

Acid Synthesis of Luminescent Amine-functionalized or Erbium-doped Silica Spheres for Biological Applications

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Abstract In this work we discuss and investigate the morphological and optical properties of luminescent silica spheres which can have interesting applications in bioimaging and biosensing. The spheres are synthesized following an acid route by the hydrolysis and condensation of tetraethylortosilicate (TEOS) and can be functionalized by incorporation of aminopropyl-triethoxysilane (APTES) during the synthesis, inducing a significant luminescence that can be attributed to a recombination mechanism from localized organic defects related to $-NH_2$ groups. It is shown that the acid synthesis route produces very regular spherical particles, but their diameter vary in the range of 200–4,000 nm. The luminescence properties have been investigated and optimized by variation of the annealing temperature for the functionalized spheres, obtaining the most efficient PL emission after a thermal treatment of 1 h at 600 °C in air. Moreover, the possibility to introduce rare earths like erbium in the spheres was also studied and the corresponding Er^3 luminescence emission at 1.53 μm is reported in terms of intensity and lifetime, pointing out that erbium can be easily and efficiently incorporated during the acid synthesis giving high PL intensity with a good lifetime of 3.9 ms.

Keywords Silica nanoparticles · Erbium · Luminescence · Biomarkers · Bioimaging

Introduction

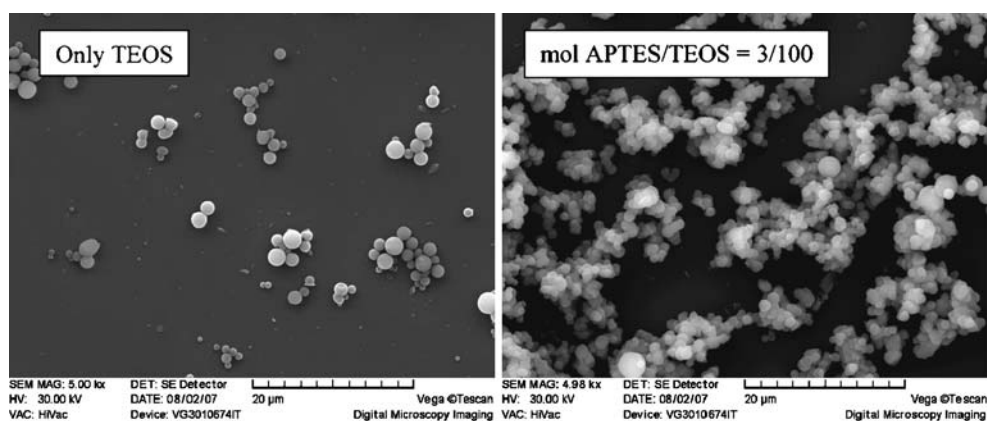
Luminescent nanoparticles are becoming more and more important in the field of biosensors and bioimaging [1, 2], leading to improvements in sensitivity, selectivity and multiplexing capacity over conventional fluorophores. Key features for their actual use for in vitro and in vivo applications are the intensity of their optical emission, the efficiency of their surface functionalization for molecular recognition and their low toxicity, possibly associated to a cheap, fast and reliable production process. For these reasons silica nanoparticles seems a very interesting choice due to an easy control of their surface ligands and biocompatibility. Silica spheres can be obtained through TEOS condensation by a Stöber basic route [3] or by an acidic route [4, 5]. Several strategies have been recently developed to make them luminescent by the incorporation of organic or inorganic emission centers such as common dyes [6], rare earths [7, 8] or quantum dots [9]. Most of these procedures allow reaching a strong photoluminescence emission but unfortunately requiring multiple processing steps and the use of expensive or toxic fluorophores.

