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Graphical abstract

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Silica nanostructures synthesis and CdTe quantum dots immobilization for photocatalytical applications

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This work proposes a new strategy for the immobilization of semiconductor nanocrystals by carrying out in simultaneous the synthesis of silica nanostructures and the encapsulation of MPA-capped CdTe quantum dots (QDs). The developed approach was bioinspired on the nature occurring biosilicification process that some organisms, like diatoms and sponges, use to produce nanostructures of silica, and prevents the use of harsh and environmentally unfriendly conditions such as toxic and expensive organic solvents, high temperature and pressure and extreme pH values. Different parameters that could influence the silica particle synthesis and the QD immobilization, such as silica precursors, catalysts and ions concentration, pH and amount of QDs were evaluated. Encapsulation efficiency, size and the shape of biosilica structures were also determined by the experimental conditions. Immobilization and QDs concentration has a strong impact on QDs fluorescence and on the silica-QDs nanostructures stability.

Introduction

Colloidal semiconductor nanocrystals or quantum dots (QDs) are monodisperse crystalline clusters of atoms with a size normally comprised between 1 and 10 nm that exhibit fascinating optical and electronic properties due to quantum confinement effects [1]. Among these, the high photoluminescence quantum yield, broad excitation profiles, narrow/symmetric emission bands and high photostability [2-6] are those more frequently highlighted and the most promising concerning the application of QDs in areas as different as the biological sensing and imaging [7, 8], chemical analysis [9] light-emitting devices [10], photonic crystals [11], etc.

In order to preserve QDs chemical and luminescent properties and to reduce their cytotoxicity by blocking heavy metal release from the semiconductor core, some techniques have been described for QDs confinement within a protecting shell of inert material [12-14].

Silica coating is one of the most widely used methods for the surface recovering of QDs [6, 15, 16]. This kind of encapsulation provides a chemical and physical shielding from

the environment, improves stability by avoiding nanocrystals aggregation, and reduces the release of toxic heavy metal ions from the core [6].

The most common methods for QDs incorporation into a silica matrix are based on the Stöber method [17] or in the use of a reverse microemulsion system [18]. In the first case, QDs act as seeds for silica growth in an ethanol-water mixture, while the second one resorts to water-in-oil (W/O) reverse microemulsion system: the silica precursor hydrolysis and condensation occurs in the W/O interface or in the water phase, and a non-ionic surfactant is used to promote the water droplets stabilization in the hydrophobic phase. Along with the incorporation of QDs into silica, distinct approaches to immobilize QDs on silica surfaces by covalent linkage were also described. These immobilizations relied either on carbodiimide chemistry or on the utilization of glutaraldehyde as crosslinker, for preparation of QDs-functionalized substrates. The former permitted the formation of amide and ester bonds between carboxylic and amino groups [19, 20], while the latter used two nucleophilic additions to link the two carbonyl groups of glutaraldehyde to both the amino-modified silica and to the a-amine of the QD capping agent [21].

In this work, we developed a bioinspired assembly of silica nanoparticles based on the biosilicification process carried out in nature by diatoms and sponges, providing the means for the efficient encapsulation of semiconductor nanocrystals into a hybrid structure that enables the preservation of their photochemical properties. In nature, and at both low temperature and pressure and near neutral pH [22], the referred organisms are able to take up silicon from the environment in a soluble form as silicic acid, to store it in the cell and to catalyze its polymerization into silica with a precise structural architecture down to a nanoscale [23, 24]. The production of nanostructured biosilica in natural systems has been considered a process to mimic [25], assuming the biomimetic synthesis an increasing relevance in the last few years when this phenomenon has been an inspiration for the development of novel fabrication procedures in nanobiotechnology [22]. By mimicking these procedures it is possible to use reaction conditions similar to the original ones, avoiding the use of harsh and environmentally unfriendly circumstances such as toxic and expensive organic solvents, and minimizing the long reaction times and the multiple steps of complex protocols [23, 26]. In fact, several ways to mimic the biosilica synthesis have been reported in order to develop new materials for different applications [27]. These have been used, for example, in sensors [28, 29], biocatalysis [30-32], drug delivery [33, 34], and for supporting metals and functional metallic nanoparticles [35, 36] and QDs. The carrying out of a mild biosilica synthesis, with a simultaneous QDs encapsulation, at ambient pressure and temperature and with a pH near the neutrality, enhanced the stability of the immobilized QDs.

