

## PREPARATION OF FLUORESCENT MICROBEADS USING DIFFERENT TYPE OF SURFACTANTS

Gospodinka Gicheva<sup>1</sup>

<sup>1</sup> University of Mining and Geology "St. Ivan Rilski", 1700 Sofia, E-mail: e\_gospodinka@yahoo.com

**ABSTRACT.** Fluorescent core-shell CdSe/CdS quantum dots are encapsulated into polystyrene microparticles using an emulsification/solvent evaporation method. An emulsion of different non-ionic surfactants in water is prepared which serves as the medium for the mixture of polymer solution containing the QDs. When the solvent evaporates, a polymer beads possessing bright fluorescence are formed. Here the effect of surfactant type and concentration on the polymer beads size and size distribution is studied.

**Key words.** CdSe/CdS, core/shell, nanoparticles, nanocrystals, quantum dots, polystyrene, latex, microbead.

### ПРИГОТВЯНЕ НА ФЛУОРЕСЦЕНТНИ МИКРОСФЕРИ С ПОМОЩТА НА РАЗЛИЧНИ ВИДОВЕ ПОВЪРХНОСТНО АКТИВНИ ВЕЩЕСТВА

Господинка Гичева<sup>1</sup>

<sup>1</sup> Минно-геоложки университет "Св. Иван Рилски", 1700 София, e-mail: e\_gospodinka@yahoo.com

**РЕЗЮМЕ.** Квантови точки от типа ядро/ обвивка CdSe/CdS са вградени в полистиренови микросфери чрез метода на емулгиране и последващо изпарение на разтворителя. За целта са приготвени емулсии от различни повърхностно активни вещества (ПАВ), които служат като среда за емулгирането на полимерния разтвор съдържащ квантовите точки. След изпарението на разтворителя се получават полимерни сфери притежаващи ярка флуоресценция. В настоящата работа е проследен ефекта на вида ПАВ и неговата концентрация върху размера и разпределението по размер на получените полимерни сфери.

**Ключови думи.** CdSe/CdS, ядро/обвивка, наночастици, нанокристали, квантови точки, полистирен, латекс, микросфери.

### Introduction

The semiconductor nanoparticles (quantum dots) attract a lot of attention due to their interesting optical and electronic properties [P. Hawrylak, 2001]. One of their most promising applications involves their incorporation in a polymer matrix [T. Jamieson *et al.*, 2007] with respect to their use in the field of biology and medicine [Gao *et al.*, 2005] as bright fluorescent markers. The polymer nanocomposites with quantum dots QDs combine the advantages of both polymer matrix mechanical strength and the QDs unique optical and electronic properties, thus resulting in novel hybrid materials with improved performance. For that purpose a QDs have been embedded in various polymers like poly(laurylmethacrylate) [Lee *et al.*, 2000], poly(methylmethacrylate) [Khanna *et al.*, 2007], polystyrene [Tamborra *et al.*, 2004] and polyisoprene [Yin *et al.*, 2007]. The embedding of QDs in silica monoliths [Selvan *et al.*, 2001; Correa-Duarte *et al.*, 2001], sol-gel films [Bullen *et al.*, 2004] and silica microbeads [Nann *et al.*, 2004; Mokari *et al.*, 2005] represents an alternative use of inorganic polymers. A direct approach for the incorporation of QDs in polymer microparticles is the emulsion polymerization of the monomer in the presence of QDs [Han *et al.*, 2001; Yang *et al.*, 2004], by infiltration of the QDs into the polymer particles [Sheng *et al.*, 2006, Cao *et al.*, 2006] or by using layer-by-layer technique [Ma *et al.*, 2007]. Instead, we have used the so-called emulsification/solvent evaporation technique for the preparation of polymer microparticles [Gicheva *et al.*, 2009]. We have demonstrated a successful incorporation of QDs in

polystyrene microparticles that results in a bright stable fluorescent polymer beads. In this method oil-in-water emulsion is prepared with the oil droplets containing the polymer with QDs and the water phase containing the surfactant stabilizing the colloidal dispersion. Further evaporation of the organic solvent from the droplets forms the polymer microbeads with QDs. To make this convenient method, here we investigate the effect of surfactant type and concentration on the preparation of QDs-containing polystyrene microparticles.

