Quantum dots in multiplex mycotoxin analysis

Holder: Dr. E. Speranskaya
Project leader: Prof. Dr. S. De Saeger
Faculty of Pharmaceutical Sciences, Laboratory of Food Analysis,
Ghent University

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INTRODUCTION

Nowadays the amount of publications devoted to the simultaneous determination of several analytes is constantly rising. Together with chromatography, screening methods which are cost-effective, simple to perform and not laboratory-based become increasingly popular. Most of these multiplex techniques are immunochemical such as ELISA, fluorescent polarization immunoassay, lateral flow dipsticks, immunochips, microarrays. As a rule, these techniques do not require complicated sample pretreatment and only involve a one-step extraction or/and dilution. Although on-site tests are less precise and specific than chromatography, the best examples allow to operate in the pg mL^{-1}- ng mL^{-1} range and they are suitable for preliminary on-site screening of large amounts of samples.

All earlier described immunoassays for simultaneous detection of multiple analytes (multiplex immunoassays) were developed by placing immunoreagents, specific towards different analytes, on separate spots (or test-zones, or tubes, or wells) within one test system. This approach allows single pretreatment of each sample followed by its distribution over a series of wells. This multiplex approach was widely common because the signals provided by two enzymes cannot be separated. Separation of the signals provided by two organic dyes requires statistical methods due to their asymmetrical unsharpened emission peaks which are broadened by a red-tail. Multiplex based on the simultaneous detection of multiple analytes on a single spot is exclusively possible through detection with quantum dots (QDs) as Cd-QDs are characterized by narrow symmetrical photoluminescent peaks. Multiplex systems based on the use of QDs which emit in different parts of the spectrum without any mathematical or statistical processing of the obtained results, were already successfully described for multicolour bioimaging, multiplex electrochemiluminescence immunoassay, multiplex luminescent microarrays.

Colloidal semiconductor nanocrystal QDs are characterized by unique size-tunable optical properties which favor their use for biomedical diagnostics: they have higher brightness and a higher signal to noise ratio compared to traditional organic fluorophores due to the combined effect of high absorption across a wide spectral range ($0.5\text{-}5\cdot10^6$ $M^{-1}cm^{-1}$) and good

photoluminescence quantum yield (QY). They possess a broad absorbance band and a narrow size-dependent symmetrical sharply defined emission peak. Thus, QDs with different colours of emission (e.g. with different core size) can be simultaneously excited with a single wavelength light source which dramatically simplifies the realization of multicomponent analysis. Therefore, their use in multiplex analysis is obvious and prospective.

In this project Cd-based hydrophilic QDs with different colour of emission were synthesized for further use as fluorescence labels in immunoassay. Two types of multiplex fluorescent-labeled immunosorbent assay (FLISA) were developed: single-analyte multiplex (SAM) FLISA in which the specific antibodies were immobilized into separate wells and double-analyte multiplex (DAM) FLISA in which two antibodies specific to different analytes were placed into the same well for simultaneous fluorescent detection of two analytes: aflatoxin B1 (AfB1) and zearalenone (ZEN). Simultaneous detection of five mycotoxins (fumonisin B1 (FB1), ZEN, AfB1, T2-toxin (T2), deoxynivalenol (DON)) in one plate (quantitative format) and simultaneous determination of four analytes (DON, ZEN, AfB1, T2) in one test column (qualitative format) were realized by the SAM FLISA.

METHODS AND RESULTS

Synthesis of hydrophilic CdSe-based quantum dots with different colours of emission

CdSe QDs are the most extensively used QDs in biochemistry and immunoassay because their size-tunable emission lies in the visible region (450-650 nm). CdSe-based QDs with different colour of emission were synthesized by high-temperature synthesis in organic solvent following their hydrophilization by polymer encapsulation. CdSe quantum dots were prepared via a rapid hot-injection method in octadecene. By changing the reaction time, CdSe QDs with fluorescence peaks at 500÷650 nm could be produced (Fig. 1). The uncoated CdSe QDs have a relatively low fluorescence QY. The fluorescence QY and stability can be sufficiently improved by growing an inorganic shell of the wide band-gap semiconductor around the cores. Zinc sulfide is the best shell material due to its large band gap (3.8 eV for bulk ZnS, 1.74 eV for bulk CdSe). Small CdSe cores (with d up to ~2.5 nm) can be covered directly with a ZnS shell. The growing of ZnS shell results in a small (less than 10 nm) red shift of absorption and fluorescence spectra (Fig. 2A). In the case of larger CdSe cores an additional layer of CdS semiconductor with intermediate lattice parameters is grown between the CdSe core and the ZnS outer shell to reduce

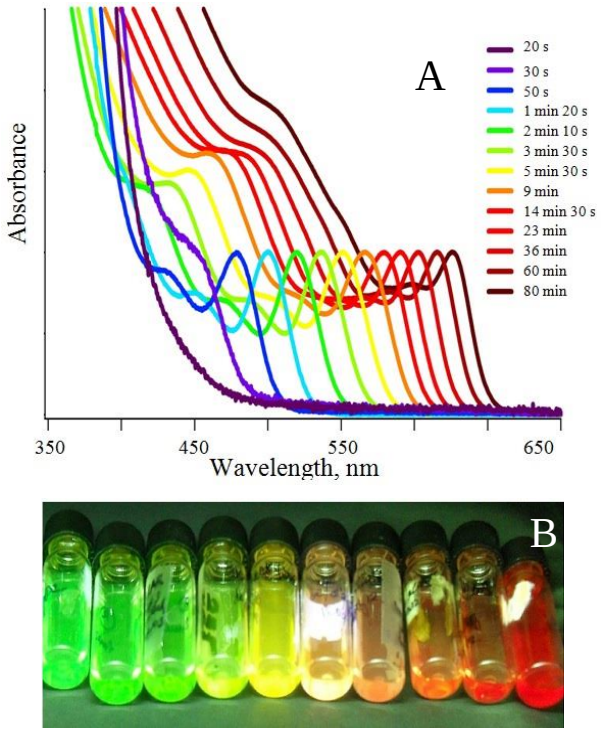

Fig. 1 The change of absorption spectra (A) and colour of fluorescence (B) of CdSe QDs during synthesis

