Analytical Techniques for Detection of Quantum Dots

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Since the discovery of Quantum Dots (QDs) by Alexey I. Ekimov in 1981, the interest of researchers in that particular type of nanomaterials (NMs) with unique optical and electrical properties has been increasing year by year. Thus, since 2009, the number of scientific articles published on this topic has not been less than a thousand a year. The increasing use of QDs due to their biomedical, pharmaceutical, biological, photovoltaics or computing applications, as well as many other high-tech uses such as for displays and solid-state lighting (SSL), has given rise to a considerable number of studies about its potential toxicity. However, there are a really low number of reported studies on the detection and quantification of QDs, and these include ICP–MS and electrochemical analysis, which are the most common quantification techniques employed for this purpose. Keeping in mind both human health and environmental risks of QDs as well as the scarcity of analytical techniques and methodological approaches for their detection, the adaptation of existing techniques and methods used with other NMs appears necessary.

Keywords: QDs ; properties ; detection

1. Electroanalytical Techniques: Voltammetry

Voltammetry is the general term for all techniques in which the current is measured as a function of electrode potential. Voltammetric-sensing techniques are characterised by simplicity, high sensitivity, good stability, low-cost instrumentation and small sample requirements. To the researchers' knowledge, the recently published literature on the detection of Quantum Dots (QDs) reports the use of Square Wave Anodic Stripping Voltammetry (SWASV), Square Wave Voltammetry (SWV) and Anodic Stripping Voltammetry (ASV) techniques.

1.1. Square Wave Anodic Stripping Voltammetry (SWASV)

A reliable and low-cost three-electrode microchip, produced by screen-printing technology, was used for the SWASV detection of CdS QDs, such as lab-made solutions [1]. CdS QDs are adsorbed on the working electrode, where Cd^{2+} ions are reduced to Cd^{0}. The subsequent oxidation of Cd^{0} to Cd^{2+} allows its electrochemical detection in NaCl 0.05 M solutions (acetate buffer solution pH 4.6). The authors report a linear range in the graph with concentrations of CdS QDs between 50 and 8000 ng mL^{-1}, a relative standard deviation (RSD) of 6.5% and a sensitivity of 0.0009 μA/(ng mL^{-1}). The proposed sensor allows the use of much lower sample volumes than other devices, which opens the way to a variety of applications in microreactions, separations or pre-concentrations.

More recently, Sýs et al. proposed an SWASV method to determine several heavy metals likely have originated from the dissolution of QDs, which suggests the possible indirect determination of QDs [2]. This possibility became true shortly afterwards, when the detection of CdSe/ZnS QDs and PbS QDs by the same technique was published [3]. After studying the conditions for the determination of each type of QDs separately (linearities ranged from 2.5 to 15 nM CdSe/ZnS QDs and from 0.5 to 10 nM PbS QDs), the simultaneous detection of both QDs without any signal overlaps was achieved. The application of these findings to the determination of biomolecules, using QDs as sensitive tags bonded to them, seems to be at hand in the near future.

1.2. Square Wave Voltammetry (SWV)

Square Wave Voltammetry has been reported as an inexpensive procedure for the rapid screening, field analysis and development of electrochemical biosensors. The SWV detection of CdS–GSH QDs was performed at pH values of 3.0 and 7.0 [4]. Different screen-printed electrodes produced reproducible signals (RSD 6.7%), although better responses were achieved by using one electrode for each measurement. Calibration was possible over the range 0.5–14.0 × 10^{16} QDs mL^{-1} (detection limit of ca. 2 × 10^{14} QDs).

The SWV detection of CdSe/ZnS–biotin QDs in an ammonia solution has been reported [5]. The authors report a linear range in graph with concentrations of CdSe/ZnS–biotin QDs between 0.05 and 2 nM (detection limit of 37 pM).

1.3. Anodic Stripping Voltammetry (ASV)

The ASV detection of CdSe/ZnS-biotin QDs in an ammonia solution has been reported [6]. The method takes advantage of the catalytic effect of the QDs on the electrodeposition of Ag at the surface of QDs. The final stripping of the electrodeposited Ag was carried out by differential-pulse voltammetry. The direct relationship between the voltammetric signal and the concentration of CdSe/ZnS-biotin QDs allows its determination.