A biosilica synthesis process for QDs immobilization has been previously referred [37], but it involved CdSe/ZnS quantum dots and was a laborious time-consuming method. Conversely, in this work we have used CdTe quantum dots, which for the best of our knowledge were for the first time immobilized in biosilica, and the developed process was simpler and much faster than those already available. The developed work also involved the study of the QDs influence on the silica nanostructures properties, and of the reciprocate impact of immobilization on QDs stability and fluorescence, and the appraisal of the experimental conditions effect on the encapsulation efficiency and on the size and shape of biosilica nanoparticles. The impact of the silica precursors and catalysts, their concentration, solution pH and anions concentrations, was also studied.

Materials and methods

Reagents and solutions

All solutions were prepared with analytical reagent grade, high purity water (milli Q) with a specific conductivity <0.1 μ S cm⁻¹. Phosphate buffer solutions (PBS), at different pH values, were prepared by mixing adequate volumes of 0.6 mol L⁻¹ NaH₂PO₄ (Fluka) and of 0.6 mol L⁻¹ Na₂HPO₄ (Sigma). QDs solutions were obtained by dissolving the appropriate amount of the synthesised nanocrystals in water.

Hydrolyzed tetramethyl orthosilicate (TMOS) solutions resulted from mixing appropriate volumes of commercial TMOS (Fluka) and 1 mmol L⁻¹ HCl (Merck) for 20 minutes. The 1.0 mol L⁻¹ hydrolyzed tetraethyl orthosilicate (TEOS) solution was prepared by mixing an appropriate volume of commercial TEOS (Fluka) and 1 mmol L⁻¹ HCl (Merck) for 10 minutes under ultrasonication. The two previous solutions were daily fresh prepared. A 1 mol L⁻¹ sodium silicate solution was also daily prepared by diluting the required volume of commercial solution (Sigma) in water. Polyethylenimine (PEI) solutions (molecular weight ~10,000 from Polysciences, Inc and molecular weight ~25,000 from Aldrich), were prepared by dissolving appropriate amounts in water. 1 mol L⁻¹ protamine (Sigma) and 1 mol L⁻¹ cysteamine (Sigma) solutions was prepared by solubilisation in water.

Synthesis and characterization of MPA capped CdTe Quantum dots (MPA-CdTe QDs)

MPA-capped CdTe QDs were synthesized as described by Zou et al. [38] with minor modifications. Briefly, tellurium was reduced with NaBH₄ in N₂ saturated water to produce a solution of NaHTe. Following tellurium conversion, the resulting solution was transferred into a second flask containing 4.0×10^{-5} mol of CdCl₂ and 6.8×10^{-3} mol of MPA in a 100 mL N₂ saturated solution. The pH of the solution was adjusted to 11.8 with 1.0 mol L^{-1} NaOH. The molar ratio of Cd^{2+} : Te^{2-} : MPA was fixed at 1:0.1:1.7. The reaction mixture was then refluxed at 100 °C under atmospheric conditions for 40 minutes. The obtained CdTe-MPA QDs where purified by precipitation with absolute ethanol. The precipitate was subsequently separated by centrifugation, vacuum dried, kept in amber flasks and protected from light, for posterior use. The particle size of the synthesized QDs was calculated by the following expression (eq.1) [39]:

$$D = (9.8127 \times 10^{-7})\lambda^3 - (1.7147 \times 10^{-3})\lambda^2 + (1.0064)\lambda - (194.84)$$

where *D* is the diameter (nm) and λ (nm) the wavelength maximum corresponding to the first excitonic absorption peak of the crystal.

The CdTe-MPA QDs molar weight was possible after establishing the extinction coefficient (ε) using the expression (eq.2) [39]:

$$\varepsilon = 3450 \Delta E(D)^{2.4}$$

where ΔE is the transition energy corresponding to the first absorption peak (in eV).

Transmission electron microscopy (TEM) was performed on a JEOL JEM-1400 at an acceleration voltage at 100 kV. X-Ray powder diffraction (XRD) was measured by wide-angle X-ray scattering, using a Siemens D5005 2808 X-ray powder

diffractometer equipped with graphite monochromated highintensity Cu-Ka radiation (1.54178Å). XRD samples were prepared by depositing NC powder on a piece of Si (100) wafer.

