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Labeling of Biocompatible Polymer Microcapsules with Near-Infrared Emitting Nanocrystals

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ABSTRACT

Microcapsules consisting solely of biocompatible components were prepared in water by a colloidal templating technique on soluble carbonate cores using alginic acid sodium salt, protamine sulfate, dextrane sulfate, and chitosan. These microcapsules, as well as capsules made from synthetic polyelectrolytes, were labeled with water-soluble CdTe nanocrystals (NCs) emitting in the visible and, for the first time, with $Cd_xHg_{1-x}Te$ or HgTe NCs emitting in the near-IR. The luminescence efficiency of NCs at physiological conditions remained stable for two weeks in the case of CdTe and at least for a month for CdHgTe and dropped by 80% for HgTe because of the shift of the luminescence band outside the water transmission window. Biocompatible microcapsules labeled with $Cd_xHg_{1-x}Te$ NCs emitting at 750−1200 nm might be of special interest for monitoring the drug delivery processes.

Recently, the attachment of luminescent CdSe/CdS[1] and CdSe/ZnS[2−4] core−shell nanocrystals (NCs) to DNA molecules[1−3] and proteins[4] has been shown to be promising for biological imaging experiments. Multicolor optical coding for biological assays has also been demonstrated using polymer microspheres tagged with specific and identifiable combinations of CdSe/ZnS NCs.[5] The use of NCs emitting in the near-IR has been declared as the next very important step in biolabeling,[1,5] being especially important for medical diagnostics in blood.[6] In fact, the optical imaging, especially in vivo, requires the use of excitation and emission wavelengths that are not absorbed by tissue (water, hemoglobine).[7] In our previous publication,[8] we reported on microcapsules made from synthetic polyelectrolytes and labeled with water-soluble CdTe NCs luminescing in the spectral range of 510−730 nm. Both single-color and multicolor tagging with controlled emission intensity ratios was demonstrated, being important for the further use of labeled microcapsules in encoding combinatorial libraries.

In this communication, we introduce a new type of microcapsules consisting solely of biocompatible components and extend the labeling procedure, spreading for the first time the wavelength of the luminescent code into the near-IR by using water-soluble $Cd_xHg_{1-x}Te$ and HgTe NCs.

The microcapsules used in this work were of two types, referred to below as model and biocompatible. The model capsules consisted of a shell made from alternate layers of oppositely charged synthetic polyelectrolytes (four layers of a polyanion sodium poly(styrene sulfonate) and four layers of a polycation polyallylamine hydrochloride, PAH) and a liquid core containing positively charged PAH with a concentration of aminogroups of ∼0.1 M.[9] The encapsulation of negatively charged NCs in this case is promoted by electrostatic forces between them and the oppositely charged polyelectrolyte.[8]

Biocompatible microcapsules were prepared using carbonate particles as templates. Nearly spherical $MnCO_3$ particles 3.7 μm in diameter (Figure 1, top) carrying positive surface charge[10] were synthesized by mixing the equal amounts of aqueous solutions of $MnSO_4$ (0.016 mol/L) and NH_4HCO_3 (0.16 mol/L), both containing 0.5% of ethanol, which played the role of a size-regulating agent and allowed us to obtain

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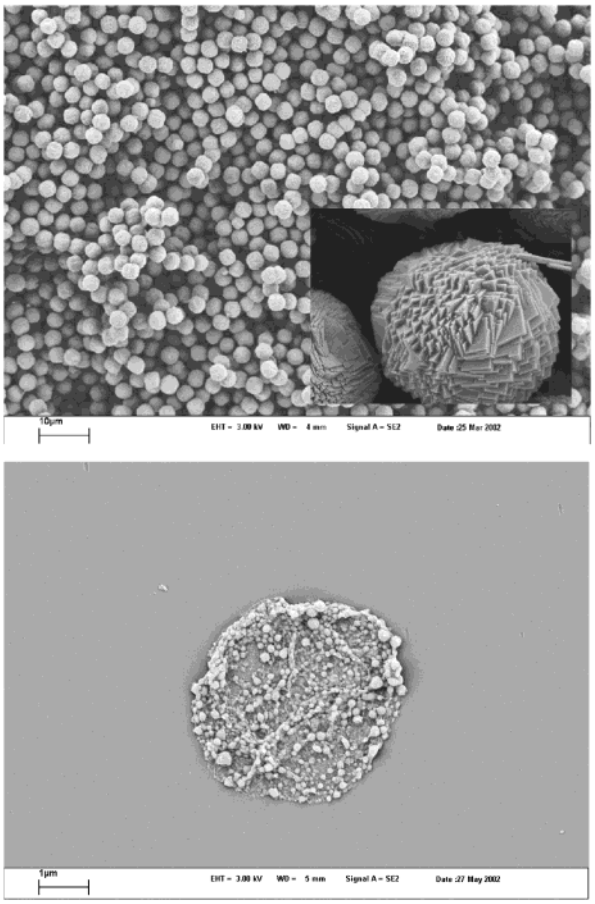