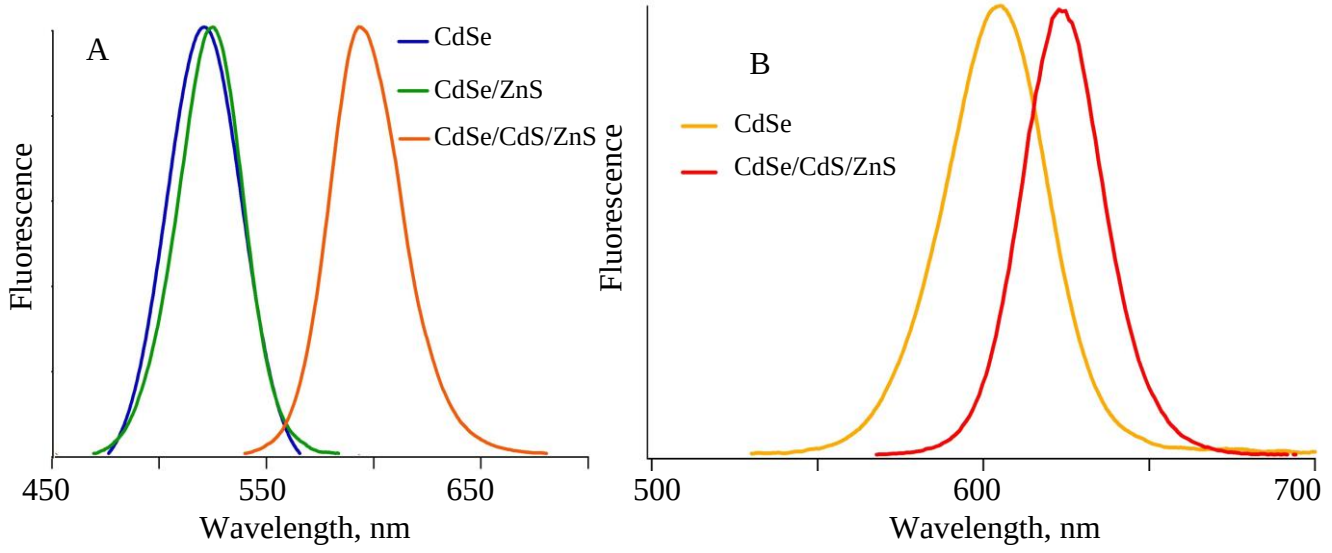

Fig. 2 Fluorescence spectra of CdSe core QDs and core-shell QDs: d(CdSe) = 2.3 nm (A); d(CdSe) = 4.1 nm (B). All spectra are normalized on peak maximum

the strain inside nanocrystals. The difference between band gaps of CdSe (1.74 eV) and CdS (2.42 eV) is not enough for complete confinement of electrons and holes inside the CdSe core. Therefore the formation of CdS shell around CdSe cores results in a sufficient red shift of both the first absorption maximum and fluorescence (Fig. 2B). In Fig.2A the shift of fluorescence spectra of CdSe covered directly with ZnS and with the use of an additional CdSe layer is shown. The conditions of both approaches (time, temperature, number of layers) were optimized. The QYs of the produced QDs are shown in table 1. Transmission electron microscopy (TEM) images of initial CdSe cores and core-shell QDs are presented in Fig. 3.

Table 1. Quantum yields of core and core-shell QDs in organic and aqueous solutions

Sample	Solvent	Ligand	λ(fluoresc), nm	Quantum yield
CdSe cores	toluene	oleic acid	520	1.5%
CdSe/ZnS	toluene	oleylamine, oleic acid	524	35%
CdSe/ZnS	water	oleylamine, PMAO-Jeffamine M1000	540	30%
CdSe/CdS/ZnS	toluene	octadecylamine, oleic acid	585	30%
CdSe/CdS/ZnS	water	octadecylamine, PMAO-Jeffamine M1000	594	25%
CdSe cores	toluene	oleic acid	605	1.5%
CdSe/CdS/ZnS	toluene	octadecylamine, oleic acid	630	33%
CdSe/CdS/ZnS	water	octadecylamine, PMAO-Jeffamine M1000	642	28%

