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Abstract

Construction of an Array of Antibody–Gold Nanoparticle Conjugates for Their Comparative Assessment on Multiplex Lateral Flow Test to Detect Mycotoxins †

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A multiplex lateral flow immuno-biosensor for rapid, simple, and ultrasensitive on-site quantification of aflatoxin B1, type B-Fumonisin, and zearalenone in food requires the conjugation of their anti-toxin antibodies to the gold nanoparticles of various colors and sizes to obtain different color test lines. The biosensor we developed integrates a multiplex indirect competitive lateral flow immunoassay (LFIA) based on the colorimetric detection of antibody-conjugated toxins.

Bio-functionalized gold nanoparticles with anti-toxin antibody conjugates have unique physical, biochemical, and optical properties that can greatly improve the performance of biochemical assays because they can enhance the signal intensity of analyte, improve signal transduction, have higher sensitivity of analyte detection, and provide simple colorimetric signal readouts. The surface properties of gold nanoparticles, along with various antibody conjugates, play a significant role in terms of functionalizing gold nanoparticles, and the strategy for surface modification is of great significance to the application of gold nanoparticle-mediated biochemical assays. Spherical gold nanoparticles are among the most used reporter molecules in lateral flow assays. However, using identical coloration for test strips and control zones can be misleading for the interpretation of the assay's results. We propose an immunochromatographic test strip with lines of different colors. For this purpose, gold nanoparticles of different shapes were used, namely blue, red, and purple gold nanospheres, nanoflowers, and nanodiamonds. A detailed synthesis procedure for gold nanoparticle synthesis and their conjugates (anti-toxin antibodies) was considered under the influence of different physicochemical reaction parameters for optimal results. Three different-sized gold nanoparticles were prepared to conjugate with an aflatoxin B1, type B-fumonisin, and zearalenone antibodies for a gold nanoparticle-based immunochromatographic assay. This study focuses on the conjugation efficiency of aflatoxin B1, type B-Fumonisin, and zearalenone antibodies with different-sized gold nanoparticles under different physicochemical reaction conditions. The effect of various physicochemical reaction factors such as pH value, concentration, and the ratio of antibodies, as well as the role of NaCl as an aggregating agent, has been discussed using UV-visible spectra and particle size analysis methods. It was found that different-sized gold nanoparticles had different conjugation efficiencies under different pH conditions and concentrations of antibodies. Consequently, different-sized gold nanoparticles should be labeled with antibodies under optimal pH value and with the optimal concentrations of antibodies to improve the sensitivity and the reproducibility of results. The findings of this study will be helpful for the application of antibody-labeled gold nanoparticles in the fields of environmental analysis, clinical diagnosis, and other related fields in the future.

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