The ASV detection of CdSe/ZnS QDs and CdSe QDs in organic medium in water after derivatisation with an amphiphilic polymer has been also reported [7]. The detection limits achieved with the selected experimental conditions were 3.0 × 10^{12} nanoparticles mL^{-1} for CdSe QDs dispersed in organic medium and 6.0 × 10^{12} nanoparticles mL^{-1} for water-solubilised CdSe/ZnS QDs. The method can be applied to other QDs, and since the results are expressed in number of QDs per unit volume, the procedure can be useful for the study of toxicological and bioanalytical uses and risks of QDs.

A very sensitive method was applied to the ASV determination of CdSe/ZnS-streptavidin QDs and CdSe/ZnS-biotin QDs [8]. The use of a magnetoelectrochemical supports for screen-printed electrodes to improve the anodic stripping voltammetry of cadmium due to the generated magnetohydrodynamic (MHD) effect has been reported. To create a significant MHD effect, Fe^{3+} was added at mM concentrations to the solution. The reduction in iron(III) simultaneously with the cadmium deposition on the electrode surface allowed the production of a high cathodic current, which generated a large Lorentz force capable of exerting a convective effect on the solution in the presence of the magnetic field. The authors report a linear range in the graph with concentrations of CdSe/ZnS-biotin QDs between 0.05 and 5 nM (detection limit of 0.05 nM).

The detection of organic-capped CdSe QDs via cadmium deposition and ASV measurement was achieved down to the highly dilute concentration of 15 pM [9]. It has been reported that the use of a large capping agent alters the mass transport and solubility of an electroactive species adjacent to the electrode. The altered mass transport regime adjacent to the electrochemical interface can be utilized to afford a highly sensitive analytical signal for the detection of CdSe QDs.

The determination of the number of Zn and Cd atoms in CdSe/ZnS QDs was achieved from ASV measurements [10]. The comparison of the results obtained from ASV and atomic absorption spectrometry (GFAAS) showed good coincidence. In contrast, a comparison of the results with concentrations obtained by UV–vis spectrophotometry revealed large discrepancies. The authors attribute this to the different nanocrystals' absorption cross sections from different synthesis routes. ASV has been also applied for the determination of novel Ag_2S QDs with two different surface coatings: 3–mercaptopropionic acid (MPA) and boronic acid (BA) [11]. The LODs achieved were 4.10 × 10^{10} QDs mL^{-1} for MPA–Ag_2S QDs and 5.70 × 10^{10} QDs mL^{-1} for BA–Ag_2S QDs, both with good precision and with a wide linear response range (10^9 to 10^{12} QD mL^{-1}).

2. Atomic Spectrometry Techniques

2.1. Inductively Coupled Plasma-Mass Spectrometry (ICP–MS)

ICP–MS is a technique useful to determine low concentrations (µg L^{-1}) and ultra-low concentrations of elements (ng L^{-1}). Atomic elements are led through a plasma source where they become ionised. Then, these ions are sorted on account of their masses. The advantages of the ICP–MS technique over AAS (Atomic Absorption Spectrometry) or ICP–OES (inductively coupled plasma optical emission spectrometry) are lower detection limits, larger linear ranges and possibilities for detecting the isotope composition of elements. The ICP–MS technique has a multi-element character and a high sample throughput (similarly to ICP–OES), but it allows the performance of more sensitive measurements. The disadvantages and weaknesses of the ICP–MS are the occurrence of spectral and non-spectral interferences and the high costs.

ICP–MS can identify the nature and/or concentration of dissolved ions released into water during QD-polymer composites degradation. When combined with centrifugal filtration separation techniques, ICP–MS can also quantify the ratio of released nanoparticles to released ions. Single-particle ICP–MS (sp–ICP–MS) can discriminate and quantify ions and nanoparticles. However, sp–ICP–MS can only discern the presence of nanoparticles with an element-dependent size limitation of 11–20 nm for cadmium, 21–80 nm for zinc and > 200 nm for selenium.