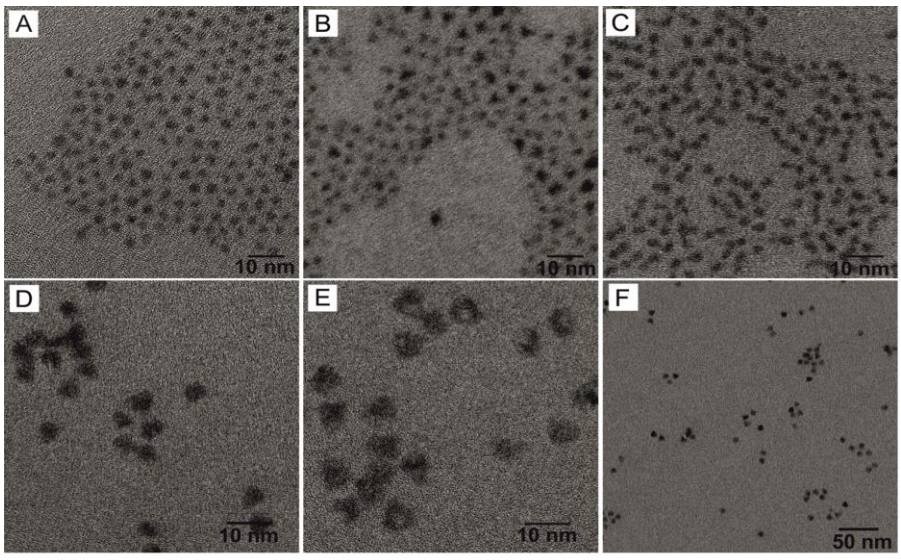

Fig. 3 TEM images: CdSe cores with fluorescence peak at 520nm (A) and corresponding core–shell QDs: CdSe/ZnS (B), CdSe/CdS/ZnS (C); CdSe cores with fluorescence peak at 605 nm (D) and corresponding core–shell CdSe/CdS/ZnS QDs (E) (all in toluene solution); hydrophilic CdSe/CdS/ZnS QDs (λ(em.)=594 nm) (F)

The initially hydrophobic QDs were transferred to an aqueous solution by polymer encapsulation. Encapsulation of QDs with amphiphilic polymers is a preferable way for QDs hydrophilization because the original hydrophobic ligands are not removed from the QDs surface in this process, thus better maintaining the initial QD brightness. The amphiphilic polymer (Fig. 4) was synthesized by dissolving Jeffamine M1000 (2 g) in chloroform (~15 mL) and then added drop by drop to a flask containing 1 g of poly(maleic anhydride-alt-1-octadecene) (PMAO) powder. For encapsulation, QDs and the amphiphilic polymer were mixed in chloroform and stirred overnight at room temperature (molar ratio of QD:polymer was ~1:40). Then an equal volume of 0.1 M $NaHCO_3$ aqueous solution (pH 8.6) was added and the chloroform was slowly removed by rotary evaporation. The excess of polymer was removed by ultracentrifugation on a sucrose gradient. First QDs were transferred from $NaHCO_3$ solution to distilled water by buffer exchange using Pierce Protein Concentrators (9K MWCO) at a centrifugation speed of 4000 rpm. Then the QD solution was carefully put on a sucrose gradient and was ultracentrifuged. The gradient density range and the speed of ultracentrifugation were varied. The best results were obtained at the following conditions: 275 000 *g*, 1.5 hour, room temperature, sucrose gradient consists of two layers – 1 ml of 50% sucrose and 0.7 ml of 10 % sucrose. To control the behavior of the PMAO-Jeffamine polymer, it was transferred from chloroform to distilled water in the same way as the QDs to be ultracentrifuged on a sucrose gradient at the same conditions as the aqueous QD dispersions. The polymer fluoresces blue under UV excitation and could be easily detected (Fig. 5). After ultracentrifugation QDs stayed in 10% sucrose layer and the polymer did not penetrate to this layer. The layer of QDs was taken out with a needle and was purified from sucrose by three rounds of centrifugation using Pierce concentrator (20ml/9K MWCO, Thermo Scientific) at room temperature. After the purification step, the QDs were transferred to phosphate buffered saline (PBS) solution by buffer exchange. The obtained hydrophilic QDs covered with the polymers retained up to 85% of their fluorescence after the phase transfer (Table 1) and showed stability over a wide pH range.

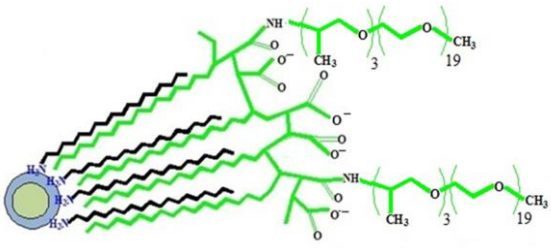

Fig. 4 The structure of hydrophilic QDs covered with the polymer

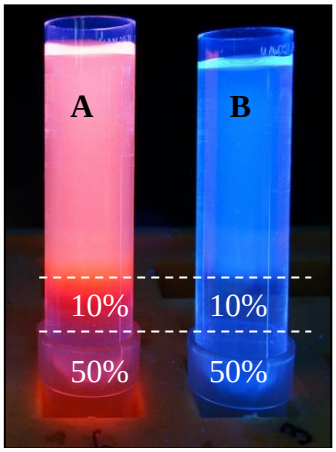

Fig. 5 QDs (A) and polymer (B) solutions after ultracentrifugation

QDs covered with PMAO-Jeffamine M1000 polymer posses free carboxylic groups for further conjugation of QDs with analytes. To produce QDs with amine groups QDs covered with

PMAO-Jeffamine M1000 polymer were bound with Jeffamine ED 2003 polymer through activation of carboxylic groups with ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (Fig. 6). The best EDC/QDs ratio was determined by gel electrophoresis and the initial QDs moved with different rates through the gel.