### Experimental procedures

Here we repeat the same experimental procedure of microbeads and QDs synthesis as described in ref. [Yordanov *et al.*, 2005]

**Chemicals.** Polystyrene (Mw~180000), Pluronic F-68, Tween 80 and Triton X-100 were purchased from Fluka. Cadmium oxide (CdO, 99 %), sulfur (S, purum, 99.5 %) and pulverized selenium (Se, pure, 99.5 %) were from Fluka. Tributylphosphine (TBP, 97 %) was from Aldrich. Liquid paraffin and stearic acid were from R.A.M.Oil SpA (Italy) and Hatkim SA (Turkey), respectively. Chloroform was of analytical reagent grade from Labscan Ltd (Ireland). Toluene, chloroform, methanol and acetone were purified by distillation. All other chemicals and solvents were used as received, without additional purification.

**Quantum dots (QDs).** QDs can be prepared by water-based syntheses of [Lianos *et al.*, 1987], but in our case we utilized the so called “hot-matrix” syntheses in liquid paraffin to produce high quality CdSe cores [Yordanov *et al.*, 2008]. Building the shell of CdS over the core of CdSe was carried out by a known method [Yordanov *et al.*, 2009]. As-prepared CdSe/CdS QDs were dispersed in chloroform.

The CdSe cores were synthesized by mixing CdO (50 mg, 0.39 mmol), stearic acid (0.600 g, 2.1 mmol) and liquid paraffin (15 ml) and then heating them up to ~260 °C. A solution of tributylphosphine selenide, TBP-Se (1.0 ml, 0.125 M), was then fast injected into the mixture in order to form CdSe QDs. The reaction mixture was then cooled down by dissolution in cold toluene. After centrifugation, the orange precipitate of CdSe was washed with toluene and liquid paraffin.

For the growth of CdS shells, CdO (50 mg) was dissolved in a mixture of stearic acid (600 mg) and liquid paraffin (5 ml) at 180 °C on open air and then cooled down to 120 °C. CdSe cores, obtained from the synthesis above, were transferred into the mixture. Tributylphosphine sulfide (TBP-S) solution (2 ml; 0.125 M) was injected at 100 °C and the reaction temperature was raised up to 250 °C within 8-10 min. The hot reaction mixture was then dissolved in toluene and cooled down to room temperature to form orange-red precipitate of CdSe/CdS QDs. The precipitate was extracted with toluene to remove the excess of TBP-S precursor, stearic acid and liquid paraffin. The core/shell QDs CdSe/CdS were redispersed in chloroform for further use.

Figure 1 shows the HR-TEM image of core-shell QDs of CdSe/CdS obtained by High Resolution Transmission Electron Microscope. The average nanoparticle size, estimated from the image, is ~4 nm.

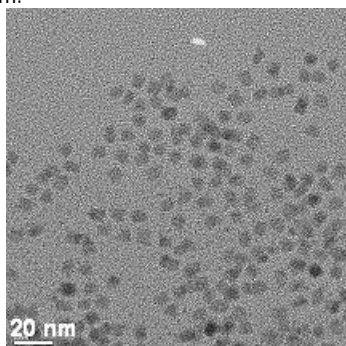


Fig. 1. HR-TEM image of CdSe/CdS QDs used for the preparation of QDs-polystyrene microparticles

Figure 2a depicts the absorbance spectra of QDs. It is seen that the spectrum of core-shell nanoparticles CdSe/CdS is red-shifted in comparison with the spectrum of CdSe cores. The calculated size of CdSe cores (from the spectrum) is ~3 nm.