The Cd concentration from a small planktonic crustacean (*Daphnia magna*) exposed to of amphiphilic polymer coated CdSe/ZnS QDs was determined by ICP–MS [12]. Fluorescence confocal laser scanning microscopy was used to visualise and spectrally distinguish QDs from competing autofluorescent signals arising from the daphnia themselves and their food sources. Fluorescence emission help localise QDs within organisms and to assess their elimination and accumulation in the digestive tract.

Since the toxic effects of QDs are mainly ascribed to the release of ions derived from their chemical components and to the generation of reactive oxygen species (ROS) [13][14][15], the determination of the elements likely to be released from QDs has been carried out from different points of view. A suspension of polyethylene glycol-coated CdSe/CdS QDs (PEG–CdSe/CdS QDs) was applied locally to the skin of mice (in some individuals after the removal of epidermis), and Cd was determined by ICP–MS in sentinel organs (liver tissue and lymph nodes) [16]. The Cd levels found showed that damaged skin enabled the penetration of QDs, which is toxicologically relevant and enables the determination of QDs, as stated by Sewell et al. [17]. These authors determined QDs by means of ICP–MS determination of Cd and Se from streptavidin-functionalized CdSe/ZnS QDs. They found that Cd and Se were independent of the QDs functionalisation, and the QDs concentrations were not different from those obtained by UV–Vis spectrophotometry. Another approach to the study of citotoxicity of CdSe QDs [15] was the incubation of human hepatocellular carcinoma cells (HepG2) for 24 h with CdSe QDs, followed by magnetic solid phase microextraction (MSPME) and a subsequent ICP–MS sensitive determination of Cd and Se (with LODs of 2.2 and 21 ng L^{-1}, respectively).

Given the described toxic effect of the elements released, other authors forced that process. The concentration of Cd released into solution as a result of an artificially accelerated photodegradation of CdSe/ZnS and CdSe QDs polymer composites was measured using ICP–MS [13], and cadmium-containing species, <0.45 µm in size, were detected with ICP–MS.

The single-cell ICP–MS (SC–ICP–MS) method was established to determine intracellular carboxyl-coated CdSeS QDs in single cells after exposure. The results were compared and validated by flow cytometry and cell digestion methods. In contrast to other methods, SC–ICP–MS can directly detect QDs and their degradation products via elements [18]. Cells are sprayed in sequence into a high temperature plasma, where each cell is desolvated, and its constituents are atomised and ionised. The resulting ions are then detected by mass spectrometry. In the mass spectra, the intensity of each transient signal corresponds to atomic constituents in a single cell, and the frequency of transient signals is directly related to the number of cells. The number of cells detected by ICP–MS (F_{cd}) during acquisition time (t) can be calculated by the following equation:

$$\mathrm{F_{cd}} = \varepsilon \cdot \mathrm{Q_{sam}} \cdot \mathrm{N_{cd}} \cdot \mathrm{t} \quad (1)$$

where ε is the transport efficiency, Q_{sam} is the sample uptake rate (mL s^{-1}), N_{cd} is the cell number density (mL^{-1}), t is the acquisition time (s) and F_{cd} is the number of cells detected. It is necessary for SC–ICP–MS analysis that only one cell enters the plasma at a time so that each transient signal in the mass spectrum corresponds to a single cell.

In addition, the use of ICP–MS as a detector in hyphenated techniques has also been reported. Size-exclusion chromatography coupled with ICP–MS as a detector (SEC–ICP–MS) was used to separate and quantify CdSe/ZnS QDs and their dissolved metal cations (Cd^{2+} and Zn^{2+}). This was made on lab-prepared suspensions and in spiked water samples (river water, moderately hard groundwater and a secondary effluent from a municipal wastewater treatment plant) [19]. The method enabled the determination of QDs at 1 µg L^{-1} or more, and the results agreed with the achieved with the conventional method involving previous separation of dissolved cations by centrifuge ultrafiltration. Moreover, regarding the release of cations from the QDs, the authors observed that the QDs leached ca. 50% Zn^{2+} and 20% Cd^{2+} over 4 days within natural river water contained within a 2 mL HPLC autosampler vial. Moreover, SEC–ICP–MS was the technique applied for the speciation of four CdSe/ZnS QDs in HepG2 cells: cells were incubated with QDs and later fractioned by SEC–ICP–MS [14]. The method was based on metallomics, a novel omics science, which was developed in recent years by focusing on the amount, speciation, distribution, structure and function of metals in biological systems. This is quite interesting, because little is known about the QD species present in media after their degradation. Eventually, two types of chemical form, named as QD–1 and QD–2, were found in HepG2 cells. QD–1 and QD–2 were confirmed to be a type of QD-like nanoparticles and a type of Cd-metallothionein complex, respectively.