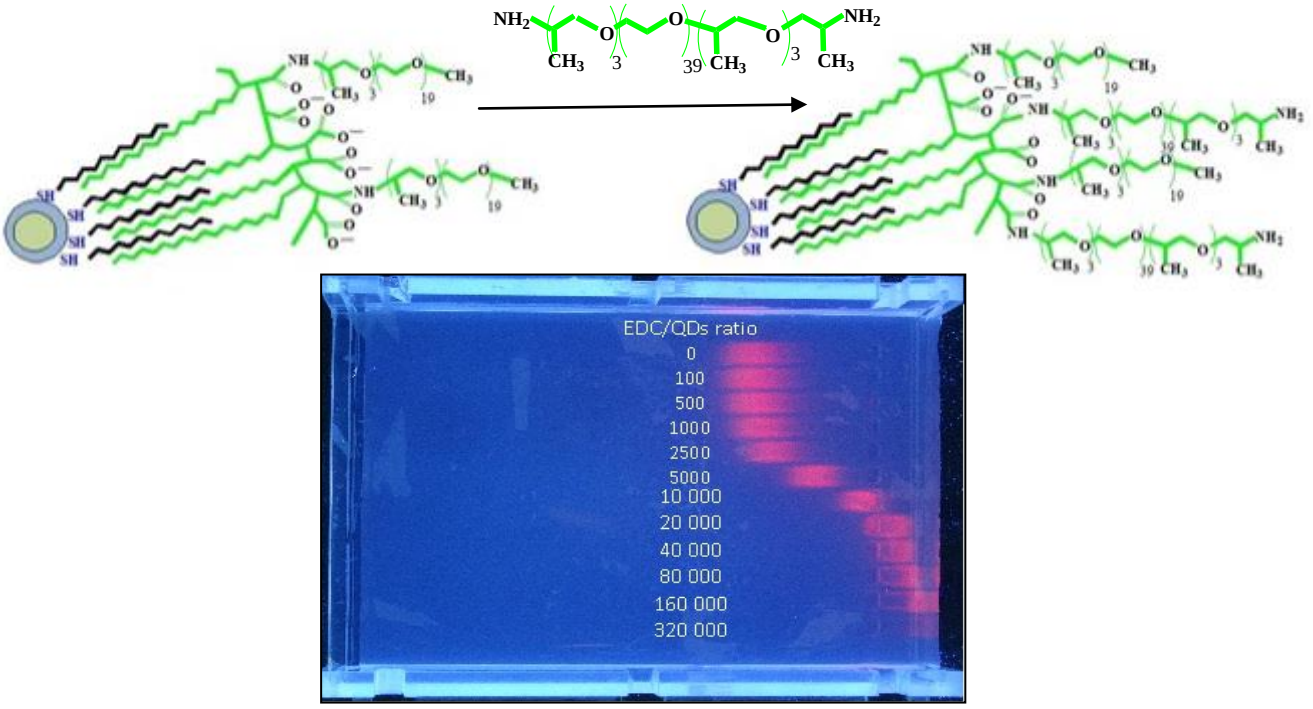

Fig. 6 Scheme of hydrophilic QDs interaction with Jeffamine ED 2003 and electrophoresis of the QDs produced with different amount of EDC

Conjugation of QDs with analytes

QDs were conjugated with the following mycotoxins: FB1, ZEN, AfB1, T2, DON. All mycotoxins were preliminarily conjugated with proteins (ovalbumin (OVA) or bovine serum albumin (BSA)).

T2-OVA, ZEN-OVA and FB1-OVA were conjugated with QDs covered with PMAO-Jeffamine M1000 polymer through activation of the QDs' carboxylic groups. The ratio NHS/EDC/QDs/analyte equal to 50/50/1/7.5 was used for QDs-labeling. The QD solution (~3.2×10^{-4} µmol in PBS) was dropwise added to the mixture of NHS and EDC, stirred for 2 hours at RT and incubated overnight at 4°C. Afterwards analyte solution in PBS was added and the reaction mixture was gently stirred during 3 hours at RT, and then again incubated overnight at 4°C. The unbound low-molecular substances were removed through dialysis against PBS (2 days, 4 °C). A tenfold excess of 1M glycine in PBS was added for reaction termination by blocking of the activated COOH-groups. The mixture was incubated during 2 hours at 37° C and dialyzed (4 °C, 2 days, against PBS).

DON-OVA, AfB1-cBSA were conjugated with NH_2-containing QDs. For coupling thiolation of DON-OVA (or AfB1-cBSA) with SPDP was done. In parallel, sulfo-SMCC was used to modify NH_2-containing QDs. SPDP (100 nmol in ethanol) was added dropwise to DON-OVA (AfB1-cBSA) solution (20 nmol in sodium phosphate, pH~7.5). The reaction mixture was incubated for 30 min at RT under constant stirring, and after, excess of SPDP was removed by the protein concentrator tube. The residue was reconstituted in sodium acetate buffer (pH~4.5). For pyridine-2-thione release, dithiothreitol (250 nmol in water) was added dropwise to the modified protein conjugate. The reaction mixture was incubated for 30 min at RT under constant stirring, and then excess of dithiothreitol was removed by the protein concentrator tube. The modified conjugates were redissolved in sodium phosphate solution. In parallel, sulfo-SMCC (2 mg) was added dropwise to the QD solution (1 nmol). The reaction mixture was stirred for 1 h at RT. Excess of sulfo-SMCC was removed by gel-filtration. The modified QDs were added dropwise to the thiolated DON-OVA (AfB1-cBSA). Reaction was continued 2 hours under constant stirring at RT, afterwards, the mixture was completed by addition of 50 mM cysteine to block excess of maleimide-reactive groups. The obtained conjugates were purified by gel-filtration.