In this work we follow a different approach for synthesizing luminescent silica spheres. It consists in the calcinations of hybrid aminopropylsilica spheres by using a procedure similar to that of van Blaaderen [10] and Jakob [11]. However, unlike these authors, who followed a basic route, we work here in an acid pH environment. Moreover, the possibility to introduce rare earths like erbium in the

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Fig. 1 SEM images of the acid synthesized silica particles with and without APTES



silica spheres was also investigated. Lanthanide doped silica spheres in fact would offer two different strategies for increasing the signal with respect to the background noise in biological analysis, mainly due to the self fluorescence of the biological material. The first is by exciting the biological system in the visible and performing delayed measurement of the PL emission. In this way the long lifetime PL emission of the rare earths (from hundreds of microseconds up to milliseconds) can be well discriminated from self fluorescence intensity, which decreases in few nanoseconds. The second strategy would be to excite in the infrared (typically 980 nm for Yb–Er rare earths) and look at the visible emission which is generated by an upconversion process due to a sequential absorption of two or more photons. In this case the self fluorescence is also very low because the biological material is almost transparent in a wide window of wavelengths. In fact, most animal cells and tissues are considered to be nearly transparent in the spectral range from about 700 to 1,100 nm [12]. Indeed, the three major tissue and cellular fluorophores are water, oxygenated haemoglobin and melanin and in the specified region the global absorption coefficient can be as low as 1, while in the visible region it rises up to 100. At the same time it is possible to use cheap and efficient visible light detectors, which offer much better performances than infrared detectors.

In this work the photoluminescence of the silica spheres has been studied in terms of excitation, emission and time-resolved spectroscopy for different synthesis routes and preparation conditions. The results obtained show the possibility of realizing good luminescent amino-functionalized erbium doped silica spheres by following the described procedures. Moreover, their cheap and easy synthesis, stability in water, possible surface functionalization and bio-compatibility makes them important alternatives to the use of quantum dots or organic dyes in biological imaging and other applications.

Experimental

Reagents were purchased either from Sigma or Fluka and used without further purification. Amino-functionalized silica spheres were obtained by adding 90 ml of glacial acetic acid to 16 ml distilled water in a flask together with a magnetic stirring bar. In a separate flask a solution of 45 ml of TEOS and APTES, in a molar rate APTES/TEOS of 3:100, was prepared and subsequently added to the previous solution and stirred overnight. The final colloidal solution was precipitated by centrifugation (5,000 rpm, 10 min) and rinsed at least three times in ethanol in ultrasonic bath.

The introduction of erbium in the spheres in spite of APTES was obtained by adding $\text{ErCl}_3 \cdot 6\text{H}_2\text{O}$ (powder) during the synthesis and using only TEOS as reagent. In particular a molar amount of 1.2% of erbium was added with respect to the molar TEOS quantity.

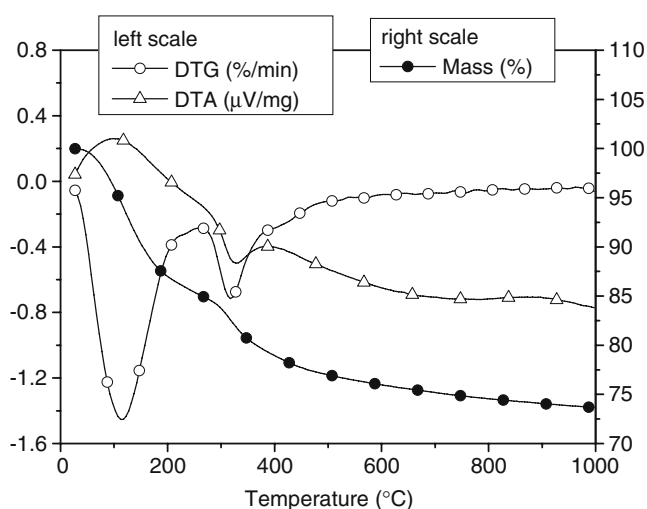
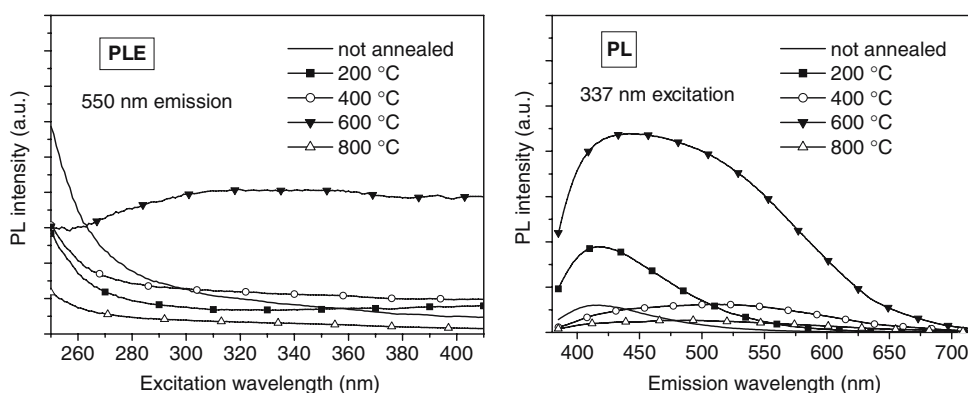


