and energy dispersive spectroscopy (EDS) were studied under the different parameters of deposition potential and time. The morphology and electrochemical characterizations of the immunosensor were also investigated by atomic force microscopy (AFM) and CV. The developed immunosensor demonstrated good linearity between highly sensitive current response and AFB_1 concentration in two ranges of 0.2–2 ng mL^{-1} and 2–30 ng mL^{-1}, with a detection limit of 0.12 ng mL^{-1} (S/N = 3). For assay of AFB_1 in wheat matrix, the linear range from 1.6 to 32 ng mL^{-1} was obtained considering the extraction of the sample.

1. Introduction

Aflatoxins, as one of mycotoxins, are secondary metabolites produced mainly by moulds of the *Aspergillus* family, which are naturally occurring contaminants in agriculture commodities and food, such as peanuts, maize, cottonseed and tree nuts [1]. Especially, aflatoxin B_1 (AFB_1) is considered a very dangerous food safety risk given its occurrence in the food chain because of its potent toxicity, carcinogenicity, mutagenicity and teratogenicity in exposed humans [1]. Due to the fact that climate in the production areas and conditions in the storage are both the most important factors for *Aspergillus moulds* growth on crops pre-, during, and post- harvest [2,3], contamination of crops by aflatoxins is unavoidable in most instances, which has been a very important issue for global food security. Aflatoxins contamination of food not only has severe negative consequences for human health but also for global agriculture products trade. On a worldwide basis as of 2003, specific regulations for regulatory limits of aflatoxins in food and/or feed had been established in at least 99 countries that had about 87% of the world's populations [4]. For example, the Food and Drug Administration (FDA) of USA has mandated the maximum limit of 20 μg/kg for aflatoxins in all foods [5], and the maximum levels of AFB_1 and sum of aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) strictly set by European Commission are respectively 2 μg/kg and 4 μg/kg in some kinds of cereals and the products derived from these cereals [6].

Reliable and sensitive analytical methods for detection of aflatoxins are essential to prevent exposure to aflatoxins. Various well-established methods for determination of AFB_1 in real samples are based on chromatographic technique and immunoassay method. Chromatography-based methods, such as high performance liquid chromatography with fluorescence detector (HPLC-FLD) [7] or coupled to tandem mass spectrometry detector (HPLC–MS or HPLC–MS/MS) [8,9] have been officially accepted for simultaneous confirmatory analysis of aflatoxins.

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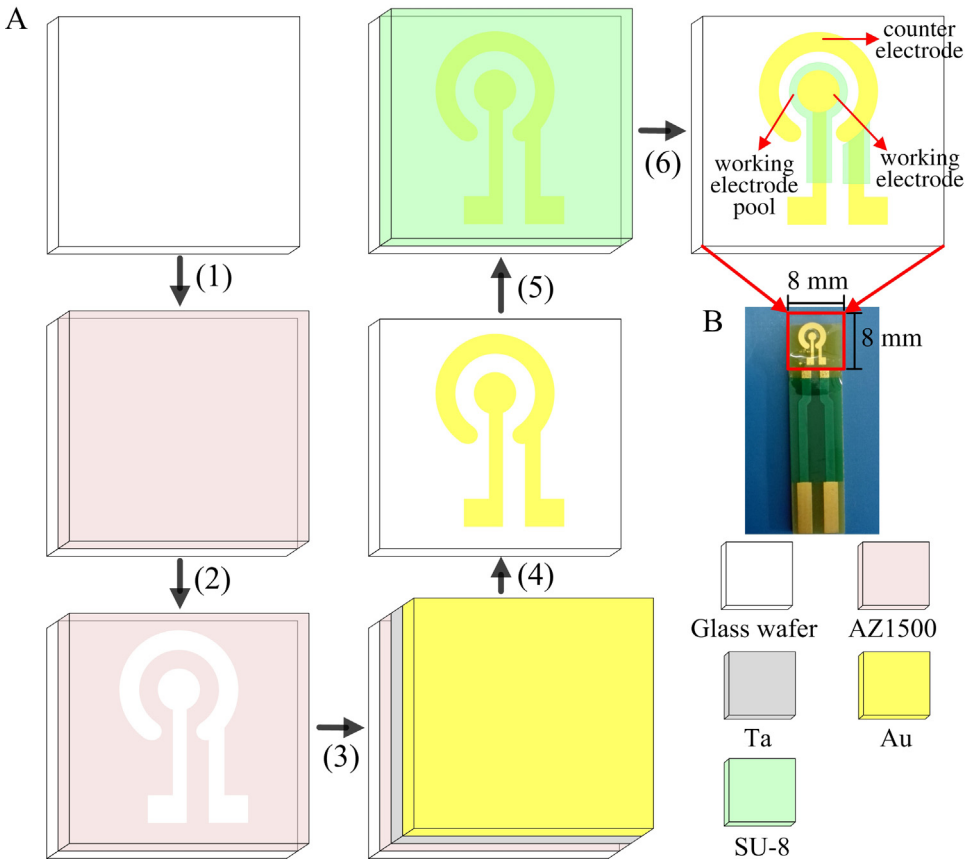

