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A fluorescent immunochromatographic strip test using Quantum Dots for fumonisins detection

F. Di Nardo [a], L. Anfossi [a,*], C. Giovannoli [a], C. Passini [a], V.V. Goftman [b,c], I.Y. Goryacheva [b], C. Baggiani [a]

[a] Department of Chemistry, University of Turin, Via Giuria, 5, I-10125 Turin, Italy
[b] Department of General and Inorganic Chemistry, Chemistry Institute, Saratov State University, Astrakhanskaya 83, 410012 Saratov, Russia
[c] Faculty of Pharmaceutical Sciences, Ghent University, Ottergemsesteenweg 460, 9000 Ghent, Belgium

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ABSTRACT

A fluorescent immunochromatographic strip test (ICST) based on the use of Quantum Dots (QD) was developed and applied to detect fumonisins in maize samples. A limit of detection for fumonisin B1 of 2.8 μg L^{-1} was achieved, with an analytical working range of 3–350 μg L^{-1}, corresponding to 30–3500 μg kg^{-1} in maize flour samples, according with the extraction procedure. The time required to perform the analysis was 22 min, including sample preparation. Recovery values in the range from 91.4% to 105.4% with coefficients of variation not exceeding 5% were obtained for fortified and naturally contaminated maize flour samples. To evaluate the possible improvements due to the use of QD for ICST technology, we performed a direct comparison of the proposed QD-ICST to a gold nanoparticles- and a chemiluminescent-ICST previously developed for fumonisins detection, in which the same immunoreagents were employed.

1. Introduction

Rapid diagnostic assays have been in use for decades in the clinical and medical area. Nowadays rapid immunoassay-based tests are widely applied in clinical, drug, food [1–3], forensic [4] and environmental [5] analysis.

The immunochromatographic strip test (ICST), also known as lateral flow immunoassay, offers several advantages when compared to other immunoassay methods, such as an easy-to-operate format, rapid detection, no requirements of technical expertize and relatively low cost. To perform a test with the ICST technique, no instrumentation is needed. It is thereby considered as particularly feasible for using outside the laboratory. An ICST device is based on immunoassays in which the sample flows by capillary forces along an analytical membrane that contains immobilized immunoreagents. Traditional ICSTs employ colloidal gold to generate visual signals and usually provide a binary yes/no answer that corresponds to the presence of the analyte over or under a certain concentration in accordance with a specific or mandatory cut-off level. To reach a better sensitivity and an easier objective interpretation, however, new labels need to be explored.

The use of fluorescent labels instead of colorimetric labels leads to a significant lowering of the detection limit in different analytical methods [6,7]. Fluorescent labels, moreover, have found widespread use in biosensing applications including immunoassays, nucleic acid detection, resonance energy transfer studies, diagnostic assays, cellular labeling and others [8–13].

Fluorescent materials could represent, therefore, an obvious choice also for the ICST development. However, many of the organic dye- and protein-based fluorophores currently in use suffer from serious chemical and photophysical liabilities, such as the pH dependence, the self-quenching at high concentrations, the susceptibility to photo-bleaching, the short-term stability in aqueous media, and the short lifetimes of the excited state. These limitations have been partially solved by the development of the fluorescent semiconductor nanocrystals (Quantum Dots, QDs) [14].

Since their first description in a biological context [15,16], QDs have attracted the interest of the biosensing community due to their unique luminescent properties. These inorganic compounds are mainly composed of elements of the II–VI or III–V groups (e.g., CdSe, CdTe, CdS, ZnSe, or core/shell systems such as CdSe/ZnS and CdSe/ZnSe, among others) with sizes comprise between 2 and 10 nm. The QDs' shape is generally spherical, and they contain hundreds or thousands of atoms, depending on their final size. Compared to conventional fluorophores, QDs have excellent fluorescent properties, such as high quantum yields, size-tunable fluorescence and broad absorption spectra, narrow and symmetric emission spectra, large molar extinction coefficients, strong

* Corresponding author.
E-mail address: laura.anfossi@unito.it (L. Anfossi).

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fluorescence emission, and high resistance to photobleaching. The special properties of these nanomaterials are explained by the strong confinement of electrons when the radius of the particle is smaller than the Bohr exciton radius, which results in a quantization of the electronic energy levels. The band gap of QDs increases as their size decreases. The distance between the discrete energy levels, which defines the absorption and photoluminescence characteristic, can be tuned through the choice of the material and the particle size [17].

Since high-fluorescent QDs are usually synthesized at high temperature in organic solvents, they are only soluble in non-polar organic solvents, which are not suitable for biological applications. For water compatibility and bioconjugation, QDs are covered with hydrophilic shells.

Among the different fluorescent labels for ICST, QDs are the most promising. Recently, several non-competitive QD-based ICSTs have been developed, which aimed at detecting *Staphylococcus aureus* in food [18], alpha fetoprotein and carcinoembryonic antigen simultaneously [19], alpha fetoprotein [20], foodborne pathogens [21], syphilis [22], and *Mycobacterium* species [23]. Nevertheless, very limited research on competitive QD-based ICSTs [24–26] has been reported in the literature.

In the present study, we described the use of QDs for the development of a fluorescent competitive ICST for fumonisins (FMs) detection in maize flour.