Fig. 2 TG measurement (*right scale*), its first derivative DTG (*left scale*) and differential thermal analysis DTA (*left scale*) on acid synthesized silica powders

Fig. 3 Photoluminescence excitation (PLE) and emission (PL) of the amine-functionalized, acid-synthesized silica particles for different annealing temperatures



For the following thermal treatments the silica spheres were dried in air and put in a furnace for 1 h at 200 °C, 400 °C, 600 °C or 800 °C. Thermogravimetry (TGA) and differential thermal analysis (DTA) were used to characterize the response of the material under thermal treatments and were performed using a STA 409 PC Luxx (Netzsch) instrument.

The morphology of the synthesized particles was investigated in terms of dimension, shape and aggregation state by means of Scanning Electron Microscopy, performed in a VEGA TS 5130 LM (Tescan) microscope under 30 kV acceleration conditions. For this analysis the spheres were dispersed in ethanol (6 mg/ml) and deposited by drop casting on a silicon wafer.

Finally, the photoluminescence excitation and emission properties of untreated and thermally treated nanoparticles in the UV and visible regions (300–850 nm) have been measured in a Fluoromax system (Horiba Jobin Yvon) and in the near IR (up to 1,700 nm) by Ar laser excitation at 488 nm.

Amino-functionalized silica spheres

The SEM images reported in Fig. 1 show the silica spheres obtained by using TEOS alone (left) or with APTES (right). The non functionalized spheres have a very regular shape, but with variable diameter in the range of 200–4,000 nm. On the other hand the APTES functionalized particles show an irregular shape and some aggregation between particles.

The TGA and DTA measurements are reported in Fig. 2 together with the first derivative of the TGA curve, which shows the maximum mass loss velocity of the sample. In particular there are two points of transition for these silica powders. The first is related to an endothermic reaction which occurs at about 100–120 °C and corresponds to physical desorption of water and humidity from the sample and from its pores [13]. The second is an exothermic

reaction which occurs at about 310–330 °C and corresponds to the removal of organic groups, to processes of polymerization and condensation of the matrix [13]. These processes lead to a more compact and dense structure, which is energetically more stable, for this reason the net energy balance is positive. After thermogravimetric analysis we decided to treat the silica powders at 200 °C, 400 °C, 600 °C and 800 °C, expecting the possibility of observing a different optical behaviour for the samples not heated and heated at 200 °C with respect to the samples heated at 400 °C or higher temperature.

The excitation photoluminescence analysis (PLE) and the emission photoluminescence analysis (PL) of the silica particles treated at the different temperatures are reported in Fig. 3. The PL curves, shown in the right image, were obtained by 337 nm excitation and using a long wavelength pass filter with cut off at 380 nm. It can be observed that the shape of the curves is completely different when the annealing temperature is 400 °C or higher and can be attributed to the structural modifications of the glass matrix. In particular the PL emission spectrum of the high temperature annealed samples is very broad and covers

