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Abstract. The unique optical properties of Quantum Dots have attracted a great interest to use these nanomaterials in diverse biological applications. The synthesis of QDs by methods from the literature permits one to obtain nanocrystals coated by hydrophobic alkyl coordinating ligands and soluble in most of the cases in organic solvents. The ideal biocompatible QD must be homogeneously dispersed and colloidally stable in aqueous solvents, exhibit pH and salt stability, show low levels of nonspecific binding to biological components, maintain a high quantum yield, and have a small hydrodynamic diameter. Polymer encapsulation represents an excellent scaffold on which to build additional biological function, allowing for a wide range of grafting approaches for biological ligands. As these QD are functionalized with poly(ethylene)glycol (PEG) derivatives on their surface, they show long term stability without any significant change in the optical properties, and they are also highly stable in the most common buffer solutions such as Phosphate Buffer Saline (PBS) or borate. However, as biological studies are normally done in more complex biological media which contain a mixture of amino acids, salts, glucose and vitamins, it is essential to determine the stability of our synthesized QDs under these conditions before tackling biological studies.

1. Introduction

The unique optical properties of Quantum Dots (QDs) have attracted a great interest to use these nanomaterials in diverse biological applications as fluorescent probes [1-2]. In contrast to common organic fluorophores, QDs have broad absorption spectra enabling excitation by a wide range of wavelengths, and their emission spectra are symmetric and narrow. The most widely used and best characterized QDs are CdX/ZnS (X = Se, Te) nanocrystals, where the CdX core is overcoated with a few atomic layers of a material with a larger bandgap such as ZnS, which improves its luminescence properties while protecting surface atoms from oxidation and preventing chemical degradation [3-4]. The synthesis of QDs by methods from the literature permits one to obtain gram quantities of nanocrystals with a narrow size distribution (Standard deviation < 5 %), coated by hydrophobic alkyl coordinating ligands but soluble only in organic solvents [5-6]. The ideal biocompatible QD must be homogeneously dispersed and colloidally stable in aqueous solvents, exhibit pH and salt stability, show low levels of nonspecific binding to biological components, maintain a high quantum yield, and have a small hydrodynamic diameter. To disperse QDs in aqueous solvents it is necessary to manipulate the surface chemistry, and three main strategies have evolved during the past few years: i)

ligand exchange, ii) silica encapsulation and iii) polymer encapsulation. Given that these different capping strategies address the requirements of various potential applications, the advantages of each method have to be careful weighted against the drawbacks. For instance, our investigations suggest that with ligand exchange methods the QD photoluminescence is often compromised and that the growth of a thin, uniform silica shell that encapsulates a single QD is at present nontrivial. By contrast, polymer encapsulation represents an excellent scaffold on which to build additional biological function, allowing for a wide range of grafting approaches for biological ligands. We have shown that the commonly synthesized QD in organic solvents transfer well using a poly(stryrene-*co*-maleic anhydride) polymer and that the resulting biocompatible QD is homogeneously dispersed and colloidaly stable in aqueous solvents, exhibits pH and salt stability and maintains a high quantum yield [7].

As these QD are functionalized with poly(ethylene)glycol (PEG) derivatives on their surface, they show long term stability for more than 6 months without any significant change in the optical properties (absorbance and fluorescence spectra), and they are also highly stable in the most common buffer solutions such as PBS or borate. However, as biological studies are normally done in more complex biological media which contain a mixture of aminoacids, salts, glucose and vitamins, it is mandatory to determine the stability of our synthesized QDs under these conditions before embarking into any *in vitro* or *in vivo* studies.

2. Stability tests

We tested a sample of CdSe/ZnS Core/Shell QDs with first absorption maximum at 535 nm. These particles have 50 % of azide groups on their surface, QD_{50} indicates coating of Jeffamine M1000 and $H_2N\_PEG\_N_3$ in a 50:50 ratio. Samples were incubated at two different concentrations 1 µM and 10 µM of QDs, and stock solutions were previously prepared in order to keep constant the added volume to the different media. Sample were incubated at 37 ºC in four different media: water, Dulbecco's modified Eagle's medium (DMEM), DMEM supplemented with 10 % fetal bovine serum (FBS) and piperazine-N,N′-bis(2-ethanesulfonic acid) (PIPES). The stability was monitored by Ultraviolet-visible (UV-vis) and fluorescence spectroscopies, fluorescence images, Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM) for periods of time up to 48 hr.

