

Fluorescent quantum dots (QDs) find a wide application in immunoassay due to their unique optical properties and high chemical, photo and physical stability. The ability of QDs to form an aqueous colloid and the presence of surface functional groups suitable for bioconjugation significantly simplifies their adaptation for immunoassay. In **Chapter 1** we made an overview of the most common methods of immunoassay and applied nanoparticles. The basic principles, modern applications, advantages and disadvantages of each method were briefly reviewed. Examples of the application of immunochemical methods for the detection of mycotoxins were introduced due to the high impact of these substances on food safety and food production. Mycotoxins are very toxic for human and animals, and therefore negatively affect health and economy. The European Commission as well as other parts of the world national and international organizations have established maximum limits for mycotoxins in food and feed products. The necessity to control mycotoxins leads to the development of new detection methods, including immunoassays. Antibody analogues were also described as a future replacement in immunoassays of such a demanding biological compound. Their structure, properties and variety of applications are based on the different physical and optical properties of nanoparticles. Here we especially paid attention to gold nanoparticles (GNs) and QDs, which were applied in our work. Nanoparticles properties are extremely important for physical processes, such as Förster resonance energy transfer (FRET). We looked in the principle of energy transfer and in particular QDs application for FRET as an acceptor and in Chapter 4 as a donor to show the QDs uniqueness.

In **Chapter 2**, the objectives of the PhD thesis were described in which we showed the possibilities for the application of QDs in heterogeneous and homogeneous immunoassays. Both of them were targeted on the detection of mycotoxins and were relatively fast in comparison with standard methods. In the next two chapters the two newly developed immunoassay analytical methods were described in detail.

A new lateral flow immunoassay (LFIA) was presented in **Chapter 3** with the application of QDs for visual detection of mycotoxins based on the QD's emission. We carefully designed each step of the process to create the brightest and most easy detectable signal. CdSe/CdS and CdSe/CdS/ZnS core-shell heterostructures were synthesised with different core size and shell thickness. By optimising the reversed microemulsion method, we designed a hydrophilic silica coating with different surface composition allowing to preserve a fluorescent quantum yield as high as 70 % in aqueous media. The final silanised QDs were equipped with epoxy and carboxy surface groups and applied for bioconjugation with specific mycotoxin monoclonal antibodies. Red and orange fluorescence conjugates were used in a multicolour LFIA for simultaneous determination of zearalenone and deoxynivalenol (DON). The developed test was validated

through the analysis of 34 naturally-contaminated maize and wheat samples and results were confirmed with liquid chromatography tandem mass spectrometry (LC-MS/MS).

QDs were used for non-radiative energy transfer in **Chapter 4**. We applied the decreasing of the QDs fluorescence in the presence of an energy acceptor. GNs as energy acceptor showed a maximum quenching effect when a Förster distance is less than 12.9 nm. The QDs and GNs donor-acceptor pair was combined with an antibody-antigen pair. QDs were conjugated with antibodies and due to the immunocomplex formation with the GNs antigen conjugate, FRET was realised. Due to the sensitivity of the FRET to the distance between donor and acceptor, we studied different sizes of the immunocomplex by changing the protein which was connected with the antigen. The FRET allowed to develop a fast homogeneous format of immunoassay. We used this system for the detection of DON as an example of a fast method for high-throughput control of mycotoxins with a limit of detection of 28 µg/kg of DON in spiked wheat samples.

In the last part we present a global overview of the commercial applications of QDs in different fields of life. Scientific groups from all of the world are working on the development of QDs and their applications. The future of QDs lies in the creation of new compositions of nanoparticles. Improvement of the hydrophilic shell around QDs will allow a more confident application in biosensors.