Figure 1. Top: scanning electron microscopy (SEM) image of $MnCO_3$ particles used as cores for the preparation of biocompatible microcapsules. Scale bar = 10 μm. Bottom: SEM image of a biocompatible capsule templated on a $MnCO_3$ sphere. Scale bar = 1 μm. Clumps are attributed to chitosan; the rest of the core material was not found by elemental analysis on Mn.

highly monodisperse samples.[11] The cores were covered by a shell consisting of three alternate layers of a polyanion alginic acid sodium salt (whose aqueous solution with a concentration of 1 mg/mL was used) and a polycation protamine sulfate (5 mg/mL) utilizing the layer-by-layer assembly technique on colloidal templates,[12] following by coating with a monolayer of dextrane sulfate (5 mg/mL) and a monolayer of chitosan (2.5 mg/mL) to improve the stability of the structure.[13] The resulting core/shell particles were thoroughly washed by repeated centrifugation and redispersion in water at pH 7, and the cores were dissolved by decreasing the pH to 3.5 through the addition of HCl, providing stable microcapsules (Figure 1, bottom). They contain some amount of polyelectrolyte in a core as the model capsules do, which was confirmed by the labeling experiments with red-emitting CdTe NCs. Under a confocal microscope, not only did the shells of biocompatible microcapsules appear strongly luminescent because of the captured NCs (Figure 2) but moderate emission from the interior of the capsules was seen as well. Confocal microscopy of the model microcapsules labeled with CdTe NCs showed almost uniform luminescence both from the interior and from the shells, indicating the presence of oppositely charged poly-

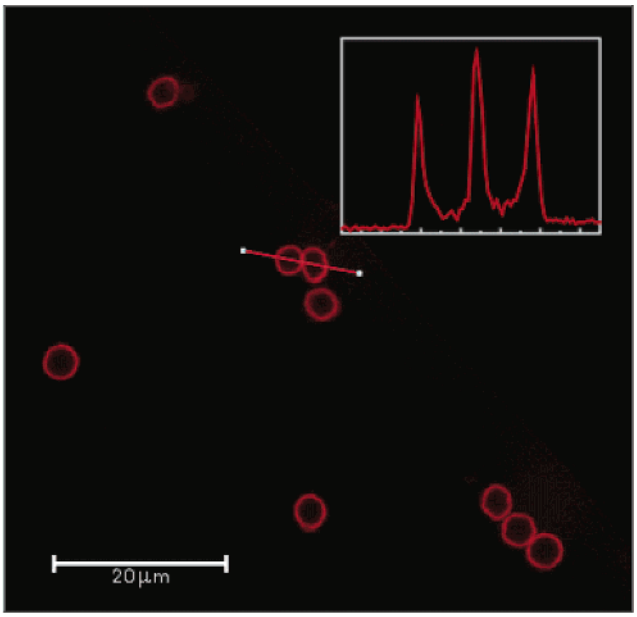

Figure 2. Confocal microscopy image (confocal laser scanning microscope TCS Leica, excitation wavelength 476 nm) of biocompatible microcapsules labeled with red-emitting CdTe NCs. Inset: luminescence profile indicating the preferential attachment of NCs to the walls of capsules and a partial filling of the capsule interior.

electrolytes in the capsule interior.[8] The presence of oppositely charged polyelectrolytes in the interior of both the model and biocompatible capsules was additionally proved by confocal microscopy utilizing polyelectrolytes that were chemically labeled with dyes as described in ref 14. The NCs are captured and irreversibly adsorbed by the capsules mainly because of the electrostatic interactions, as was shown before in the multiple examples of polymer/NC composites prepared by the layer-by-layer assembly.[8,15]