The absorption spectra of QD_{50} at a concentration of 1 µM showed no significant variation in most of the cases except in DMEM, indicating in this case a major nanoscale aggregation or surface degradation after 48 hr (Figure 1A). By contrast, for samples of 10 µM concentration of QDs incubation in all the media showed a great stability, including the case of DMEM (Figure 1B). This result is somewhat surprising since concentration determines the chances of two particles interacting with each other and subsequently aggregate. The higher stability at higher concentrations of QDs is likely to occur because the stability is majorly due to steric (stabilized by PEG chains) instead of electrostatic interactions. For a given concentration of salt at low concentration of QDs the screening of the fixed surface charges is more effective, but with high concentration of QDs there is also a greater number of PEG chains in solution which interact more efficiently preventing the formation of a diffuse double layer around the particles.

Figure 2A shows fluorescence images of 547 nm emitting QD_{50} samples incubated in the four different media and taken after certain periods of time. After 48 hr in DMEM for a 1 µM concentration of QDs there is evidence of colloidal instability seen by the aggregation of the QDs, which crashed out from solution. The photoluminescence intensities for the cases of 1 µM concentration of QDs were also lower after incubation in DMEM + 10% FBS and PIPES for 48 hr when compared to water, and this was drastically reduced for the case of DMEM (Figure 2B). In comparison with its analogue sample incubated in DMEM, the higher colloidal stability observed by UV-vis and fluorescence spectroscopies of QD_{50} samples at 1 µM concentration incubated in DMEM + 10% FBS after 48 hr is likely due to the serum proteins sticking on the surface of the QDs and forming a protein corona which stabilizes the particles.

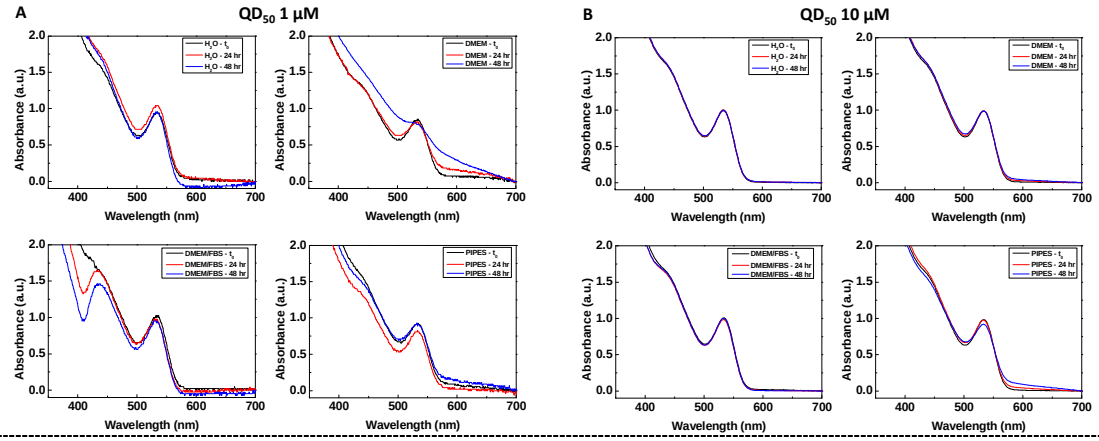

Figure 1: Normalized absorbance of two different concentrations 1 µM (A) and 10 µM (B) of QD_{50} in H_2O, DMEM, DMEM + 10% FBS and PIPES.