Biosilica synthesis and quantum dots encapsulation

Biosilica synthesis, with simultaneous QDs encapsulation, was carried out by the successive mixing of 700 μ L of QDs solution, 100 μ L of 5.0x10⁻⁴ mol L⁻¹ PEI solution, 100 μ L of 0.3 mol L⁻¹ PBS pH=6 and 100 μ L of 1.0 mol L⁻¹ TMOS solution (which was pre-hydrolyzed in 1 mmol L⁻¹ HCl for 20 min). Nanoparticles formation started immediately after the addition of the freshly hydrolyzed TMOS solution and was completed in 15 minutes. Afterwards, the nanostructures were separated by centrifugation at 10000 rpm for 3 minutes, washed 4 times by re-dispersion in water, re-centrifuged and dried. The supernatant of the initial centrifugation was reserved for utilisation in the assessment of the loading efficiency.

Scanning Electron Microscopy (SEM) and Electron Diffraction X-ray Spectroscopy (EDS)

The determination of morphology and size and of the elemental composition of nanoparticles was performed by Scanning Electron Microscopy (SEM) and Energy dispersive X-ray spectroscopy (EDS), respectively. These analysis were performed using a High resolution (Schottky) Environmental Scanning Electron Microscope with X-Ray Microanalysis and Electron Backscattered Diffraction analysis: Quanta 400 FEG ESEM / EDAX Genesis X4M. Samples were mounted on aluminum stubs and coated with a Au/Pd thin film, by sputtering, using the SPI Module Sputter Coater equipment.

Attenuated Total Reflection Fourier Transform Infrared

Attenuated total reflection Fourier transform infrared (ATRFTIR) spectra were run with a FTIR PerkinElmer Frontier spectrometer.

Loading efficiency

As it was previously referred, loading efficiency was appraised by taking into account the fluorescence of the original QDs solution (used in the synthesis) and the residual fluorescence of the supernatant obtained from centrifugation of the synthesis precipitate. By using a Jasco FP 6500 spectrofluorometer these were related according to the equation (eq. 3):

Loading efficiency (%) =
$$(1 - \frac{F_s}{F_o}) \times 100$$

where F_s and F_o are the supernatant and the original QDs fluorescence, respectively.

Electron paramagnetic resonance spectroscopy (EPR)

EPR spectra were recorded using a Bruker ELEXSYS E500 spectrometer operating at 9 GHz (X-band) using the following experimental conditions: modulation frequency of 100 kHz, microwave power of 10 mW, modulation amplitude of 2 G and 40 dB of receiver gain, acquisition time of 100 s, 5 scans. The Spin Hamiltonian parameters were obtained using the computer suite program Brucker WinEPR/SimFonia. Samples for EPR analysis were prepared by mixing 100 μ L of QDs loaded and non-loaded silica nanoparticles dispersion and BMPO solution (100 μ L, 50 μ M). The suspension was then irradiated with visible light for 18 min, using a photo-excitation unit (LED-PEU) [40] equipped with a high-power LED lamps (Parathom® R50 40 daylight). One aliquot was collected from the reacting mixture and transferred into the capillary placed in the quartz EPR tubes. Samples were analyzed at room temperature.

Results and discussion

Characterization of CdTe-MPA quantum dots

Taking into account the first excitonic transition of the UV/Vis absorption spectra (Figure 1) [39], at 532 nm, the size of the synthesized MPA-QDs was calculated to be 3 nm. Moreover, the emission spectra showed an almost symmetrical band with a maximum of emission at 564 nm, with a full-width at half-maximum (FWHM) of about 57 nm, indicating that the asprepared CdTe QDs population exhibited a homogeneous size distribution.



Fig. 1. UV-Vis absorption spectra (-) and fluorescence emission spectra (- - -) of MPA-CdTe quantum dot.

The theoretically calculated size was confirmed by TEM (Figure 2), which also revealed the formation of nearly spherical and well dispersed CdTe nanocrystals (NCs). Powder X-ray diffraction (p-XRD) patterns for CdTe NCs (Figure 3) confirmed the characteristic peaks of zinc blend cubic CdTe, which resulted broadened due to the finite crystalline size.

