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Applications of quantum dots in Food Science and biology

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ABSTRACT

Quantum dots are inorganic semiconductor fluorescent nanoparticles with a size in the range of 1–10 nm. There are many semiconductor combinations that are used but the most popular and widely used consist of a CdSe core and can be functionalized in different ways when coated with polymers. Their application in Biological sciences has been widespread but few articles report the use of quantum dots in Food Science. The results obtained so far in limited applications show a promising future. In Food Science, they have been used mostly to detect pathogenic bacteria, and proteins. The applications in biological sciences are reviewed since they constitute a path of applications that can be emulated in other fields and the initial uses in Food Science are reviewed to encourage others in the field to take a closer look and benefit from the opportunities offered. Limitations and opportunities in the methods used in Food Science are discussed in order to simplify the work for researchers interested in using quantum dots as markers. The two methods used to attach quantum dots to bacteria and proteins in Food Science were broadly used previously in biological systems. The aim of this review is to show how quantum dots have been used in Food Science, and enhance their use.

1. Introduction

The use of Quantum dots (QDs) in biology as fluorescent tagging tools have been illustrated in many world class scientific publications in Biology with the use of resulting vibrant color images for more than a decade. There are scientific papers presenting very sharp images, from a tagged nucleus in a particular cell (A. M. Derfus, Chan, & Bhatia, 2004a, 2004b), up to, an entire mouse tissue where the QDs were followed through the complete blood system (Ballou, Lagerholm, Ernst, Bruchez, & Waggoner, 2004). QDs have been preferred instead of organic dyes because of their well-known properties (stability, broad excitation spectra, narrow emission spectra), which allow multiple and long term imaging with a very long shelf life (Fitzpatrick et al., 2009; Jamieson et al., 2007). In the last two decades, hundreds of studies have been performed using QDS as fluorescent nano-markers in biological imaging. The different methods used to attach/bind the QDs to specific targets have been constantly improving. Two of the most applied methods in biological imaging include biotin-avidin crosslinking and antibody/antigen crosslinking. These methods are now being used in different areas of Food Science. Biotin-avidin cross-linking, is reported to be a highly sensitive and specific method when detecting pathogenic bacteria in foods (Huang et al., 2014). Antibody-Antigen cross-linking can be used with a monoclonal antibody when more specificity is required, or with a polyclonal antibody, which gives a more intense signal for detection. To use a polyclonal antibody, a Western Blot needs to be performed in order to insure that the QDs will not attach to an undesired protein. This Antibody-Antigen method has also been used to detect pathogenic bacteria, such as, *Escherichia coli* O157:H7, *Salmonella typhimurium* and *Listeria monocytogenes* (Wang, Li, Wang, & Slavik, 2011). The Antibody-Antigen method has also been used to attach QDs to food proteins, specifically gliadin (from gluten) in bread and dough samples (Ansari et al., 2015; Bozkurt et al., 2014; Sozer & Kokini, 2014). By knowing the distribution of the protein in dough a deeper understanding of its function and characteristics can be achieved. This last application seems to be a new opportunity for food scientists not only to investigate the location of proteins in a food matrix, but to monitor the proteins through different food processes, such as mixing.

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2. Quantum dots

2.1. What are quantum dots

Quantum dots are nanocrystals made of a semiconductor materials, often composed of atoms from groups II–VI or III–V elements in the periodic table, and are defined as particles with physical dimensions smaller than the exciton Bohr radius (the radius of the first orbit of an atom) (Chan et al., 2002). The light they produce is much more saturated than light from other sources, and its color will depend upon the size of the Quantum Dot, which can vary from 2 nm up to 10 nm (Henglein, 1989). These inorganic semiconductor fluorophores consist of a core, which is most commonly made of cadmium selenide (CdSe), cadmium telluride (CdTe) or Indium phosphide (InP) (Resch-Genger, Grabolle, Cavaliere-Jaricot, Nitschke, & Nann, 2008). The QDs mostly used are the ones made with a cadmium selenide (CdSe) core, because they have displayed more quantum yield (Bang & Kamat, 2009). Quantum yield is an indicator of the brightness of a fluorescent molecule and it is expressed as the proportion of the light emitted to light absorbed by a fluorophore. This CdSe core is generally covered by a zinc sulfide (ZnS) shell (Fig. 1-a), to increase the resistance to photobleaching and increase the quantum yield (Bang & Kamat, 2009; Mattoussi et al., 2000). The fluorescence is generated when an excited electron relaxes to the ground state and combines with its respective hole (every excited electron leaves a hole in the ground state). The electron emits energy when traveling back from the excited state to the ground state. The energy necessary from jumping from the ground state to the excited state comes from an external source such as UV light. The distance that the electrons have to travel from the excited state to the ground state is known as band gap. When the band gap is larger, the electron emits more energy. Smaller QDs have a larger band gap, thus, their light is bluer because higher frequency wavelength (more energy) is emitted. Bigger QDs have a smaller band gap, so, the light they emitted is redder because lower frequency is emitted (Fig. 1-b) (Visser & Rolinski, 2010).