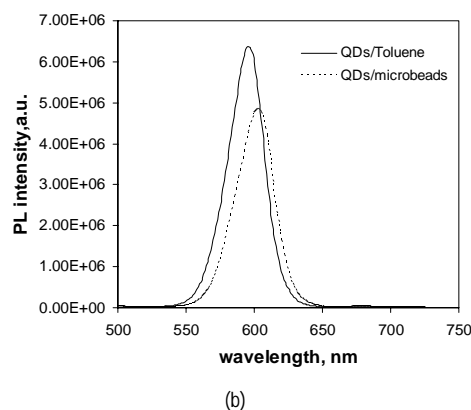
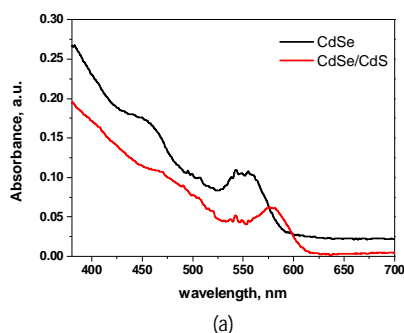


Fig. 2. Absorption and fluorescence spectra of CdSe/CdS QDs incorporated in QD-polystyrene microparticles. (a) Absorption spectra of CdSe core QDs and core-shell CdSe/CdS QDs. (b) Fluorescence spectra of the initial QDs in toluene and QD-polystyrene microparticles (excitation wavelength 375 nm). The fluorescence spectrum of the QD-polystyrene nanocomposite microparticles is found to be slightly red-shifted.

**Preparation of QD-polystyrene particles.** Polystyrene solution in toluene (5 wt. %) was mixed together with QD-chloroform dispersion (0.8 ml). An amount of 150 µl of solution of QDs in polystyrene was added to 10 ml of surfactant solution in water of Triton X-100 (Merck), Pluronic F-68 and Tween 80 with a different concentration (depending on CMC) upon intensive magnetic stirring (1000 rpm) at room temperature. The resulting emulsion was stirred for 24 hours on open air to form the QD-polystyrene particles. All experiments were carried out at all other conditions constant. The obtained QD-polystyrene microparticles can be purified by washing out with distilled water and centrifugation. Also, one can isolate more monodisperse in size QD-polystyrene microparticles by means of standard fractional centrifugation.

**Methods for analysis.** The optical absorption spectra of QDs were measured by UV-vis spectrophotometer Jenway 6400. The fluorescence spectra were measured by a Carl Zeiss monochromator, equipped with a photo-multiplying tube (excitation at 375 nm). The QD-polystyrene microparticles were observed by a Scanning Electron Microscope (SEM) JSM-5510 (JEOL), operated at 10 kV of acceleration voltage. Samples for SEM were prepared on a glass substrate and coated with gold by JFC-1200 fine coater (JEOL). The QDs were imaged by HR-TEM JEM-2100F (JEOL) operated at 200 kV of acceleration voltage, equipped with an EDS (energy dispersive spectroscopy) probe. Fluorescent Microscope Leica, equipped with a CCD camera, was used to visualize the fluorescent QD-polystyrene microparticles (excitation wavelength 375 nm). The size distribution of polystyrene microbeads was estimated from the respective SEM images by counting the number of species of diameters in a specified interval of sizes.

## Results and discussion

We use core-shell QDs of CdSe/CdS for incorporation in polymer latex particles, because they possess a high quantum yield of fluorescence (about 60 %), which is much higher than the one in respective CdSe core QDs (about 25 %). The CdS shell built over the core of CdSe protects it also from oxidation, which results in higher stability of the fluorescence with

respect to various factors of the matrix around the QDs. The CdSe/CdS QDs, used in our experiments have an average diameter  $\sim 4$  nm. These QDs are further dispersed in a solution of polystyrene in toluene to form a dispersion. Since the QDs are hydrophobic, we expect them to remain entrapped in the organic phase (the emulsion droplets) after the emulsification of dispersion in a water solution of the surfactant. Toluene is a volatile organic solvent and after its evaporation, the organic emulsion droplets form polymer microparticles with the QDs embedded inside.

We have prepared water solutions of three different surfactants: Triton X-100, Pluronic F-68 and Tween 80 with five different concentrations defined by their critical micelle concentration (CMC) for each one of them (Table 1): CMC, 5CMC, 15CMC and 30CMC.

Table 1.