Synthesis of biosilica nanoparticles and CdTe QDs encapsulation

In this work, was evaluated not only the effect of different reaction conditions on the size and morphology of the biosilica-

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QDs nanoparticles but it was also appraised how the structure of the resulting nanoparticles could affect QDs immobilization and stability. Bearing in mind that QDs, likewise enzymes, could shape the biosilica nanoparticles acting as templates during the synthesis process [32], MPA-capped QDs were selected for these studies as they have never been used before in similar circumstances.



Fig. 2. Transmission electron microscopy (TEM) images of MPA-CdTe quantum dot.



Fig. 3. Powder X-ray diffraction (p-XRD) patterns for CdTe NCs.

Given that the size of biosilica nanostructures could be influenced by ions concentration, and that QDs stability is affected by ionic strength, one of the first parameters assessed in this study was the buffer concentration. By using PBS concentrations between 0.1 and 0.8 mol L^{-1} it was observed that for 0.3 mol L^{-1} and above concentrations the obtained results were similar in terms of loading efficiency and particles size and morphology. On the other hand, for a concentration of 0.1 mol L^{-1} the loading efficiency of QDs was lower, which could be explained by the nanoparticles morphology (Figure 4), since these, although showing improved size homogeneity (Figure 4A) were definitely smaller.



Fig. 4. Scanning electron microscopy (SEM) images of biosilica synthesized with phosphate buffer solution concentration of 0.1 mol L^{-1} (A), and 0.3 mol L^{-1} (B).

Analysis of the smaller particles by EDS confirmed these results revealing low values of Cd and Te, therefore a minor load of QDs. Accordingly, a PBS concentration of 0.3 mol L⁻¹ was selected for the posterior experiments. Since the synthesis of biosilica nanostructures is favoured in neutral conditions the pH of the buffer solution was evaluated between 6 and 8. It was verified that for lower pH values (up to 6.5) the QDs loading efficiency was near 100%. However, for higher values the loading efficiency decrease to 95% (pH=7) and to 85% (pH=8). In addition, QDs leakage from the nanostructures increased with the increment of pH (around 3% for pH=6 and about 32% for pH=8, after 11 days of storage).

The influence of the silica precursor on the nanoparticles synthesis was assessed by using TMOS, TEOS and sodium silicate [41-44], at a concentration of 1.0 mol L^{-1} . Sodium silicate solution had a dramatic effect on QDs stability causing a complete loss of fluorescence. On the other hand, the QDs loading onto the formed nanostructures provided by TMOS was 6% higher than the one observed with TEOS. A comparison of the nanoparticles obtained with these two precursors by SEM showed that with TMOS, the nanoparticles structures were much more regular and defined than with TEOS (Figure 5). So, the higher loading efficiency and the more regular structures obtained with TMOS, dictated the selection of this precursor for the subsequent synthesis.

5%.

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mol L⁻¹ decreased the QDs immobilization for approximately

Fig. 5. Scanning electron microscopy (SEM) images of biosilica synthesized with TEOS (A), and TMOS (B).

The evaluation of the most adequate precursor concentration

was carried out between 0.5 and 2.0 mol L⁻¹ and it was found

that a concentration value of 1.0 mol L⁻¹ afforded the highest

loading efficiency (about 98%) while a concentration of 0.5

With the purpose of selecting the most appropriate nucleation catalysts compounds such as protamine, cysteamine and PEI (two different molecular weights of 10000 and 25000), at a concentration value of 1.0x10⁻³ mol L⁻¹, were evaluated. It was verified that protamine led to the formation of a gel, instead of a solid, while cysteamine did not yield the formation of neither the gel nor the solid. One the other hand, PEI promoted the formation of solid nanoparticles. The loading efficiencies of the QDs onto the nanostructures were similar for the two polyethylenimines assayed, although the one with higher molecular weight (PEI 25000) yielded not only nanoparticles with larger volume but also with enhanced size uniformity as verified in the analysis by SEM (Figure 6).



Fig. 6. Scanning electron microscopy (SEM) images of biosilica synthesized with PEI 10000 (A), and PEI 25000 (B).