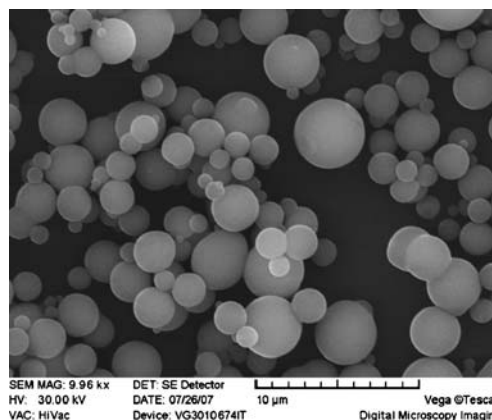
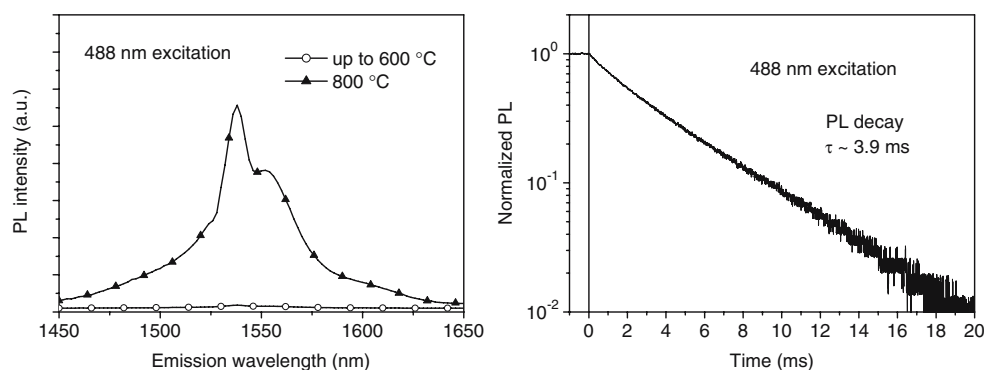


Fig. 4 SEM image of the acid-synthesized erbium-doped silica particles

Fig. 5 PL emission under 488 nm excitation of the acid-synthesized Er-doped silica powder treated at different annealing temperatures (*left*). 1.53 μm PL decay curve for the 800 $^{\circ}\text{C}$ annealed sample (*right*)



almost the entire visible range, up to about 650 nm. On the other hand, the low temperature or untreated samples shows a narrower blue emission, up to about 500 nm. In analogy to what reported in other papers related to amino-functionalized silica particles synthesized under basic conditions we can attribute the intense photoluminescence emission as associated to a radiative recombination mechanism from localized organic defects related to $-\text{NH}_2$ groups and the electron-hole recombination occurring in the siloxane cluster [14]. A thermal treatment is needed for efficient photoluminescence activation thanks to the removal of defects and $-\text{OH}$ groups within the material, which are responsible for a strong reduction of the optical efficiency due to non-radiative recombination processes. In fact the highest signal intensity is obtained for the 600 $^{\circ}\text{C}$ annealing. However at 800 $^{\circ}\text{C}$ the organic defects are degraded and their radiative emission is removed. The PLE excitation spectra of the same samples is reported in the left image of Fig. 3 and show a broad excitation band for the 600 $^{\circ}\text{C}$ annealing. More investigations are being pursued to better understand these aspects and to optimize the synthesis parameters.

Erbium-doped silica spheres

In Fig. 4 the SEM image of the Er-doped sample shows a uniform spherical shape of the silica particles. These particles are very similar to the undoped ones, reported in the left image of Fig. 1.

In analogy to what already shown for the amine-functionalized particles, different thermal treatments were done in these erbium-doped silica spheres: 200 $^{\circ}\text{C}$, 400 $^{\circ}\text{C}$, 600 $^{\circ}\text{C}$ and 800 $^{\circ}\text{C}$. In contrast and as shown in the left image of Fig. 5, an intense erbium PL emission in the near-infrared region (the maximum occurs at 1.53 μm) is detected only for the highest annealing temperature. For lower annealing temperatures the signal is non detectable or very low. These measurements were done by exciting the system by an argon laser at 488 nm. A thermal treatment of

at least 800 $^{\circ}\text{C}$ is needed for the removal of the $-\text{OH}$ groups and of the other non radiative defects and to promote the formation of Er^{3+} oxidized form, which is the optically active species. In the right image of Fig. 5 the 1.53 μm PL temporal decay curve is reported for the 800 $^{\circ}\text{C}$ annealed sample, showing a quite long lifetime of about 3.9 ms, which indicates an efficient removal of non-radiative recombination centers from the material.