Further functionalization of capsules with biomolecules is possible by utilizing the layer-by-layer approach, as was recently shown in the example of latex microspheres coated with synthetic polymers, following by an outermost layer of anti-immunoglobulin G to render them biospecific.[16]

Details of the aqueous synthesis of thiol-stabilized CdTe, $Cd_xHg_{1-x}Te$, and HgTe NCs have been reported previously.[17−19] These NCs are stable against aggregation and photobleaching and have sufficiently narrow emission peaks that are tunable with the particle sizes and the particle composition in the wide spectral range from green to near-IR because of the quantum confinement effect (Figure 3). The form of the emission spectrum of the NCs emitting in the near-IR is strongly affected by the transmission window of water, which has a cutoff at 1.35 μm (Figure 5), so that only the smallest HgTe nanocrystals emitting from 1.1−1.2 μm can be used for biolabeling. The room-temperature luminescence quantum yields of the NCs are typically in the range of 10−25% and can reach values of 40−50%.[17−19] The NCs used in this work carried a negative surface charge due to the stabilizers used (thioglycolic acid or thioglycerol).

To be used in biological systems, NCs themselves and their luminescence efficiency in particular should be stable at physiological conditions.[20] We chose the phosphate-

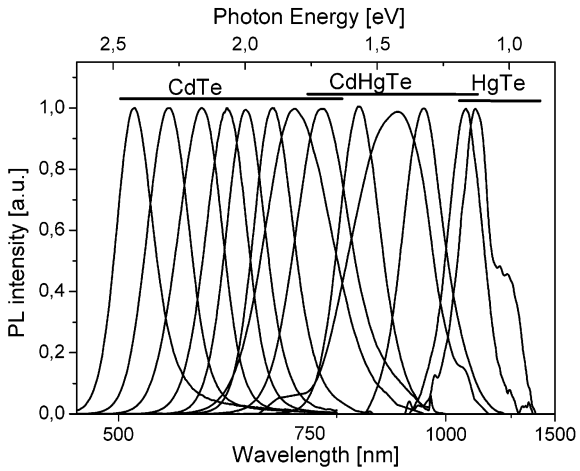

Figure 3. Size tunability of the photoluminescence spectra of thiol-capped CdTe (2−6-nm size range), CdHgTe (3−6-nm size range), and (the smallest available) HgTe NCs (2.5 nm) through the visible and near-IR spectral region. Thioglycolic acid and thioglycerol were used as capping agents for CdTe NCs and for CdHgTe and HgTe NCs, correspondingly.

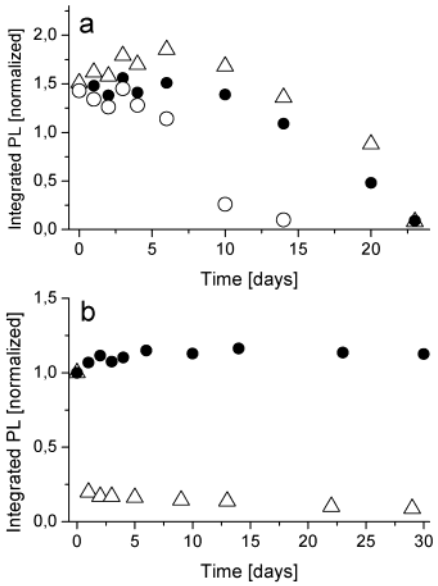

Figure 4. Temporal evolution of the integrated photoluminescence intensity of the strongly diluted (optical densities of 0.02−0.03 at the wavelength of excitation) colloidal solutions of (a) CdTe NCs in pure water (△), PBS (●), and PBS additionally containing 0.137 mol/L NaCl (○). (b) CdHgTe (●) and HgTe (△) in PBS solution.