After synthesis, the presence of all QD-based conjugates was confirmed by gel electrophoresis: the obtained QD-analyte conjugates and free QD moved with different rates through the gel.

Development of QD-based multiple fluorescent-labeled immunosorbent assay (FLISA)

Two types of multiplex FLISA were developed: SAM FLISA in which the specific antibodies were immobilized into separate wells and DAM FLISA in which two antibodies specific to different analytes were placed into the same well for simultaneous fluorescent detection of two analytes: AfB1 and ZEN.

The following protocol was used for FLISA: The 96-well opaque black microtiter plates were coated with rabbit anti-mouse antibody (5 μg/mL; 100 μL/well in 0.05 M sodium carbonate buffer, pH 9.6) for 2 h at 37 °C. Then the plates were washed three times with phosphate buffer saline (PBS) containing 0.05 % (v/v) Tween 20 (PBST) and blocked for 1 h at 37 °C. As blocking solutions, PBS containing 3% skim milk (w/v) was chosen. Further, the plates were washed two times with PBST, and specific primary antibody (100 μL/well) was added. The plates were incubated for 2 h at 37 °C and then washed three times with PBST. Standard mycotoxin solution (in the range of 0.001 – 1000 ng mL^{-1} in PBS) was added simultaneously with QDs-labeled antigen. After 1 hour-incubation the plates were washed with PBST. To the content of each well 100 μL of PBS was added, and luminescence was measured.

For SAM FLISA, the strips specific for different analytes were within one plate and different QDs-conjugates were added depending on the strip. For the DAM format, the mixture of anti-

ZEN and anti-AfB1 antibodies was immobilized in each well and the mixture of ZEN-QDs and AfB1-QDs (1/1, v/v, in the appropriate dilutions) was added as labeled conjugate.

Analytical characteristics and calibration curves of SAM FLISA and SAM ELISA are given in Table 2 and Fig. 7. It was found that application of QDs as a label in competitive immunosorbent assay led to a significant increase of the method sensitivity: IC_{50} values for FLISA were in the range of 0.11-11 ng mL^{-1}, whereas those for ELISA were 1.5-62 ng mL^{-1} (~3-12 times decrease depending on the mycotoxin).

Table 2. Analytical characteristics for mycotoxins' determination by ELISA and single-analyte multiplex (SAM) FLISA in buffer solutions (n = 5).

	LOD, ng mL^{-1}		IC_{50}, ng mL^{-1}		Linear range, ng mL^{-1}	
	ELISA	FLISA	ELISA	FLISA	ELISA	FLISA
DON	1.4	0.16	27±2	11±1.4	6.5-80	2-29
ZEN	0.2	0.03	1.5±0.3	0.13±0.03	0.45-3.63	0.06-0.23
AfB1	0.12	0.01	1.34±0.05	0.11±0.02	0.37-3.28	0.03-0.26
T2	5	0.5	62±5	9±1.1	16-160	2-24
FB1	0.2	0.02	2.4±0.07	0.3±0.06	0.6-5.9	0.08-0.89

But as SAM FLISA only demonstrated an increased sensitivity as a benefit over the traditional ELISA, DAM FLISA resulted in a substantial simplification of the analysis procedure. Since two specific antibodies (anti-ZEN and anti-AfB1) were immobilized into the same well, the DAM format allowed addition of a mixture of AfB1-QDs and ZEN-QDs (1/1 v/v, in appropriate dilutions). This is in contrast with SAM FLISA, where different labeled conjugates were added into different wells separately. The conjugates were labeled with QDs which luminesce in different parts of the spectrum: ZEN was labeled with green-emitting QDs and AfB1-with orange-emitting QDs. Since the spectra of QDs slightly overlapped (wavelength of maximum of emission peak of green QDs was at 540 nm, whilst orange QDs emitted with the maximum at 594 nm), the analytical signal could be detected for both analytes separately without any statistical tools by double scanning of the plate with different emission wavelengths.

Because the mixture of labeled conjugates was added into the same well, the number of washing steps after the addition of conjugate increased to four instead of three to obtain complete removal of unbound QDs-conjugates. This could lead to a decrease in analytical signal intensity and subsequently to an ambiguous interpretation of the results. Therefore, the concentrations of the labeled conjugates were increased in comparison to SAM FLISA in order to enhance the analytical signal. This resulted in a lower DAM FLISA sensitivity compared to SAM FLISA:

IC_{50} values of 13 µg kg^{-1} and 10 µg kg^{-1} for DAM multiplex instead of 2.6 µg kg^{-1} and 2.2 µg kg^{-1} for SAM multiplex for ZEN and AfB1 respectively. Calibration curves for mycotoxin detection by DAM FLISA are presented in Fig. 8. It should be emphasized that an interaction between the labeled conjugates was not be detected. The limits of detection for the simultaneous determination of ZEN and AfB1 by this technique were 1.8 and 1 µg kg^{-1}, respectively.

