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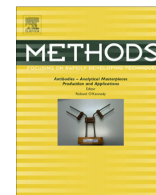

Fluorescently labelled multiplex lateral flow immunoassay based on cadmium-free quantum dots

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ABSTRACT

A sensitive tool for simultaneous qualitative detection of two mycotoxins based on use of non-cadmium quantum dots (QDs) is presented for the first time. QDs have proven themselves as promising fluorescent labels for biolabeling and chemical analysis. With an increasing global tendency to regulate and limit the use of hazardous elements, indium phosphide (InP) QDs are highlighted as environmentally-friendly alternatives to the highly efficient and well-studied, but potentially toxic Cd- and Pb-based QDs. Here, we developed water-soluble InP QDs-based fluorescent nanostructures. They consisted of core/shell InP/ZnS QDs enrobed in a silica shell that allowed the water solubility ($QD@SiO_2$). Then we applied the $QD@SiO_2$ as novel, silica shell-encapsulated fluorescent labels in immunoassays for rapid multiplexed screening. Two mycotoxins, zearalenone and deoxynivalenol, were simultaneously detected in maize and wheat, since the two $QD@SiO_2$ labelled conjugates emit at two different, individually detectable wavelengths. The cutoff values for the simultaneous determination were 50 and 500 μg kg^{-1} for zearalenone and deoxynivalenol, respectively, in both maize and wheat. Liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) was used to confirm the result.

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1. Introduction

Colloidal quantum dots (QDs) have emerged as a new class of fluorescent labels for biomedical diagnostics [1,2], molecular imaging [3–5] and chemical analysis [6,7]. QDs are characterized by unique optoelectronic properties such as a size-tunable light emission, broad absorption spectra that enable the simultaneous excitation of multiple fluorescence colors, high photoluminescence quantum yield (QY), low susceptibility to photobleaching, and excellent chemical, thermal, and photo-stability [8,9]. Until recently, research has focused mainly on Group II-VI (e.g., CdSe, CdTe, HgS) or Group IV-VI (e.g., PbSe, PbS) types of QDs [10,11]. Despite their properties being well suited for imaging at the single-molecule level and for multiplexed biomedical diagnostics, their high toxicity remains a major concern for responsible, real-world applicability [10]. Although surface coatings such as ZnS can significantly reduce cytotoxicity, it is not completely eliminated [12].

Currently, $CuInS_2$, Ag_2S and InP QDs are considered the main alternatives to the toxic Cd- and Pb-based QDs for biophotonic applications [13–16]. InP QDs are one of the most promising candidates [17]. Indeed, InP QDs offer a comparable or even broader emission wavelength coverage than Cd-based nanoparticles (size-tunable emission from blue to NIR) [18], high absorption coefficients, high emission QY [14,19], and an absence of toxic elements [13,20–23]. To date, there are very few studies reporting the use of InP QDs in bioimaging applications [24,25] due to the difficulties in obtaining good quality QDs [25].

Abbreviations: EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; OVA, albumin from chicken egg white; BSA, bovine serum albumin; CEST, carboxyethylsilanetriol; DON, deoxynivalenol; ELISA, enzyme linked immunosorbent assay; FLISA, fluorescent labelled immunoassay; InP, indium phosphide; LFIA, lateral flow immunoassay; LC–MS/MS, liquid chromatography coupled to tandem mass spectrometry; MAb, monoclonal antibody; S-NHS, N-hydroxysulfosuccinimide sodium salt; CMO, O-(carboxymethyl) hydroxylamine hemihydrochloride; PBS, phosphate buffered saline; Brij L4, polyethylene glycol dodecyl ether; QD@SiO2, QDs enrobed in a silica shell; QDs, quantum dots; QY, quantum yield; RT, room temperature; TEOS, tetraethyl orthosilicate; TEM, transmission electron microscopy; ZEN, zearalenone.

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Nowadays, the amount of publications covering easy-to-operate immunochemical tests for simultaneous determination of several analytes is constantly rising. Rapid methods, like lateral flow immunoassays (LFIA), do not require any sophisticated instruments, complex operations, long analysis time, or skilled personnel to manipulate. LFIA can provide fast and robust detection of multiple co-occurring mycotoxin analytes in different matrices, enabling prompt corrective action when contaminants are detected. However, LFIA is considered to be less sensitive than instrument-based immunoformats (enzyme linked immunosorbent assay (ELISA), biosensors, fluorescent labelled immunoassay (FLISA)). Increasing LFIA sensitivity is possible by changing the label/signal reporter used to enable visual detection. Various nanoparticles have been used as labels in LFIA, such as carbon nanoparticles [26], fluorescent dyes [27], liposomes [28], magnetic nanoparticles [29], colloidal gold [30,31]. Cd-based QDs are widely applicable labels both for single-analyte LFIA [32,33] and multiplex formats [34]. Application of non-toxic non-Cd QDs is a prerequisite for further progress in this research field.

In this study, the synthetic conditions for obtaining orange and yellow core/shell QDs enrobed into a silica shell were investigated. We used these water-soluble fluorescent labels to develop a rapid qualitative immunofluorescent test for the simultaneous determination of two mycotoxins: zearalenone (ZEN) and deoxynivalenol (DON) (Fig. 1). Mycotoxins are low-molecular-weight toxic secondary metabolites produced, under appropriate environmental conditions, by filamentous fungi mainly *Aspergillus spp*., *Penicillium* spp. and *Fusarium* spp. They are common contaminants of many grains like wheat, barley, maize, and rice, and can evoke a broad range of pathologies [35]. Mycotoxins have significant impact on human and animal health and can contribute to economic losses. Around a quarter of the world's food crops are contaminated with mycotoxins and globally more than 30% of food and feed samples are co-contaminated [36]. Fusarium mycotoxins, such as ZEN and DON are widely distributed in the food chain worldwide. This shows the importance of mycotoxin control in food and feed, and they should be considered as a major food safety concern. To the best of our knowledge there are no multiplex immunochemical techniques based on InP QDs currently developed and described.