buffered saline (PBS) solution (0.137 M NaCl, 0.0027 M KCl, 0.01 M phosphate buffer, pH 7.4) as a medium for doing stability tests for both the NCs and the labeled capsules. The samples were stored in darkness at room temperature and taken for measurements at periodical time intervals. The CdTe and CdHgTe NCs showed excellent stability of optical properties in PBS solutions for months, being at relatively high particle concentrations $\{(0.5-2.5) \times 10^{-5}$ M$\}$, which were also used by us for labeling the capsules. The photoluminescence of HgTe NCs appeared to be strongly red-shifted in the PBS solution. This low-energy shift outside the water transmission window led to an approximately 5-fold decrease in the effective photoluminescence intensity because of the light reabsorption. To compare the stability of NCs in aqueous medium and in PBS, the samples were intentionally diluted (particle concentration 5×10^{-7} M). At such a low concentration, NCs slowly degrade in time because of the partial release of thiol molecules from the capping shell. As seen from Figure 4, the stabilities of CdTe NCs in pure water and in a PBS solution are comparable. The influence of the ionic strength on stability became pronounced only when the NaCl concentration was greater than twice the physiological one. At the same time, CdHgTe and HgTe NCs appeared to be very stable even at such low concentrations, although the abovementioned red shift of HgTe luminescence led to the undesirable decrease in the detected luminescence intensity due to the reabsorption by solvent.

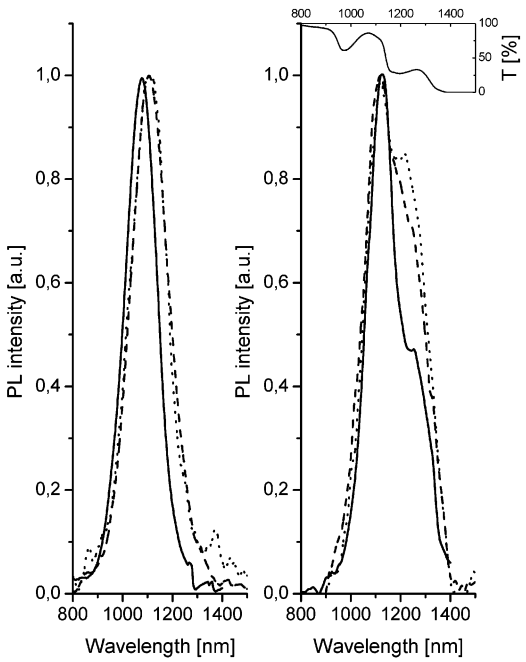

Figure 5. Photoluminescence spectra ($\lambda_{ex} = 500$ nm) of CdHgTe (left panel) and HgTe (right panel) NCs taken from the initial aqueous colloids used for encapsulation (—), from model capsules (− −), and from biocompatible capsules (···). The inset (right panel) shows the transmission spectrum of water. For NCs in capsules, the spectra were measured in a PBS solution 5 h after encapsulation.

The encapsulation of IR-emitting NCs in both model and biocompatible microcapsules was achieved by their soaking in aqueous 0.2 M NaCl solution containing $\sim 0.5 \times 10^{-5}$ M (particle concentration) CdHgTe or HgTe for 1 h at neutral pH, following by their separation through centrifugation and redispersion in PBS solution. Figure 5 compares the photoluminescence spectra of both CdHgTe and HgTe NCs in the aqueous solution used for encapsulation with those for the NCs embedded in the model and biocompatible microcapsules. The spectra from labeled capsules were measured in quartz microcuvettes (45 μL). The positions of the emission maxima of both CdHgTe and HgTe NCs were almost identical in all cases, indicating that no or only minor aggregation occurs during the encapsulation and that the Förster energy transfer between NCs plays a minor role. The

long-wavelength part of the HgTe spectra was influenced by the transmission of water.

The mechanical and chemical stability of biocompatible microcapsules themselves during the encapsulation of NCs was monitored by means of confocal microscopy using the capsules whose external chitosan layer was chemically modified with the dye tetramethylrhodamine-5-(and-6)-isothiocyanate.[14] The capsules appeared to be stable during the whole encapsulation procedure and during multiple washings and weeks of storage in PBS.

No changes in the NC luminescence were observed for weeks when the dispersions of nanocrystal-labeled microcapsules in PBS solution were stored in a refrigerator (4−10 °C) and in the dark. No leakage of NCs into the background solution took place, as confirmed by the absence of luminescence in the supernatant when the microcapsules were removed by centrifugation.

In summary, microcapsules consisting solely of biocompatible components were prepared by a colloidal templating procedure and were labeled with NCs emitting in the near-IR. Near-IR tagging of model polymer microcapsules whose interior contains a charged polyelectrolyte has been done as well. The stability of the NC luminescence at physiological conditions has been tested. The interior of the capsules can be used for the encapsulation of drugs by applying the recently developed procedure,[21] and the capsules can be monitored by luminescence in tissue, which provides another way that they may be used in drug delivery studies.

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