Asymmetric flow field-flow fractionation (AF4) coupled with ICP–MS is another hybrid technique to be mentioned. AF4–ICP–MS has been proposed for the detection of QDs in the monitoring of bioconjugation between a protein, namely a “model” monoclonal IgG antibody (Ab) and CdSe/ZnS QDs (which were surface-coated with an amphiphilic polymer) [20]. Cd, Se and S were determined, which is a critical need for the assessment of the elution of the bioconjugated or free Ab.

The LOD obtained, as QD concentrations, was 11 ± 3 nM. Later, through the determination of Zn by AF4–ICP–MS, the concentration of Mn^{2+}-doped ZnS QDs was estimated as the number of QDs per unit volume and number of surface ligands on the QDs (ligand density). These two parameters help in assessing the reliability of quantitative analytical or bioanalytical applications of the QDs [21]. L-cysteine and dihydrolipoic acid (DHLA) were compared as surface ligands, and the latter one was found to enhance the phosphorescent properties of capped QDs and their aqueous stability, while facilitates the further bioconjugation of the QDs to biomolecules.

Finally, capillary electrophoresis coupled to ICP–MS (CZE–ICP–MS) enabled the separation of L-glutathione/L-cysteine-capped CdTe QDs, and the corresponding ionic species, Cd^{2+} and TeO_3^{2-}, likely to be released from them [22]. The method was applied to buffered solutions and complex biological matrices (cell culture solution and normal rat serum sample). The interest of ions determination is their toxicity, as commented above [13][14][15]. All the analytes were completely separated, detected and quantified, with good sensitivity (LODs below 5.5 µg L^{-1}), precision and analytical recovery.

2.2. Inductively Coupled Plasma-Optical Emission Spectrometry (ICP–OES)

Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP–OES) is a multi-elemental analysis technique capable of determining and quantifying, at concentrations ranging from % to ppb, most of the elements of the periodic table, with the exception of C, N, O, H, F, noble gases, some rare earths and other rare elements. The samples are introduced in liquid form, transformed by a nebuliser into an aerosol and excited by an argon plasma. The emissions of the excited atoms are collected by an optical system based on a polychromator combined with CCD detectors, obtaining an emission spectrum for the selected lines of each element.

The research of the extent of cadmium release upon exposure to a series of environmental and biological simulant fluids has been reported, in addition to the tracking of the loss of QD-characteristic fluorescence, as a marker for chemical damage to the CdSe/ZnS nanoparticles [23]. ICP–OES was used to distinguish soluble cadmium from particulate forms. Probably due to the lower sensitivity of ICP–OES compared to ICP–MS, ICP–OES is usually not the technique of choice.

2.3. Graphite Furnace Atomic Absorption Spectrometry (GFAAS)

Although GFAAS is a powerful analytical technique for the analysis of elements present in complex samples such as biological and environmental samples by measuring the radiation absorbed by the target element, its use in the field of QDs determination is limited.

The determination of the Cd concentration by means of GFAAS was followed by the calculation of the amount of Cd atoms in each nanocrystal [10]. The CdSe QDs concentrations were determined by factoring the number of Cd atoms per nanocrystal of relevant size (AF) into the calculation. These values were calculated with the crystallographic software Diamond 3.0. The exponential fitting curve for the total amount of cadmium atoms (y) in one nanocrystal at a given emission wavelength (nm) is stated below.

$$y_{Cd} = 0.0952 \cdot 10^{0.0168\,\lambda},\ R^2 = 0.9914 \quad (2)$$

3. Molecular Spectrometry Techniques

3.1. Spectrofluorimetry

Molecular Fluorescence Spectrophotometry has been used to study aqueous solutions containing graphene QDs (GQDs), which allowed its quantification [24]. GQDs are retained in a strong anion exchange column for their preconcentration, recovered in an aqueous solution and measured in a spectrofluorimeter using the fluorescence of the GQDs as the analytical signal for the quantification. The limits of detection and quantification were 7.5 mg L^{-1} and 25 mg L^{-1}, respectively. The precision for 200 mg L^{-1}, expressed as %RSD, was 2.8%. Recovery percentages between 86.9 and 103.9% were obtained for two different concentration levels. Interferences from other nanoparticles were studied, and no significant changes were observed at the concentration levels tested.