By testing PEI 25000 at concentration values between 1.0x10⁻⁴ and 1.0x10⁻² mol L⁻¹ it was noticed that with concentrations of 5.0×10^{-3} mol L⁻¹ and above not only the QDs fluorescence was completely quenched but they were also not encapsulated into the biosilica particles. In opposition, at concentration values between 1.0x10⁻⁴ and 1.0x10⁻³ mol L⁻¹, QDs were fully encapsulated. However, in this situation an increment of the total volume of the produced nanoparticles was also observed.

Moreover, within this concentration range PEI affected the photoluminescence of the immobilized QDs by shifting the wavelength of maximum emission (with respect to the maximum emission wavelength of QDs in water). In order to minimise this effect a concentration of 5.0×10^{-4} mol L⁻¹ was selected for the posterior experiments seeing that higher concentrations promoted а blue-shift, while lower concentrations led to a red one.

Effect of encapsulation on QDs optical properties

An aspect that was also contemplated during the study of the most favourable immobilization conditions was the variation in the QDs optical properties that results from the immobilization itself. Indeed, one of the most challenging issues regarding the immobilization of photoluminescent nanoparticles is to find new strategies that prevent the occurrence of alterations in these optical properties. For semiconductor nanocrystals, and anticipating their utilisation as chemosensors, this is a matter of crucial importance.

Therefore, aiming at better understanding its mechanism and potential applications, the effect of the immobilization in the fluorescence emission was evaluated by measuring the maximum emission wavelength of silica-QDs NPs prepared with different QDs concentrations. As shown in figure 7, a progressive red-shift is noticeable for increasing QDs concentration.



Fig. 7. Effect of the concentration of QDs in the emission wavelength.

At low concentration (up to 2.5 mg mL⁻¹) the maximum wavelength was similar to the one exhibited by the original QDs solution (565 nm), indicating that no interaction occurred between the loaded nanocrystals or between these and silica. Above 2.5 mg mL⁻¹ a consistent red-shift start was observed, initially with a pronounced increase up to 10 mg mL⁻¹ and, subsequently, a more moderate one up to reaching its maximum value at 100 mg mL⁻¹, corresponding to a shift of 45 nm (28 eV). This effect was dependent on the amount of QDs loaded onto the silica particles and followed a logarithmic trend. Such behavior is typical of self-quenching phenomena [45] and is

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accompanied by a strong emission quenching. Fluorescence quenching could not be evaluated because of the intense light scattering of the loaded silica particles. Self-quenching is wellknown for NPs systems where assembly in solution or the formation of a solid NPs film from solution takes place, and it can be attributed to energy transfer (ET) from the smaller NPs to the larger ones in the NPs size distribution [45-49]. In our case this effect was dependent on the amount of QDs loaded onto the silica particles and could be explained by the increased proximity of the loaded QDs that are forced against each other by the silica matrix. When the distance between two QDs becomes small enough, energy transfer phenomenon between adjacent nanoparticles could occur. Because of the red shift observed two mechanisms are possible: a) the transmission of the exciton from a nanocrystal to the emitting trap states existing on the surface of a contiguous QDs, with an increase in defect related emission [48, 50], and b) a ET mechanisms between small and bigger QDs, since the nanoparticles population is a distribution of sizes [45]. Moreover, because of the reduced diameter of the used QDs (3 nm), tunnelling effects [48] between proximal nanoparticles could be possible. However, due to the back scattering was impossible to verify the occurrence of a red-shift in the absorption spectra. This kind effects were already described for others QDs of immobilization methods [19, 21].

Time-resolved photoluminescence measurements showed a multiexponential decay behavior for the 3 nm CdTe-MPA QDs, with a $t_{1/2}$ of 57 ns. The encapsulation onto silica nanostructures promoted a decrease in the average lifetime that depended on the QDs concentration being used: lifetime was about 52 ns for 10 mg QDs and 26 ns for 100 mg. The strong increase of the fast initial luminescence decays population (\approx 3 ns), observed for the silica nanostructures obtained with high QDs concentration, can be a corroboration of the occurrence of ET processes. For low QDs concentration the average lifetime was even lower than the one observed for the non-encapsulated QDs and it is evidence of a predominant fast decay population.