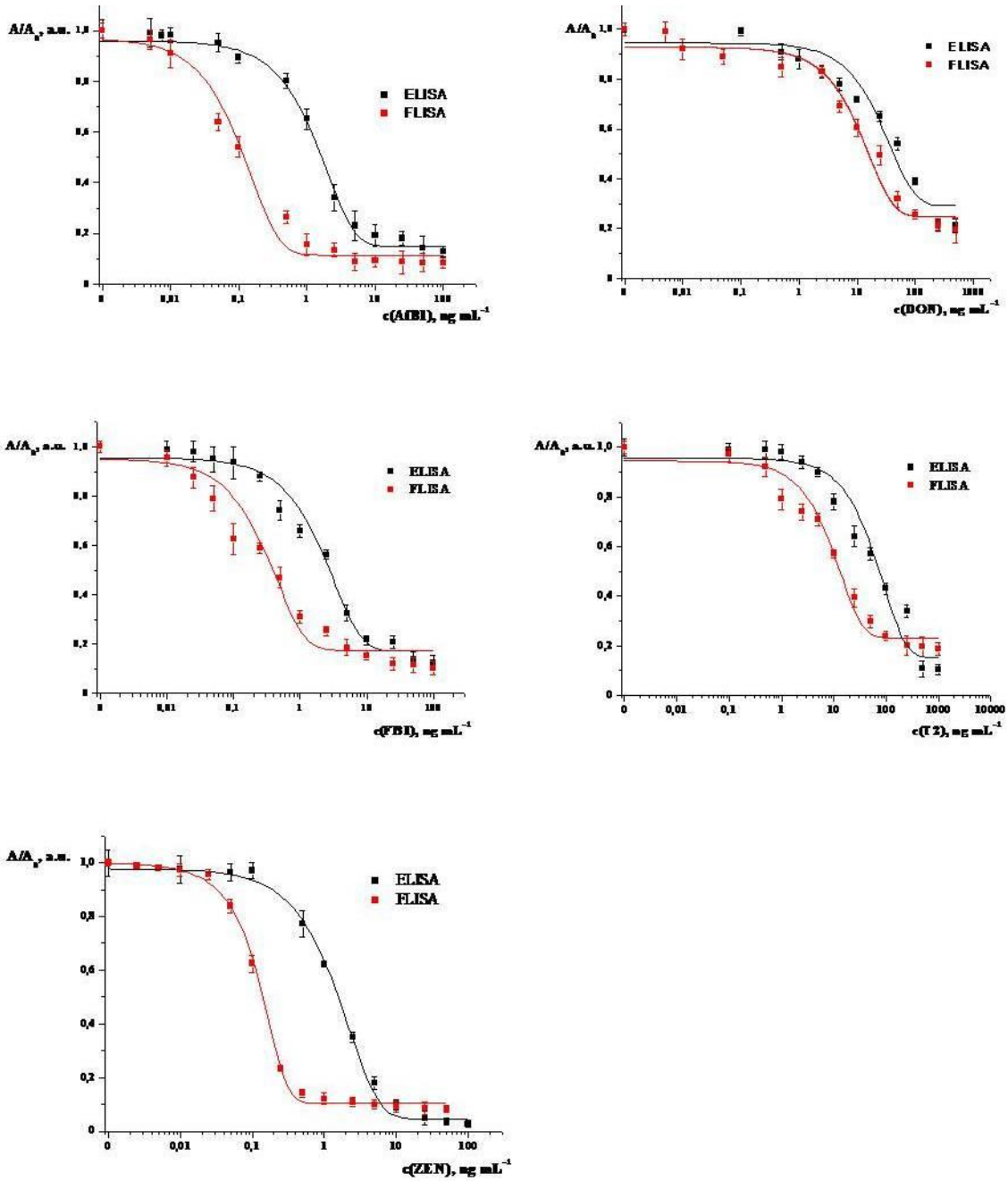

Fig. 7 Calibration curves for the mycotoxins determination by ELISA and FLISA. A/A_0 is a relative analytical signal: absorbance (λ= 450 nm) for ELISA and luminescence (λ= 540 nm for green QDs, 594 nm for orange QDs and 642 nm for red QDs) for FLISA.

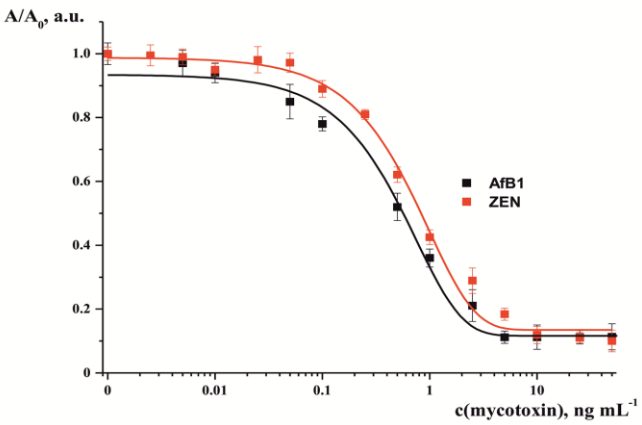

Fig. 8 Calibration curves for the mycotoxins determination by DAM FLISA. A/A_0 is the relative luminescence (λ_{em} = 540 nm for green QDs (ZEN determination), 594 nm for orange QDs (AfB1 determination)). Data indicate averages of fivefold determinations.

