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Fluorescence enhancement of CdTe MPA-capped quantum dots by glutathione for hydrogen peroxide determination

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ABSTRACT

The manipulation of the surface chemistry of semiconductor nanocrystals has been exploited to implement distinct sensing strategies in many analytical applications. In this work, reduced glutathione (GSH) was added at reaction time, as an electron-donor ligand, to markedly increase the quantum yield and the emission efficiency of MPA-capped CdTe quantum dots. The developed approach was employed in the implementation of an automated flow methodology for hydrogen peroxide determination, as this can oxidize GSH preventing its surface passivating effect and producing a manifest fluorescence quenching.

After optimization, linear working calibration curve for hydrogen peroxide concentrations between 0.0025% and 0.040% were obtained ($n=6$), with a correlation coefficient of 0.9975. The detection limit was approximately 0.0012%. The developed approach was employed in the determination of H_2O_2 in contact lens preservation solutions and the obtained results complied with those furnished by the reference method, with relative deviations comprised between −1.18 and 4.81%.

1. Introduction

The surface chemistry of colloidal semiconductor nanocrystals or quantum dots (QDs) is an important parameter determining most of their optical and physical properties, namely their reactivity, luminescence efficiency (quantum yield), photoluminescent properties stability and the solubility in a given solvent [1]. Indeed, the QDs size (usually in the range 1–10 nm) and the resultant quantum confinement effect in combination with the high surface-to-volume ratio and other surface characteristics render QDs photoluminescent properties very sensitive to any micro-environmental change or interaction with a chemical specie [2]. Morphologically, the occurrence of surface imperfections that act as charge carrier traps can impair the efficiency of electron–hole recombination, thus favoring non-radiative recombination processes which can dramatically reduce the fluorescence quantum yield (QY) of QDs [3]. In this regard, surface modification strategies are often used to eliminate trap sites and to increase solution stability preventing aggregation and leading to an enhancement of the QDs luminescent emission intensity [4]. Organic ligands used in the adaption of the surface chemistry provide electronic and chemical passivation of surface traps and enable QDs to be chemically manipulated as large molecules with solubility and reactivity defined by the ligand characteristics [5]. This adaptability has boosted the application of QDs as chemosensors in different analytical applications gaining a wide acceptance among the scientific community. Several works have studied the interaction of quantum dots surface with different substances exhibiting functional groups such as thiol (sulfhydryl) [3,6], amine [7] and phosphonate [4] that could act as enhancers of the fluorescence intensity of QDs. This enhancement was explained by the formation of covalent bonds between the donor atom of the ligand (usually nitrogen, sulfur or oxygen) and incompletely coordinated Cd^{2+} ions on the QDs surface, wherein the referred atoms acted as electron-donors and the dangling orbitals acted as electron-acceptors. Thus, the mid-gap energy states produced by dangling orbitals located between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) are effectively removed, thereby preventing the occurrence of non-radiative relaxation pathways.

Glutathione (L-γ-glutamyl-L-cysteinylglycine) is the most abundant intracellular non-protein sulfhydryl compound present in all mammalian tissues participating in numerous cellular functions mainly involving the thiol group of the cysteine residue [8]. In particular, reduced glutathione (GSH) plays an important role in detoxification of hydrogen peroxide, other peroxides, and free radicals [9]. Furthermore, GSH has been widely used as a thiol ligand in the aqueous synthesis of different semiconductor nanocrystals [10,11].

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Hydrogen peroxide is a strong oxidant often used in various industrial and household applications as a bleach or cleaning agent [12]. Owing to its broad antimicrobial activity, H_2O_2 is used for contact lens disinfection, destroying pathogens by triggering oxidative processes. However, depending on the concentration, hydrogen peroxide can be toxic to the ocular epithelium and cornea being necessary to carry out its neutralization before lens wear in order to avoid eyes irritation and possible corneal damage [13]. The wide use of hydrogen peroxide along with the need to assess the efficiency and the safety of its utilization promoted the development of suitable analytical methods for H_2O_2 determination. These include titrimetric [14], electrochemical [15,16], fluorometric [17,18], spectrophotometric [19,20] and chromatographic [21,22] techniques. Additionally, some methodologies based on different flow analysis approaches have been also proposed, including, flow injection analysis with fluorescence [23], chemiluminescence [24], spectrophotometric [25] and amperometric [26] detection. Nevertheless, to the best of our knowledge, the determination of hydrogen peroxide in lens care solutions was only performed by Vidigal et al. [27] wherein a sequential injection lab-on-valve method with spectrophotometric detection was exploited.

In the present work, and for the first time, a novel chemosensor based on GSH-induced fluorescent enhancement of MPA-capped CdTe QDs was developed for hydrogen peroxide determination. With this purpose, the surface interactions between GSH and CdTe nanocrystals were thoroughly evaluated.

The operational characteristics of multipumping flow system (MPFS) [28], namely, low reagents consumption, straightforward automation and control, high portability and versatility allowed to take advantage of particular features of the nanocrystals, such as, the versatile surface chemistry and ligand binding ability for the implementation of a simple, fast and sensitive automatic methodology for the monitoring of hydrogen peroxide in lens care solutions. The analytical methodology was based on the reduction effect of hydrogen peroxide on the GSH-induced fluorescent enhancement of MPA-capped CdTe.