Fig. 1. The fabrication processes by MEMS technology (A) and photography (B) of the disk-ring microelectrode.

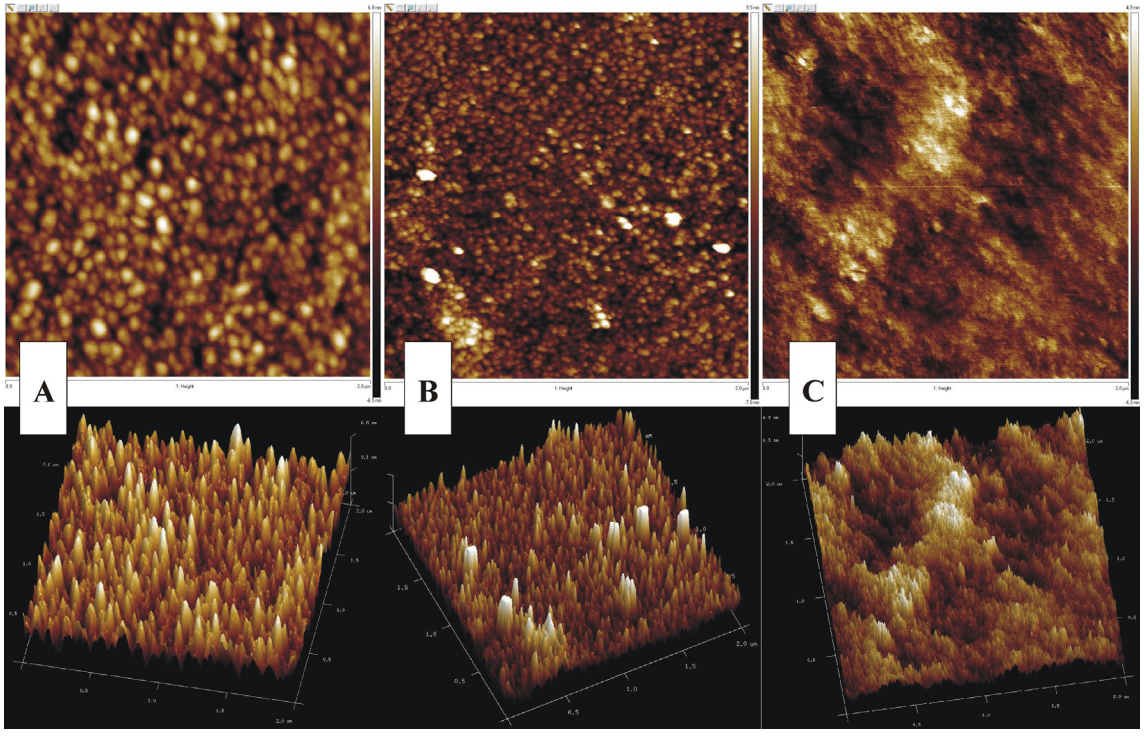

Fig. 2. AFM images of bare gold (A), chitosan-AuNPs/gold (B) and MAb/chitosan-AuNPs/gold (C) microelectrode.

Although chromatographic techniques have many advantages providing high sensitive and accurate quantification of analytes, they usually use solid-phase extraction columns or immunoaffinity columns in extensive cleanup procedures, and often require expensive laboratory facilities, well-trained operators as well as pre- or post-column derivitizations in some cases, which make