Conclusions

The present work is focalized on the synthesis and characterization of luminescent silica spheres, which can find application for bio-sensing and bio-imaging. Indeed, their actual use for in vitro and in vivo applications is related to the intensity of their optical emission, the efficiency of their surface functionalization for molecular recognition and their low toxicity. In particular we provide a clear evidence for the possibility of realizing luminescent silica spheres by an acid synthesis route through the hydrolysis and condensation of TEOS. The luminescence can be easily and cheaply obtained either by the incorporation of APTES during the synthesis or by the introduction of erbium (or other rare earths) in the silica spheres. APTES incorporation generates localized organic defects related to $-\text{NH}_2$ groups which are responsible for an intense broad-spectrum luminescence. On the other hand, erbium is well known for its near infrared 1.53 μm PL emission, for the capability of upconversion, which leads to visible emission under NIR excitation, and for the long lifetime of its excited states.

Further analysis is needed in order to find the best compromise for a nanometric size control of erbium doped silica spheres. Moreover, surface functionalization for DNA and cellular recognition is also under investigation. The present results seem to hold the promise for non-toxic, biocompatible, easily functionalizable and fairly cheap luminescent nanoparticles. These would also be of much interest in biosensing and bioimaging applications.

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References

1. Tansila NC, Gao Z (2006) Nanoparticles in biomolecular detection. *Nanotoday* 1(1):28–36
2. Seydack M (2005) Nanoparticle labels in immunosensing using optical detection methods. *Biosens Bioelectron* 20(12):2454–69
3. Stöber W, Fink A, Bohn E (1968) Controlled growth of monodisperse silica spheres in the micron size range. *J Colloid Interface Sci* 26(1):62–9
4. De G, Karmakar B, Ganguli D (2000) Hydrolysis–condensation reactions of TEOS in the presence of acetic acid leading to the generation of glass-like silica microspheres in solution at room temperature. *J Mater Chem* 10:2289–93
5. Karmakar B, De G, Ganguli D (2000) Dense silica microspheres from organic and inorganic acid hydrolysis of TEOS. *J Non-cryst Solids* 272(2–3):119–26
6. Sokolov I, Kievsky YY, Kaszpurenko JM (2007) Self-assembly of ultrabright fluorescent silica particles. *Small* 3(3):419–23
7. de Dood MJA, Berkhout B, van Kats CM et al (2002) Acid-based synthesis of monodisperse rare-earth-doped colloidal SiO₂ spheres. *Chem Mater* 14(7):2849–53
8. Moran CE, Hale GD, Halas NJ (2001) Synthesis and characterization of lanthanide-doped silica microspheres. *Langmuir* 17(26):8376–9
9. Chan Y, Zimmer JP, Stroh M et al (2004) Incorporation of luminescent nanocrystals into monodisperse core-shell silica microspheres. *Adv Mater* 16(23–24):2092–7
10. van Blaaderen A, Vrij A (1993) Synthesis and characterization of monodisperse colloidal organo-silica spheres. *J Colloid Interface Sci* 156(1):1–18
11. Jakob AM, Schmedake TA (2006) A novel approach to monodisperse, luminescent silica spheres. *Chem Mater* 18(14):3173–5
12. König K (2000) Multiphoton microscopy in life sciences. *J Microsc* 200(2):83–104
13. Brinker C, Scherer G (1990) *Sol–gel science: the physics and chemistry of sol–gel processing*. Academic, New York: Academic; 1990
14. Carlos LD, SaFerreira RA, Pereira RN et al (2004) White-light emission of amine-functionalized organic/inorganic hybrids: emitting centers and recombination mechanisms. *J Phys Chem B* 108(39):14924–32