Biosilica-QDs NPs stability and morphology

The exploitation of fluorescent nanomaterials in distinct areas, such as in photocatalysis or in bioanalytical applications, is clearly conditioned by their long term stability. To gather further information regarding the stability of the photoluminescent silica structures and the reversibility of the self-quenching effect, the maximum emission wavelength was monitored throughout time. As showed in figure 8, excluding the 100 mg sample, and independently from the QDs concentration used in the nanocomposites synthesis, only a small blue-shift (< 5 nm) was evident after 16 days of ageing. For the highest concentration (100 mg) a strong blue-shift was noticeable and probably due to an overloading of the silica structure, that was no longer protecting the QDs from surface oxidizing processes.



Fig. 8. Variation of the maximum emission wavelength of the silica NP-QD throughout time.

Another stability related issue concerned the QDs leakage from the biosilica NPs. Water suspensions of the prepared silica-QDs NPs were collected after 14 days and the supernatant fluorescence was monitored to check for the presence of CdTe nanocrystals. By establishing a relationship between the observed fluorescence and the QDs concentration it would be possible to quantify the amount of nanoparticles that leached. This procedure was repeated for all the samples prepared with different QDs concentration to better understand the likely influence of the morphology in the nanocomposite stability. For this study, the fluorescence of the QDs that leached from the silica-QDs nanoparticles into water during storage, was evaluated. The fluorescence was correlated with the fluorescence of the original QDs solution used in the synthesis, according to the procedure described in the subsection Loading efficiency. The obtained results showed that after the referred 14 days the nanocomposites could be considered to be stable, the lowest QDs retention being verified for the QDs solution of 2.5 mg (91.70%) while all other samples showed a retention \geq 99.48% (Table 1). These higher values of retention allowed to presume that the QDs retention in the silica structures was done by encapsulation. When adsorption phenomena take place the possibility of leaching of the QDs would be higher.

Table 1. QDs retention after 14 days in water for silica-QDs nanocomposites synthesized with different QDs concentration.

QDs concentration (mg)	2.5	5	7.5	10	20	60	100
QDs retention (%) after 14 days	91.70	99.80	99.85	99.48	99.95	99.99	99.99

An analysis of the morphology of the silica nanocomposites synthesized with different QDs concentrations (Figure 9) revealed a progressive change in the silica organization. At the lowest nanocrystals concentration the main structure is represented by spheres while, at increasing QDs concentration, this organization is lost and it becames an amorphous material apparently formed by fused spheres.



Fig. 9. Scanning electron microscopy (SEM) images of biosilica synthesized with different concentrations of QDs (A: 1.25 mg mL⁻¹, B: 2.50 mg mL⁻¹, C: 5.00 mg mL⁻¹, D: 7.50 mg mL⁻¹, E: 20.0 mg mL⁻¹, F: 60.0 mg mL⁻¹).

According to these data one can conclude that the stability of biosilica-QDs NPs is generally excellent within the assayed 14 days. Nevertheless, it is apparent that QDs concentration can indirectly affect the nanocomposite stability due to its effect on the silica structure. In this regard, by using lower QDs concentration it is possible to obtain silica nanocomposites with an organized and fairly homogeneous spherical conformation. However, upon increasing the QDs concentration an amorphous material was obtained, probably because the huge amount of QDs acted as seeds for silica growth. Despite the lack in organization this amorphous material efficiently retained the QDs, preventing its leaking into solution. The retention of the QDs by the silica nanostructures was probably performed by encapsulation during the synthesis. This can be supported by the fact that if the QDs suspension was added after the formation of the silica nanostructures, the immobilization of the QDs did not occur, while if the addition of TMOS solution and consequently the instantaneous formation of the nanostructures was done after the addition of QDs suspension, immobilization happened.

Biosilica-QDs NPs characterization and encapsulation mechanism

The surface composition of the obtained biosilica-QDs NPs was studied by SEM–EDS confirming the undoubtedly presence of silica (Figure 1, supplementary information (SI)). The observed peaks of Cd and Te (Figure 1A, SI) proved that QDs were present in the nanostructures. These peaks are not visible for particles synthesized in the absence of QDs (Figure 1B, SI).

FTIR spectra of the produced biosilica nanoparticles, with and without immobilized QDs (Figure 10), were consistent with a composite of silica, since a characteristic band at about 1032 cm⁻¹, corresponding to Si-O-Si bond stretching and a band around 800 cm⁻¹ that reflects δ Si–OH vibration, were observed. The characteristic bands obtained in the FTIR spectra of the individual QDs were also observed when these were immobilized in the biosilica. Furthermore, the biosilica with

immobilized QDs spectrum did not show any new band with respect to the original QDs and silica FTIR spectra.