The size of QDs is controlled during production. QDs are called self-assembled molecules because of their manufacturing process. During CdSe QDs production, cadmium oxide, oleic acid, and 1-octadecene are mixed and heated to completely dissolve cadmium oxide and form cadmium oleate. After the cadmium oxide dissolves, the mixture is kept at 225 °C and trioctylphosphine selenide (made of selenium powder, 1-octadecene, and trioctylphosphine) is added, this allows the cadmium oleate to react with the trioctylphosphine selenide forming cadmium selenide nanoparticles (CdSe). These particles grow in size as the time of reaction is longer (keeping at constant temperature). The growth stops if the temperature is lowered, so, withdrawing some solution at regular intervals and placing it in room temperature vials stops growth and maintains particles at their actual size. The oleic acid is used to prevent nanoparticles from aggregating (Gerion et al., 2001; Nordell, Boatman, & Lisensky, 2005; Peng & Peng, 2001; Yu & Peng, 2002).

2.2. Coating and water solubilization

There are a wide selection of polymeric coatings that can be used with QDs. The application for which the QDs are used define the type of coating that is selected. In biological and food application sin particular water solubilization of QDs is generally required. QDs are typically formed in nonpolar organic solvents which make them insoluble in water. Numerous solubilization approaches have been developed in order to render them hydrophilic, leading to biocompatibility and their ability of being used *in vivo*. The fluorescent and brightness characteristics of QDs make them excellent probes for imaging. The majority of applications for imaging need water soluble probes. The need to use QDs to identify a specific molecule in a biological environment requires a conjugation process to link the QDs to substrates of interest. Different biomolecules are used to link QDs to different targets. Often water soluble QDs are needed in order to bind to biomolecules, polymeric coatings makes them water soluble.

A technique that has been used to make quantum dots water soluble is ligand displacement with molecules with thiol groups. Compact versatile ligands have been used to synthesize quantum dots with improved stability and biological functionalities (Susumu, Mei, & Mattoussi, 2009, 2007). A ligand molecule consists of three units: polyethylene glycol (PEG) to promote hydrophilicity, a functional unit to provide further modification on quantum dot surface linked to the one end of PEG (−COOH, −NH2, −OH and Biotin) and dihydrolipoic acid (DHLA) to offer anchoring on quantum dot surface. Thioctic acid (TA) is attached to one end of PEG and then, DHLA is formed by reduction of 1, 2-dithiolanein groups of TA in the presence of NaBH4 via ring opening reaction. The second approach is for the use with amphiphilic molecules (phospholipids micelles) (Dubertret et al., 2002), poly (maleic anhydride-alt-1-octadecene) (PMAO)-PEG (Yu et al., 2007), amphiphilic polymer shell (Pellegrino et al., 2004), triblock copolymers (Gao, Cui, Levenson, Chung, & Nie, 2004) to obtain biocompatible quantum dots. Amphiphilic molecules possess both hydrophobic and hydrophilic moieties. As hydrophobic molecules bind to hydrophobic ends of amphiphilic molecules via hydrophobic interactions, the hydrophilic end extends from QDs' surface towards the aqueous environment. An examination of QDs coating and water solubilization for biological applications by Smith, Duan, Rhyner, Ruan, and Nie (2006) shows that QDs should be covered with a hydrophobic bilayer if a better stability against chemical oxidation is required. For high stability under acidic conditions, QDs should be prepared using hyperbranched polyethylenimine. Finally, for stability in high salt buffers, it is preferable to have uncharged QDs. In order to obtain QDs which are uncharged they should be coated with PEG.

2.3. Stability and resistance

QDs are highly photostable compared to conventional organic fluorophores. Organic fluorophores such as Rhodamine (Rh), fluoresceinisothiocyanate (FITC) and tetramethylrhodamineisothiocyanate (TRITC), light green, and acid fuchsine bleach after only a few minutes of exposure to light, but QDs are extremely stable and can undergo repeated cycles of excitation and fluorescence for hours with a high level of brightness and without photobleaching (Alivisatos, 1996; Chan & Nie, 1998; Gerion et al., 2001; Jaiswal, Mattoussi, Mauro, & Simon, 2003). Photobleaching is the loss of intensity due to photochemical damage to the chemical structure of the dye over time. Therefore, the amount of light emitted from the organic dyes gradually decreases and lose their brightness. Besides resistance to photobleaching, QDs are also highly resistant to metabolic degradation as reported when QDs were used to label HeLa mammalian cells and *Dictyostelium discoideum* cells (Jaiswal et al., 2003). For these reasons, QDs are used for long-term imaging, which cannot be done by organic fluorophores, because they are susceptible to photodamage and to degradation (Jaiswal & Simon, 2004). The stability of QDs during few days, inclusive more than a week, has been proved with mouse lymphocytes (blood cells) were properly targeted with QDs. The cells were completely distinguishable using fluorescence microscopy and the QDs were proven to be a viable method to label blood cells in mice (Hoshino, Hanaki, Suzuki, & Yamamoto, 2004b). Long-term *in vivo*