Development of a QD-based multiplex column test

A rapid qualitative non-instrumental immunochemical test which can be performed outside the laboratory for the simultaneous screening of four mycotoxins in cereals was developed. The intention was to develop a gel-based immunoassay (GBI) with sensitivities close to the maximum limits of the target mycotoxins, established by the European Commission Regulation as 1250 and 1750 µg kg^{-1} for DON, 100 and 350 µg kg^{-1} for ZEN, 2 and 5 µg kg^{-1} for AfB1, 100 and 200 µg kg^{-1} for the sum of T2 and HT2, 4000 and 4000 µg kg^{-1} for the sum of FB1 and FB2 in unprocessed wheat and unprocessed maize, respectively. Optimization of the GBI procedure included choice of optimal antibody and QDs-conjugates concentrations, composition and amount of washing buffer. Primary antibodies were placed onto the gel via binding with the already immobilized secondary antibody (SA). This was done to ensure universal distribution of primary antibodies. Thereby, to reach the required sensitivity, three variables: SA-coupled gel dilution, primary antibody concentration and QDs-labeled conjugate dilution were simultaneously evaluated.

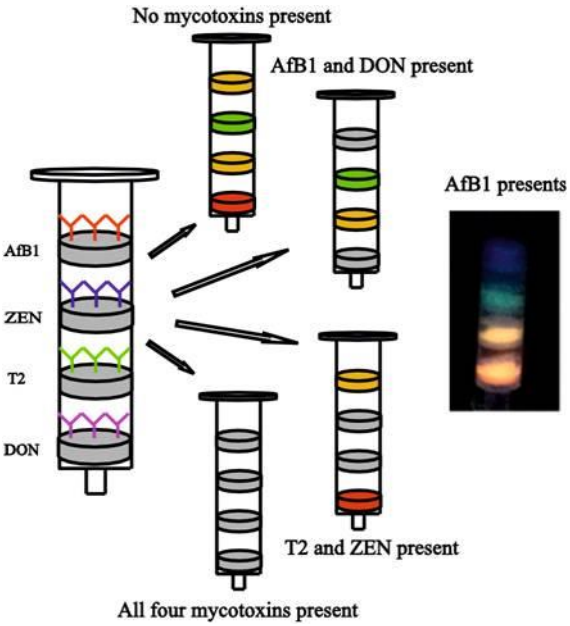

Fig. 9. Gel-based immunoassay set-up for simultaneous detection of deoxynivalenol, zearalenone, aflatoxin B1 and T2-toxin and examples of analysis outcomes

First, the column tests were developed and validated for each target mycotoxin (DON, ZEN, AfB1, T2, FB1) separately, afterwards their combination was achieved. A GBI with four test layers was designed. A test with five layers was found to be difficult to operate. Application of red-emitted QDs in the GBI was cumbersome. Polyethylene frits as well as sepharose gel showed a weak red colour under UV leading to the appearance of false-negative results. So, just one mycotoxin (DON) out of four was labeled with the red QDs. The dilution of the DON-QDs was high (1/15 for the single GBI and 1/12 for the multiplex format) to determine the analytical signal.

The main challenge was the simultaneous determination of four mycotoxins at the specified cut-off levels. A mixture of DON-QD, ZEN-QDs, AfB1-QDs and T2-QDs was used as conjugate, so the dilution of every labeled antigen was elevated in order to decrease their influence on each other and facilitate the signal recognition. Since both wheat and maize were tested, the lowest maximum limit for each toxin was taken as cut-off, while for DON a cut-off level of 500 μg kg^{-1} was chosen. Bearing in mind the extraction and dilution of sample, a GBI with cut-offs at 500, 100, 2 and 100 μg kg^{-1} for DON, ZEN, AfB1 and T2, respectively, was created. The visual contrast between the results obtained with positive samples and those obtained with negative samples was easily detected. Fig. 9 shows the results obtained with a typical multiplex assay column after analysis of a sample spiked with AfB1 in the concentration above the cut-off.

Application of the developed immunoassays for analysis of naturally-contaminated cereals

Calibration curves for both immunoassay (SAM and DAM FLISA) set ups in two different matrices (wheat and maize) led to a slight change of the assay sensitivities (IC_{50} values): 14 and 17 ng mL^{-1} for DON, 0.16 and 0.18 ng mL^{-1} for ZEN, 0.14 and 0.18 ng mL^{-1} for AfB1, 11 and 13 ng mL^{-1} for T2 and 0.5 and 0.7 ng mL^{-1} for FB1 by SAM FLISA in wheat and maize, respectively, and 0.69 and 0.74 ng mL^{-1} for ZEN and 0.65 and 0.68 ng mL^{-1} for AfB1 for DAM FLISA in wheat and maize, respectively. Hereupon, determination of mycotoxins in naturally-contaminated samples was done using calibration curves prepared in blank maize extract. A set of 19 wheat and 34 maize samples was used for validation of the developed multiplexes. As a confirmation technique LC-MS/MS was chosen.

Comparison of results obtained by LC-MS/MS and SAM FLISA is shown in Fig. 10. The SAM FLISA allowed AfB1 detection in the concentration range 0.5-18 μg kg^{-1}. Among the samples which were negative in LC-MS/MS, 17 samples tested positive for the

target mycotoxins (ZEN, AfB1, T2) in a concentration range between the LODs of the SAM FLISA and the LOQs detected by the chromatographic technique. A good agreement was demonstrated with the samples which were found to be contaminated, as determined with LC-MS/MS (r^2=0.981).

For the mycotoxins determination by DAM, an excellent correlation with the data obtained by LC-MS/MS was found: r^2=0.983 for ZEN and r^2=0.977 for AfB1. Seventeen samples which lacked ZEN and AfB1 according to LC-MS/MS analysis, demonstrated the presence of ZEN and AfB1 in the concentration range ≥LOD (DAM FLISA) and <LOQ(LC-MS/MS).