An approach based on single-molecule two-colour coincidence detection was developed to evaluate the on-state QDs in a microfluidic flow, taking advantage of the fact that single QDs exhibit the dynamic fluctuation of fluorescence intensity (i.e., blinking) with the transition between on and off states [25]. The authors have quantified the on-state QDs by detecting the coincidence signals of red streptavidin-coated CdSe/ZnS QDs associated with the green fluorescent microspheres in a

microfluidic flow, which can overcome the limitations that are inherent in the above immobilisation method and fluorescence correlation spectroscopy methods.

Spectrofluorimetric detection has been combined with poly(acrylamide) gel electrophoresis (PAGE) for the characterisation of CdSe/CdS/ZnS QDs [26].

3.2. Laser-Induced Fluorescence Spectrophotometry (LIF)

In LIF, the excitation step is produced by a laser source. The most important advantages of this technique are low background signal, high selectivity towards the analyte, the obtention of information about the rotational-vibrational structure of the ground or excited state of the sample, and time-resolved information if pulsed lasers are used. In addition, polarisation-dependent measurements are easy to implement since most laser beams are linearly polarised.

It has been reported the quantification of the 3.1 nm QDs–TOPO/TOP/SDS by using LIF detection (excitation 480 nm and emission 520 nm) [27]. The surfactants used to achieve the surface functionalisation were trioctylphosphine oxide/trioctylphosphine (TOPO/TOP) and sodium dodecyl sulfate (SDS). A log-linear regression was found to provide the following equation: $Y = -0.21 + 0.215 \log X$, $R^2 = 0.979$, where Y is the peak area, and X is the concentration of the QDs–TOPO/TOP/SDS complex. These free QDs were separated by capillary zone electrophoresis (CZE) based on the differences in the charge-to-mass ratio of the QDs–TOPO/TOP/SDS complexes, and the detection was carried out with UV–Vis and laser-induced fluorescence (LIF) techniques obtaining detection limits 5-times lower with CE–LIF.

3.3. Selective Plane Illumination Microscopy (SPIM)

SPIM is a fluorescence microscopy technique that uses a focused light sheet to illuminate the sample from the side. SPIM achieves excellent resolutions at high-penetration depths while being minimally invasive at the same time. SPIM offers a number of advantages over established techniques such as strongly reduced photo-bleaching, high dynamic range and high acquisition speed. It has been reported a setup for SPIM, which enables the detection and tracking of single streptavidin-coated CdSe/ZnS QDs in model biological systems, with the resolution of confocal microscopy and the optical penetration beyond 300 µm [28][29].

3.4. UV–Vis Spectrophotometry

The concentration determination was performed by taking the extinction coefficient into account [30]. The CdSe/ZnS QDs concentrations determined by Schmelz et al. [31] and Yu et al. [31] provided a much higher Cd concentration in the solution if compared to the ASV and AAS data. However, the extinction coefficient and, thus, the concentration determined by Striolo et al. [32] seem to be comparable to the ASV and AAS. Due to the large discrepancies from different work groups, it is conceivable that the extinction coefficient of nanocrystals in comparison to organic fluorophores is additionally a function of the crystal's quality. High crystallinity, i.e., fewer defects, guarantees higher quantum yields. Since the quantum yield of nanocrystals is strongly influenced by surfactants, their coverage rate to nanocrystals will differ not only in terms of the photoluminescence characteristic but also, conceivably, in terms of the absorption cross section. This can lead to a different extinction coefficient. Hence, nanocrystals prepared by different syntheses should be compared carefully, even if they have similarly shaped absorption characteristics.

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