*Parameters of the surfactant solutions used to stabilize the emulsions.*

	Pluronic F-68	Triton X-100	Tween 80
CMC (mM)	0.04	0.2-0.9	0.012
Mw*	8350	625	1310
HLB**	29	13.5	15
Av. Micelle Weight	-	80000	79000

\* - molecular weight

\*\* - hydrophilic-lipophilic balance

The SEM images (Fig. 3) show that the obtained QD-polystyrene microparticles are spherical. Varying the amount and type of the surfactant affects the size of the obtained latex microparticles. In all cases the microparticles are with a rather broad size distribution. Actually, the size and distribution of microparticles are expected to depend on the average size and distribution of the initial emulsion droplets. We chose this surfactants because they proven to be suitable for incorporation of QDs in polymer microbeads. Using ionic surfactants (cationic and anionic) leads to aggregation of QDs and inability to incorporate in the polymer microbeads. Where the surfactant concentration is 15 times CMC the results indicate that the average size of microbeads about  $1.5 \mu\text{m}$  in case of Pluronic F-68 and Tween-80, while using Triton X-100 leads to bimodal size distribution. Increasing the surfactant concentration up to 30 times CMC affects the size of obtained microbeads differently-where Pluronic F-68 is involved it leads to formation of smaller microbeads ( $0.75 \mu\text{m}$ ) while in the case of Triton X-100 the obtained microbeads have been more uniform in size with average diameter of  $1.5 \mu\text{m}$ . We have also tried to prepare microbeads using lower surfactant concentration – 5 and 10 times CMC, but the experiment showed that the least possible concentration for the surfactants is 15 time CMC. The results from dynamic light scattering show a double-size distribution of the droplets, which correlates to the one of the obtained microparticles. Although the as-prepared QD-polystyrene microparticles have a relatively broad size distribution it is not a limitation toward their practical utilization, because various techniques, such as conventional fractional centrifugation can be easily applied to separate the latex particles in relatively monodisperse fractions.