Fig.10. FTIR spectra obtained for QDs (-), for biosilica nanoparticles (-) and for biosilica nanoparticles with immobilized QDs (...).

These results demonstrated that the encapsulation of QDs into the biosilica nanostructures resulted from physical phenomena and no new chemical bonds were formed during the process of immobilization.

Photocathalytic potential of biosilica-QDs nanocomposites

A promising feature of QDs is their capacity to generate reactive oxygen species (ROS) in aqueous solution upon exposure to UV/Vis light. Several studies were carried out with different QDs to assess this possibility, while evaluating at the same time the influence of the semiconductor nature and of the capping ligand on the generated reactive oxygen species [51-55]. A few analytical applications were developed taking advantage of this aptitude [40, 56, 57] but all implied the use of QDs in solution, resulting in the concomitant generation of hazardous wastes. The immobilization of the QDs into a solid support, with the preparation of stable functionalized materials, is an advantageous strategy to overcome these problems and to extend the applicability of QDs.

In order to determine the photocatalytic potential of the biosilica-QDs nanocomposites EPR studies were carried out. For these experiments silica nanocomposites loaded with 7.5 mg of QDs where selected and exposed to visible light for 18 minutes in the presence of BMPO. This spin trapping reagent was selected, instead of the more widely used DMPO, because of the extended lifetime of its superoxide radical adduct ($t_{1/2} = 23$ min), much longer than the corresponding DMPO adduct ($t_{1/2} = 45$ s) [58-60]. Moreover the superoxide-DMPO adduct spontaneously decays into the DMPO-hydroxyl adduct, whose signal could contribute to misleading results.

The EPR spectra (Figure 11) obtained upon photo-excitation of the silica-QDs nanocomposites exhibits the four line signal corresponding to the interaction of the unpaired electron with the nitrogen and hydrogen atoms, typical of the hydroxyl-BMPO radical). The isotropic Spin-Hamiltonian parameters (g ARTICLE

 $_{iso} = 2.005$, a_{iso} (¹⁴N) = 13.22 G and a_{iso} (¹H) = 12.20 G) are in good agreement with the published data for the hydroxyl-BMPO adduct [59]. As shown in figure 11 the silica nanoparticles did not photo-react to produce the BMPO adduct.



Fig.11. EPR spectra obtained for silica-QDs nanocomposites (—) and for silica nanoparticles (—).

From a theoretical point of view the CdTe QDs used to synthesize the tested nanocomposites does not have a valence band energetic enough to directly generate hydroxyl radicals, but only a redox potential sufficient to convert oxygen into superoxide. Therefore, the hydroxyl adduct signal is presumably due to the conversion from superoxide.

In view of these results we can consider that the silica-QDs nanocomposites are suitable materials for photocatalytical application, with properties depending on the immobilized QDs. Moreover the permeability of the silica-QDs nanocomposites was confirmed since the generation of the active oxygen species required the diffusion of oxygen into the QDs surface. Finally, the detection of BMPO adducts is an indirect proof of the material permeability to BMPO because it implied the direct interaction between the spin trapping reagent and the radical superoxide, which has an extremely short lifetime.

Conclusions

A novel synthesis method for the production of highly stable silica-QDs NCs was successfully developed. The developed process was based on the biosilicification process that occurs in nature carried out, for instance, by diatoms and sponges. In addition, it revealed to be simpler and faster than other methods described for QDs immobilization. The study of different reaction conditions showed that parameters such as ion concentration, pH, silicic acid precursor and QDs concentration can affect the silica-QDs nanocomposite shape, size and ability to retain the semiconductor nanoparticles. Optical studies on the nanocomposites fluorescence confirmed the alteration of the optical properties of the immobilized fluorophores, in particular a QDs concentration dependent red shift that was probably due to mixed ET and tunnelling effects. Studies on the stability of the biosilica-QDs NCs in water revealed the absence of QDs leaking after 14 days, for the majority of the samples analysed. EPR studies demonstrated the ability of these silica-QDs NCs to produce hydroxyl radicals upon photoexcitation with visible light anticipating their photocatalytical potential.

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Notes and references

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