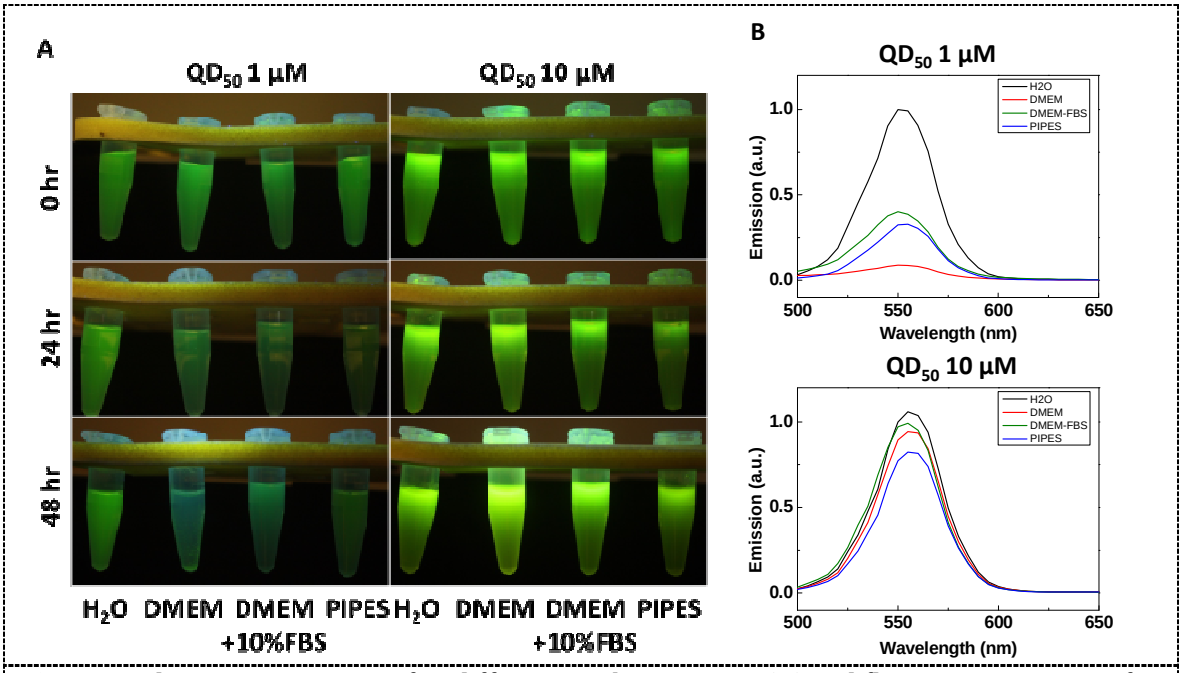

Figure 2: Fluorescence images after different incubation times (A) and fluorescence spectra after 48 hr (B) of 1 µM and 10 µM samples of QD_{50} in H_2O, DMEM, DMEM + 10% FBS and PIPES.

Most of the QD_{50} samples incubated in the different media exhibit low variations of the hydrodynamic diameter obtained from DLS measurements (~ 15-20 nm) (Figure 3). However, incubation in DMEM at a concentration of 1 µM drastically increased the Hydrodynamic Diameter (HD) to ~ 270 nm, corroborating the aggregation effect. TEM also confirmed these data, as images taken from the QD_{50} samples incubated in DMEM showed major signs of aggregation (Figure 4).

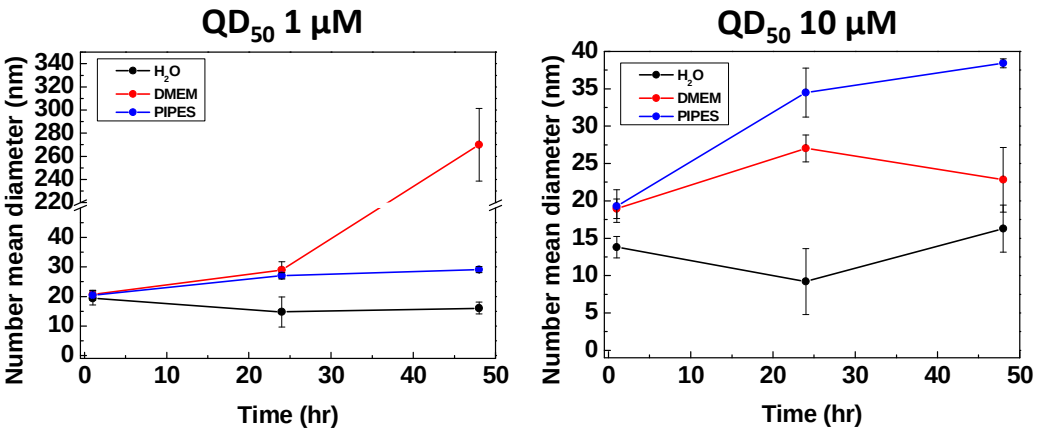

Figure 3: Size evolution by DLS (number mean diameter) at different times of 1 µM and 10 µM samples of QD_{50} in H_2O, DMEM and PIPES. Samples incubated in DMEM + 10% FBS were not analyzed by this technique as the abundance of proteins in the medium would overpower the signal of QDs present at much lower concentrations.