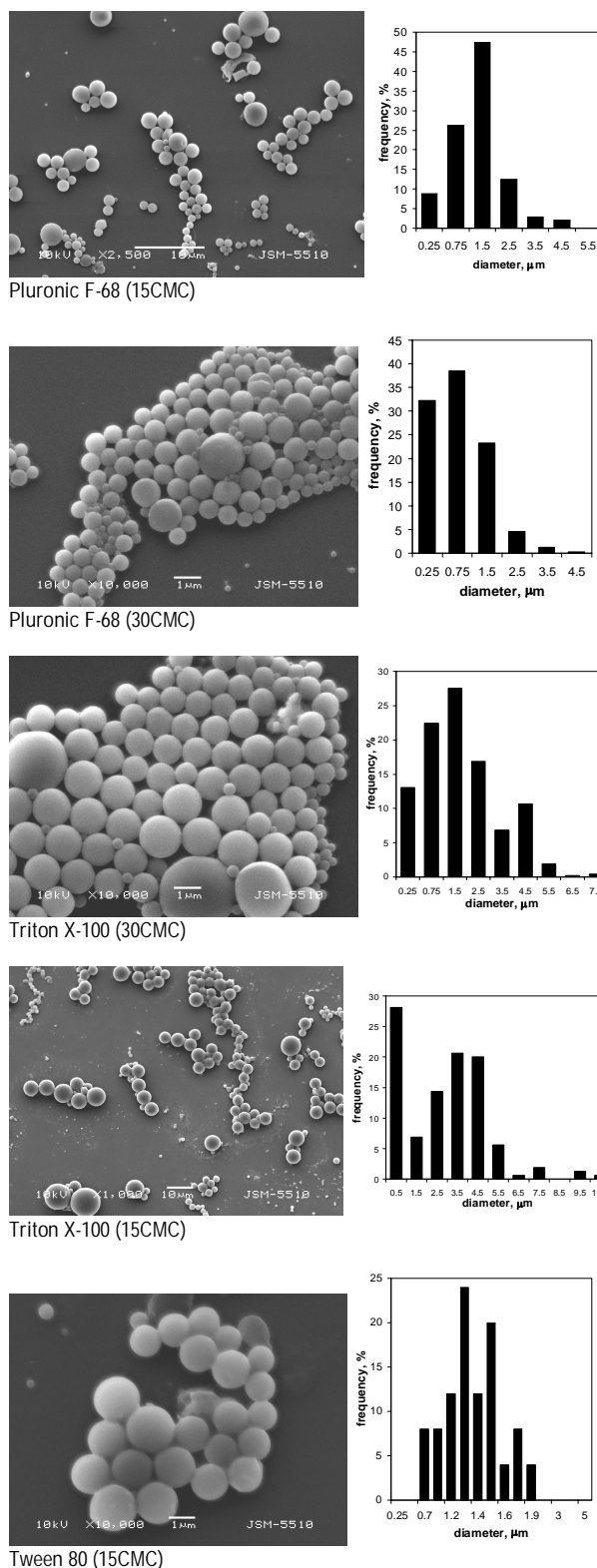


Fig. 3. Scanning electron microscopy (SEM) images of QD-polystyrene microparticles, prepared from: Pluronic F-68, Triton X-100 and Tween 80 as surfactants, emulsified in 10 ml solution with 30CMC and 15CMC for each type of surfactant. After emulsification, the toluene solvent was evaporated to form the QD-polystyrene microparticles. Beneath the SEM images a size and size distribution of QD-polystyrene microparticles is shown.

We have tried to incorporate QDs in polymer bead by emulsion polymerization, but the resulting composite latex did not show any or very low fluorescence. It is known that the fluorescence of QDs is very sensitive to radicals generated during the emulsion polymerization of polystyrene. A slight

blue-shifted fluorescence spectrum of the QD-polystyrene microparticles was previously observed in comparison with the initial QDs in toluene (Fig. 2b).

In the case of emulsification/solvent evaporation technique, which is utilized here, QDs remain fluorescent inside the polymer microparticles, as confirmed by fluorescence microscopy (Fig. 4).

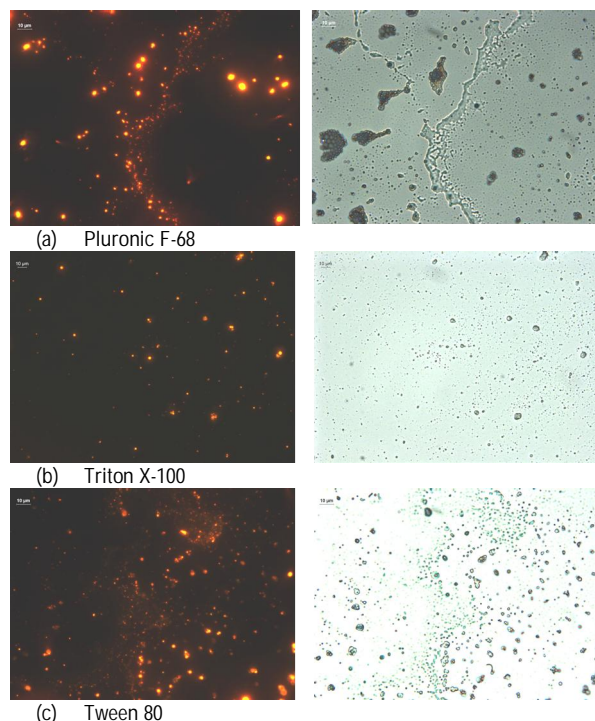


Fig. 4. QD-polystyrene microparticles (~2 micrometers in size) visualized by fluorescence microscopy (excitation wavelength 375 nm).

## Conclusions

Fluorescent core-shell CdSe/CdS quantum dots are successfully encapsulated into polystyrene microparticles using an emulsification/solvent evaporation method by using three different type of surfactants. The effect of surfactant type and concentration on the size and size distribution of QD-polystyrene nanocomposite microparticles have been studied. Since these hybrid nanocomposite particles have potential applications in biology and opto-electronics, the bright fluorescence of quantum dots embedded in polystyrene matrix is of great importance. This nanocomposite material can be used as building blocks of photonic crystals and devices, fluorescent biological markers, barcodes, etc.

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