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Applications of quantum dots as probes in immunosensing of small-sized analytes

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A B S T R A C T

Quantum dots (QDs) are semiconductor nanoparticles with very interesting optical properties, like high quantum yield or narrow and size-tuneable fluorescence spectra. Current applications of QDs are widespread, their use as fluorescence labels in bioassays being one of the most promising. These nanoparticles are usually conjugated to highly specific biomolecules like antibodies, oligonucleotides, enzymes or aptamers to improve assay selectivity. In this review, QD surface passivation, conjugation to biomolecules, and purification strategies are discussed with special emphasis to the development of QD-based immunoassays for the detection of low molecular weight compounds given the relevance of this sort of analytes in health, food safety, pharmaceutical, or environmental monitoring areas. The aim of this review is to summarise the main achievements attained so far and to initialise researchers in the field of antibody-based assays employing QDs as labels, such as fluorescence-linked immunosorbent assay (FLISA), fluorescence (or Förster) resonance energy transfer (FRET), immunochromatographic methods, and immunosensors.

Contents

1. Introduction . . . 13
   1.1. Evolution of quantum dot research . . . 13
2. Synthesis of quantum dots . . . 14
   2.1. Core/shell quantum dots . . . 16
   2.2. Quantification of quantum dots. . . . 16
3. Surface functionalization . . . 16
4. Commercial quantum dots . . . 16
5. Bioconjugation of quantum dots . . . 18
   5.1. Conjugation by active esters . . . 18
   5.2. Conjugation by activated maleimides and fragmented antibodies. . . . 19
   5.3. Indirect conjugation with avidin bridge . . . 20
   5.4. Indirect conjugation with protein G bridge . . . 20
   5.5. Conjugation using polyhistidine peptides . . . 21
   5.6. Other considerations . . . 21
6. Quantum dot-based fluorescent immunoassays . . . 21
   6.1. Fluorescence-linked immunosorbent assays . . . 21
   6.2. Fluorescence (or Förster) resonance energy transfer . . . 22
   6.3. Immunosensors and related bioanalytical systems. . . . 25
   6.4. Multiplex immunoassays . . . 27
7. Conclusions and future trends. . . . 27
Acknowledgements . . . 27
References . . . 27

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1. Introduction

Quantum dots (QDs) are semiconductor nanoparticles with particular electronic and optical properties that have been widely studied and applied in the last decade. The typical diameter of QDs is in the 1–20 nm range and they can contain from 100 to 100,000 atoms per nanoparticle. Some of the most attractive properties of QDs are high quantum yield, high molar extinction coefficients, broad absorption spectra, narrow and symmetric emission bands (30–50 nm), large effective Stokes shifts and high resistance to photobleaching and chemical degradation (Algar et al., 2010; Rosenthal et al., 2011). The special characteristics of these nanomaterials are explained by the strong confinement of electrons when the radius of the particle is smaller than the exciton Bohr radii. Different applications can be found for QDs, such as in the photovoltaic, thermoelectric or light-emitting diode industries, but their applications in life sciences have revolutionized the state of the art of many biological assays, including fluorescence-linked immunosorbent assays (FLISA), fluorescence resonance energy transfer (FRET) assays, immunosensors, DNA probes, or imaging, currently being a real alternative to the use of traditional organic dyes, enzymatic labels, or isotopic markers.