2. Experimental

2.1. Apparatus

The flow manifold comprised of four model 120SP solenoid micropumps (Bio-Chem Valve Inc. Boonton, NJ, USA), which were of the fixed displacement diaphragm type, dispensing 10 μL per stroke. All tubings connecting the different components of the flow system was made of polytetrafluoroethylene PTFE (Omnifit, Cambridge, UK), with 0.8 mm of internal diameter. Homemade end-fittings and acrylic confluence connectors were also used.

The automatic control of the solenoid micro-pumps was accomplished by a microcomputer through the lab-made software developed in Microsoft Visual Basic 6.0®. For the actuation of the micro-pumps a homemade power drive based on the ULN2003 chip was used which was controlled through communication by the computer parallel port.

The detection unit was a spectrofluorometer Jasco (Easton, MD, USA), model FP-2020/2025, equipped with a 16 μL internal volume flow cell.

For the characterization of the synthesized nanoparticles, QDs absorption spectra were obtained by using a Jasco V-660 spectrophotometer (Easton, MD, USA). The fluorescence measurements were performed on a model LS-50B Perkin Elmer luminescence spectrometer (Waltham, MA, USA). A ThermoElectron Jouan BR4I refrigerated centrifuge (Waltham MA, USA) was used for the separation of the precipitated QDs.

FT-IR spectroscopic measurements were carried out using a PerkinElmer Frontier spectrophotometer (Waltham MA, USA) equipped with an universal ATR Diamond/ZnSe support.

The zeta potential of the nanocrystals was obtained using a BI-MAS dynamic light scattering (DLS) instrument (Brookhaven Instruments, USA).

The morphology of the nanoparticles was observed by transmission electron microscopy (TEM) using an electron microscope JEOL JEM 1400 TEM (Tokyo, Japan), at an acceleration voltage of 100 kV, equipped with a Gatan SC 1000 ORIUS CCD camera (Warrendale, PA, USA).

2.2. Samples and standards

All solutions were prepared with water from a Milli-Q system (specific conductivity $\leq 0.1\ \mu S\ cm^{-1}$) and chemicals were of analytical reagent grade quality. Reagents were used as received.

For the assays, a solution containing 1.00 μmol L^{-1} of CdTe QDs was prepared by dissolving 7.84 mg of the synthesized and purified CdTe QDs, with a size of 2.48 nm, in 25 mL of water.

A glutathione solution of 0.651 mmol L^{-1} was daily prepared by dissolving 20 mg of the *L*-glutathione reduced (Sigma, 98% purity, St. Louis MO, USA) in 100 mL of water.

A 0.1% intermediate solution of H_2O_2 was daily prepared by adding, in a 100 mL volumetric flask, 333 μL of hydrogen peroxide (30% w/v, Panreac, Barcelona, Spain) solution and then the volume was made up to the mark with deionized water. The hydrogen peroxide stock solution was standardized by titration with potassium permanganate (Riedel-de Haën, 99% purity, Germany).

The working hydrogen peroxide standard solutions (0.0025–0.040%) were daily prepared by proper dilution of the above intermediate solution by transferring aliquots (1.25–20.0 mL) into a series of 50.00 mL volumetric flasks and the volume was completed to the mark with water.

Five commercially available lens care solutions were analyzed according to the proposed method and no pre-treatment was necessary prior to analysis. Sample solutions were prepared by diluting with deionized water, in 10 mL volumetric flask, an appropriate volume of the lens care solution, in order to obtain a hydrogen peroxide content included in the analytical range of the procedure.

2.3. Reagents and synthesis of CdTe quantum dots

For the synthesis of the CdTe quantum dots, tellurium powder (200 mesh, 99.8%), sodium borohydride ($NaBH_4$, 99%), cadmium chloride hemi(pentahydrate) ($CdCl_2 \cdot 2.5H_2O$, 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA); 3-mercaptopropionic acid (MPA, 99%) and absolute ethanol (99.5%) were obtained from Fluka (St. Louis MO, USA) and Panreac (Barcelona, Spain) respectively.

Five different diameters of MPA-capped CdTe QDs were synthesized as described by Zou et al. [29] with some modifications. Briefly, the first stage consists on the reduction of tellurium with $NaBH_4$ in N_2 saturated water to produce NaHTe. After all tellurium has been completely consumed the resulting solution was transferred into a second flask containing 4.0×10^{-3} mol of $CdCl_2$ and 6.8×10^{-3} mol of MPA in 100 mL N_2 saturated solution. The pH of the solution was adjusted to 11.5 with a 1.0 mol L^{-1} NaOH solution. The molar ratio of Cd^{2+}:Te^{2-}:MPA was fixed at 1:0.1:1.7. The size of CdTe QDs was controlled by changing the refluxing time.

To purify the CdTe QDs these were precipitated in absolute ethanol to remove the contaminants and the precipitate was subsequently separated by centrifugation, vacuum dried, kept in amber flasks and protected from light.