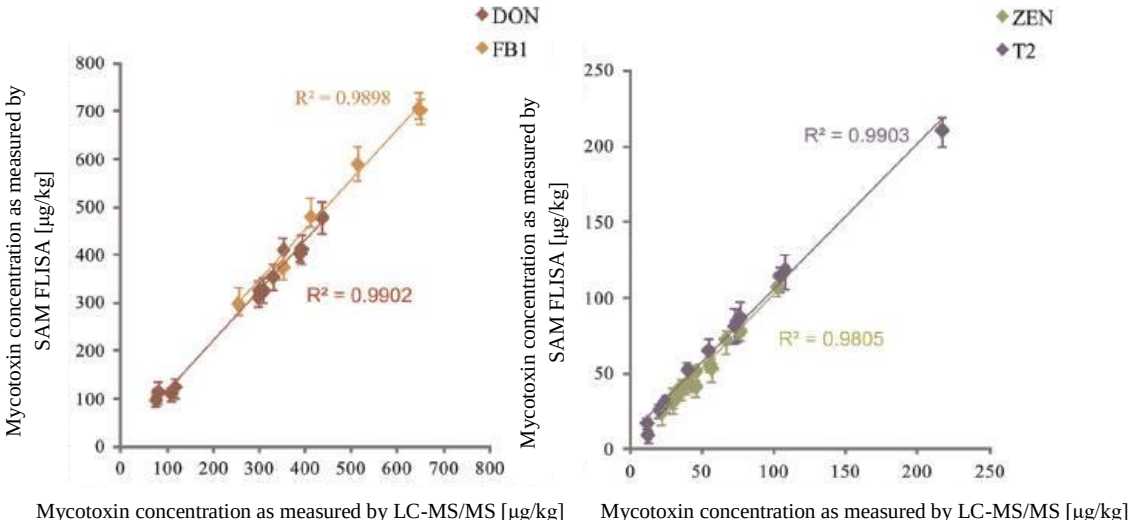

Fig. 10 Linear regression equations derived using SAM FLISA and LC–MS/MS data for mycotoxins screening in naturally-contaminated cereal samples, found to be positive by both LC–MS/MS and the single-analyte multiplex FLISA (data indicate averages of fivefold determinations)

The developed GBI test was validated using 24 naturally- contaminated cereal samples (12 wheat and 12 maize). Comparison of the GBI and LC–MS/MS results showed good agreement both for positive and negative samples. Analytical characteristics of the test were calculated and were based on these results and data of an intra-laboratory validation performed with blank cereal extracts artificially spiked with the target mycotoxins at concentrations less, equal and above the corresponding cut-off levels. The rates for false positive and negative results were always below 5 % and the specificity and sensitivity rates were >96% (Table 3). All calculated parameters fulfilled the requirements set by the Commission Decision2002/ 657/EC for a screening method hence makes the multiplex GBI suitable for mycotoxin screening in cereals.

Table 3. Analytical performance of the column gel–based immunoassay

Parameter	Value, %				
	DON	ZEN	AfB1	T2	FB1
False–positive rate, % ($N_{false\ positive}/N_{-}$) ×100)	2.1	1.9	1.4	2.6	2.4
False–negative rate, % ($N_{false\ negative}/N_{+}$) ×100)	1.5	2.2	1.2	2.8	2.0
Specificity rate, % ($N_{negative}/N_{-}$) ×100)	97.6	96.0	98.5	97.4	96.8
Sensitivity rate, % ($N_{positive}/N_{+}$) ×100)	97.8	98.3	98.0	98.1	97.2

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CONCLUSIONS

The syntheses of Cd-based core-shell QDs with different wavelength of fluorescence peak (CdSe/ZnS, λfl 524 nm; CdSe/CdS/ZnS, λfl 585 nm; CdSe/CdS/ZnS λfl 630 nm) were successfully developed. The obtained QDs were transferred to water solutions by encapsulation with PEG-containing amphiphilic polymer. Special attention was paid to purification of hydrophilic QDs from polymer excess by ultracentrifugation on sucrose gradient. The advantage of this approach is that QDs stay dispersed in solution during the procedure and do not form QD aggregates. The synthesized water-soluble QDs had carboxylic or amine groups for further conjugation with biomolecules. The synthesized QD were demonstrated to be very efficient labels for application in immunoassay.

Two different approaches for multiplex fluorescent immunosorbent assay were employed.

For the first time the immobilization of two different types of antibodies into the same well (double-analyte multiplex, DAM) for simultaneous fluorescent detection of two mycotoxins (zearalenone and aflatoxin B1) was described and compared with single-analyte multiplex (SAM) FLISA where the specific antibodies were immobilized into separate wells. Simultaneous detection of five mycotoxins (deoxynivalenol (DON), ZEN, AfB1, T2-toxin (T2) and fumonisin B1 (FB1)) in one plate (quantitative format) and simultaneous determination of four analytes (DON, ZEN, AfB1, T2) in one test column (qualitative format) were realized by the SAM FLISA. To the best of our knowledge, this is the first time that on-site QDs-based multiplex was developed for the simultaneous determination of four analytes. For detection, multicoloured (red, orange, green) CdSe-based quantum dots were used. Both FLISA formats demonstrated excellent applicability for screening of cereals.

Thus, the developed multiplex assays capable of screening analytes meet the target requirements providing cheap, simple and reliable analytical techniques.

Publications

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