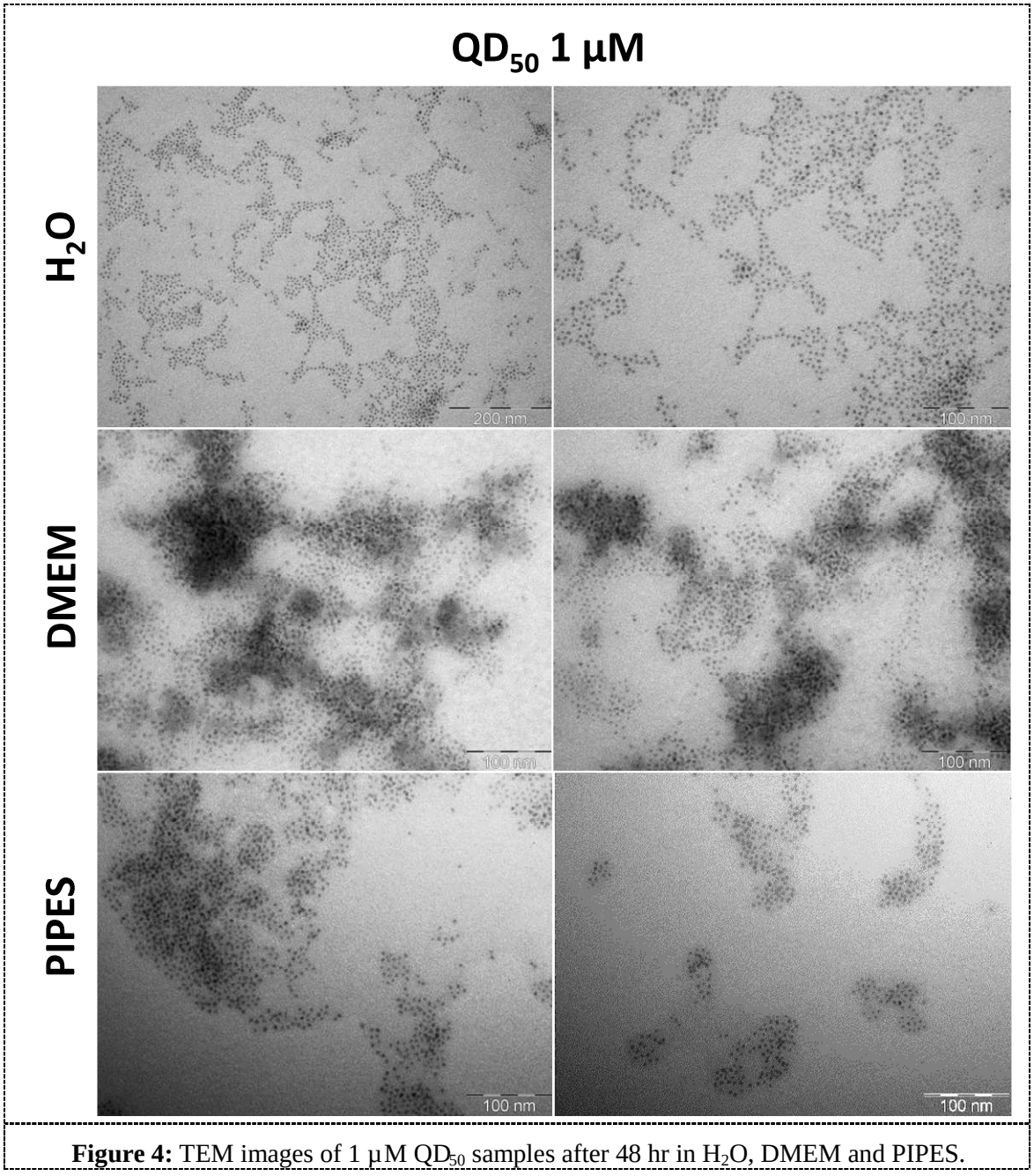

Figure 4: TEM images of 1 µM QD_{50} samples after 48 hr in H_2O, DMEM and PIPES.

3. References

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