QDs are very versatile labels because their photoluminescence emission band can be easily tuneable, from the UV to the IR regions, by the selection of the particle size (1–12 nm) and the nature and composition of the nanoparticle, which can be synthesised with binary alloys of atoms from 12–16 (ZnS, CdS, CdSe, HgS), 13–15 (GaAs, InP, InAs, GaN) or 14–16 (PbTe, PbSe) groups (see Fig. 1). Ternary alloys of CdZnS, CdSSe, InNP or InGaAs have been also synthesised with analogous properties (Medintz et al., 2005). The aforementioned versatility in the QD emission wavelength and the fact that they can be excited by a single wavelength makes possible their simultaneous use as fluorescent labels of different processes running at the same time. Moreover, the fluorescence of QDs shows a very narrow emission band, so different markers can be potentially employed in different bioassays for the detection of several compounds in multiplexing studies without spectral interferences. Most of these bioassays involve the conjugation of QDs to a selective biomolecule, like an antibody. In this sense, several QD-based immunoassays have been developed for the detection and quantification of a wide range of pathogens, proteins and toxins, but only few of them have been proposed for the analysis of small-sized analytes (Algar et al., 2010; Biju et al., 2008; Gill et al., 2008). These challenging analytical targets, immunochemically named haptens, encompass a wide range of physicochemically different compounds like veterinary drugs, pesticide and food additive residues, persistent organic pollutants, explosives and war agents, mycotoxins, environmental and industrial contaminants, numerous metabolites in biological fluids, hormones, packaging components, drugs of abuse, etc. The analytical determination of these chemicals is largely performed by accredited laboratories that use instrumental procedures, most of them based on chromatographic separations. Considering the high number of samples that are required to be analysed and the low to moderate sample throughput and high solvent and reagent consumption of those analytical methodologies, unaffordable economical and human resources are often required. The use of immunoanalytical approaches in monitoring programmes is currently deemed feasible and useful alternatives to chromatographic instrumental procedures, based on the high sample throughput, reduced sample treatment, portability, on-site analytical capability, and the high sensitivity and selectivity generally ascribed to these rapid methods (Lee and Kennedy, 2001). Moreover, the use of immunoassays agrees with the basic principles of the Green Chemistry by minimising residues and waste generation (Armenta et al., 2008). Accordingly, this review will pay special attention to the different procedures and methods employed for QD conjugation to antibodies and their application to the analysis of small-sized analytes.

1.1. Evolution of quantum dot research

Despite the first published papers concerning the properties of QDs date back to the mid-eighties, we can consider the synthesis studies by Bawendi's group as the starting point of the revolutionary use of QDs, because they first proposed a new and simple synthetic route for the preparation of semiconductor nanocrystals of uniform size and shape in macroscopic amounts (Murray et al., 1993). From that time onwards, the scientific references found in the literature concerning QDs increase almost exponentially (Fig. 2), with more than 4000 papers published each year over the last decade, and more than 6000 annually in the last five-year period (ISI Web of Knowledge database, Thomson Reuters). This huge productivity on the development and improvement of new applications involving QDs points out the wide acceptance by the scientific community of these innovative fluorescent nanoparticles due to the sound expectations of getting more sensitive, simple and robust immunosensing systems.

Later on, Alivisatos' and Nie's groups simultaneously published the coupling of QDs to different biomolecules (Bruchez et al., 1998; Chan and Nie, 1998), thus opening the door to different pioneer biosensing schemes using QDs as tracers, such as (i) the

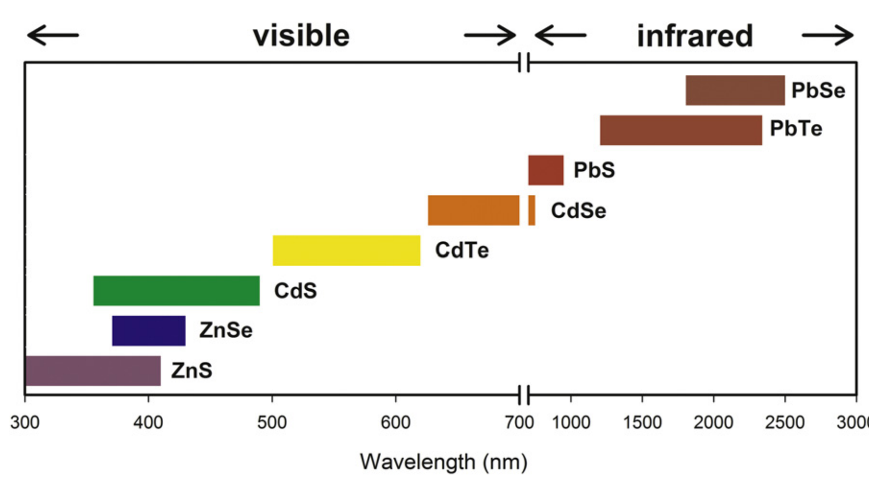

Fig. 1. Dependence of fluorescence emission wavelengths of quantum dots on their chemical composition.