FMs are mycotoxins mainly produced by *Fusarium* species growing on agricultural commodities in the field, at the harvest or during the storage [27]. There are different forms of fumonisins and among these, Fumonisin B1 (FMB1) is the most common naturally occurring form, followed by FMB2 and FMB3. FMB1, mainly found in maize and maize-derived food, is a hepatocarcinogen in rodents and a kidney carcinogen in rats [28]. It has been classified as a possible human carcinogen (Group 2B) by the International Agency for Research on Cancer (IARC) [29]. Due to adverse effects in animals and humans and to their incidence in food, FMs are regulated by European Union [30] and their monitoring is mandatory for food safety assessment.

Moreover, the use of FMB1 as a model analyte for the development of a QD-based ICST allowed us to make a well-founded evaluation of the potential of QDs as new labels for ICST, thanks to the comparison with more traditional probes (gold nanoparticles, GNP-ICST) and an ultrasensitive chemiluminescent-based ICST (CL-ICST). These assays were previously developed by our group and employed exactly the same analytical antibody (Ab vs FM, raised against the FMB1) and reagents deposited on the nitrocellulose membrane [31,32].

2. Materials and methods

2.1. Reagents

Fumonisin B1, zearalenone, aflatoxin B1, deoxynivalenol (Oekanal standard solutions), polyethylene glycol (PEG, average mw 10 kDa), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), cadmium oxide (CdO, 99.99%), selenium powder (Se, 99.99%), sulfur powder (S, 99%), zinc acetate ($Zn(OAc)_2$, 99.99%), oleic acid (90%), 1-octadecene (90%), oleylamine (70%), octadecylamine (90%), and trioctylphosphine, poly(maleic anhydride-alt-1-octadecene) (PMAO, M ~ 30 000-50 000) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Tween 20, Triton X-100 and other chemicals were purchased from Merck-VWR (Milan, Italy).

Polyclonal antibodies towards Fumonisin B1 Ab vs FM developed in rabbit and BSA-FMB1 were kindly provided by Generon srl (Modena, Italy). The γ-globulin fraction was isolated by ammonium sulfate precipitation and used without any additional treatments. Jeffamine M1000 (1000 g/mol) was kindly provided by Huntsman (Belgium).

Millipore High Flow (HF) 240, 180, 120 and 90, and cellulose adsorbent pad were obtained from Merck Millipore (Billerica, MA, USA).

Buffers and solutions used in the work: sodium hydrogen phosphate–sodium dihydrogen phosphate 20 mM pH 7.4 (phosphate buffer), sodium hydrogen phosphate–sodium dihydrogen phosphate 100 mM pH 7.4, sodium hydrogen phosphate–citric acid 20 mM pH 6.5), tris(hydroxymethyl)aminomethane hydrochloride (Tris) 20 mM pH 7.4, (Tris) 20 mM pH 8.5, sodium borate–boric acid, 20 mM pH 8.5 (borate).

2.2. QD synthesis

Hydrophilic core/shell/shell CdSe/CdS/ZnS QDs with emission at 603 nm and photoluminescence quantum yield of ~30% were synthesized as previously described [33]. Briefly, CdSe QDs were prepared using a hot-injection method, and then they were covered with three layers of CdS and two layers of ZnS by the successive ionic layer adsorption and reaction method.

The hydrophobic QDs were transferred to aqueous solutions by covering with an amphiphilic polymer. The polymer was synthesized from the PMAO and the Jeffamine M-1000 polyetheramines (molar ratio was 1:1). Purified QDs and the amphiphilic polymer were mixed in chloroform and stirred overnight at room temperature (mass ratio of the QD:PMAO was~1:7). An equal volume of $NaHCO_3$ solution (0.1 M) was added to the QDs-polymer chloroform solution. Afterwards, the chloroform was slowly evaporated with a Bunsen's water-air-jet pump, and a clear fluorescent solution was obtained. Finally, to remove the Jeffamine M1000 excess, we performed three rounds of ultrafiltration with Amicon centrifuge filters (100 kD MWCO).

2.3. Preparation of QD–Ab vs FM conjugates

The QD–antibody conjugates (QD–Ab) were prepared using the activated ester approach, in which the carboxyl groups of the polymer coating QD surface were activated with EDC. The activated carboxyl groups were subsequently reacted with amine groups of the antibodies.

Operatively, 0.1 ml of QDs diluted in 1 mL of 20 mM phosphate buffer, pH 7.4, was pre-activated with 0.05 ml of a freshly prepared aqueous solution of EDC (10 mg mL^{-1}) for 20 min at room temperature. Then, 0.15 mg of Ab vs FM was added to the pre-activated QDs and incubated for 30 min at room temperature. A second aliquot of EDC was added and the tube was transferred to 4 °C for a further overnight incubation. The molar ratio of QD:Ab:EDC in the conjugate synthesis was 1:10:4000. QD–Ab conjugates were centrifuged for four times at 14,000 rpm to remove any unbound antibodies. After the addition of 10% Triton X-100, the conjugates were stored at 4 °C, in the dark, until use.

2.4. Preparation of test strips

Assay strips were prepared from nitrocellulose membranes (Hi-flow plus 180) employing an XYZ3050 platform (Biodot, Irvine, CA, USA), equipped with two BioJet Quanti™ 3000 Line Dispenser for non-contact dispensing. In particular, from bottom to top of the strip, the FMB1-BSA conjugate (0.2 mg mL^{-1}) and the goat anti-rabbit IgG antibodies (1 mg mL^{-1}) diluted in phosphate buffer were dispensed to form the T-line and the C-line, respectively. Reagents were deposited at 1 μL cm^{-1}, keeping a distance of 4 mm between the lines.

Membranes were dried at 37 °C for 60 min under vacuum and then assembled with a cellulose pad as the adsorbent pad, with 1–