2. Materials and methods

2.1. Materials

Indium(III) chloride (99.999%), zinc(II) chloride (⩾98%), zinc(II) bromide (⩾98%), tris(diethylamino)phosphine (97%), zinc stearate (technical grade, 65%) were purchased from Sigma-Aldrich (Bornem, Belgium). Trioctylphosphine (>97%) and sulfur powder was purchased from Strem Chemicals (Newburyport, MA, USA). Oleylamine (80–90%) was purchased from Acros Organics (Thermo Fisher Scientific, Geel, Belgium). Oleylamine is stored under an inert atmosphere). Octadecene (technical 90%) was purchased from Alfa Aesar (Ward Hill, MA, USA). Tetraethyl orthosilicate (TEOS), carboxyethylsilanetriol, sodium salt 25% (CEST), polyethylene glycol dodecyl ether (Brij L4), 1-ethyl-3-(3-dimethylaminopro pyl)carbodiimide (EDC), N-hydroxysulfosuccinimide sodium salt (S-NHS), O-(carboxymethyl) hydroxylamine hemihydrochloride (CMO), Tween 20 (Tween; polyoxyethylenesorbitan monolaurate), bovine serum albumin (BSA), albumin from chicken egg white (OVA), phosphate buffered saline tablets (PBS), Tris-Borate-EDTA buffer 10x concentrate were purchased from Sigma-Aldrich (Bornem, Belgium). Agarose was purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). ZEN and DON were supplied by Fermentek (Jerusalem, Israel). Methanol and the protein concentrators (9 K, 20 mL) were purchased from Biosolve (Valkenswaard, The Netherlands) and Thermo Scientific (Erembodegem, Belgium), respectively. All other chemicals and solvents were of analytical grade. Polyclonal rabbit anti-mouse immunoglobulins (2.1 g L^{-1}) were provided by Dako Cytomation (Heverlee, Belgium). Chloroform, sodium sulfate, different nitrocellulose membranes (Hi-Flow (HF) plus 09002XSS, HF13502XSS, HF18002XSS, HF24002XSS), glass fiber conjugate pad and sample pad were supplied by Merck Millipore (Darmstadt, Germany). Membrane 'Fusion 5' and agarose were purchased from GE Healthcare (Diegem, Belgium). Monoclonal anti-ZEN and anti-DON antibodies were prepared at the Laboratory of Food Analysis at Ghent University, Ghent, Belgium. Cross-reactivity of the ZEN monoclonal antibody was 69% with α-zearalenol, 42% with α-zearalanol, 22% with zearalanone and none at all (<1%) with β-zearalenol and β-zearalanol. Cross-reactivity of the DON monoclonal antibody was 147% with 3-acetyldeoxynivalenol, 65% with 15-acetyldeoxynivalenol, and minor cross-reactions with DON-3-glucuronide (31%) and deepoxy-deoxynivalenol (28%), but no cross-reactivity towards the other trichothecenes. The synthesis of DON-OVA was performed according to [37], whereas the synthesis of ZEN-OVA was presented in [38].

Size distribution of the particles was measured by dynamic light scattering (DLS) using the Zetasizer Nano ZS (Malvern, England). All measurements were carried out at 25 °C. Bright-field transmission electron microscopy (TEM) images were taken using a Cs-corrected JEOL 2200 FS microscope (Peabody, MA, USA) operating at 200 kV. UV–vis absorption spectra of QDs were measured by a

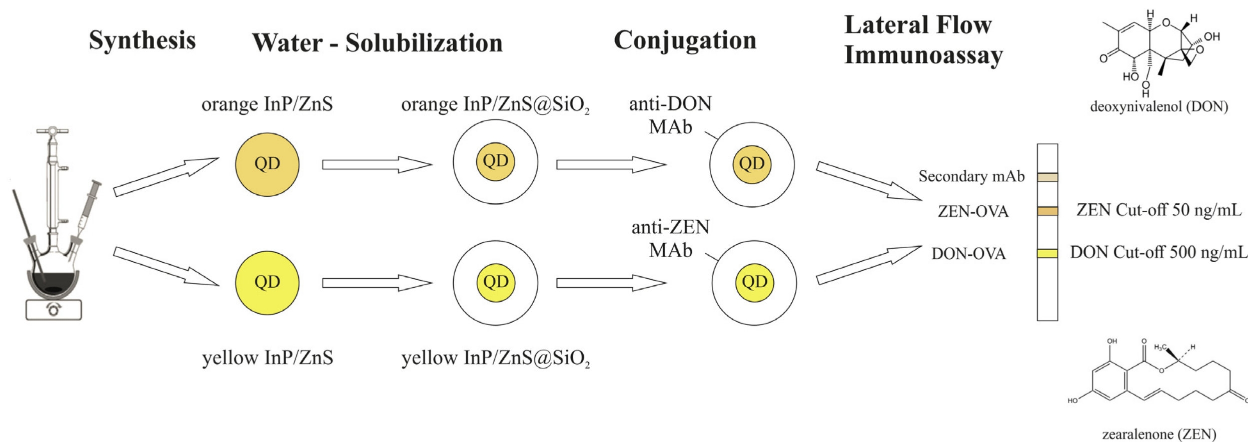

Fig. 1. Scheme of the investigation.

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