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Ultra-fast synthesis of magnetic and luminescent silica beads for versatile bioanalytical applications

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Abstract

A method for the synthesis of both spherically shaped micro/nano silica particles and silica hybrid particles using a novel inverse sol–gel suspension technique was developed. The technique enables the synthesis of beads within seconds and provides a simple basis for quantum dot and biosubstances encapsulation. The carriers can be used as DNA adsorbents, individually addressable optical codes for bioassays and biomolecule library screening as well as photonic crystals.

Keywords: Silica; Synthesis; DNA separation; Encapsulation; Quantum dots; Photonic crystals; Optical codes; Inverse sol–gel suspension technique; Silica hybrids

1. Introduction

Silica sol–gel technology provides a versatile platform in material sciences for the manufacturing of innovative products such as biomimetric materials, biosubstance encapsulated carriers, luminescent devices, photochromic coatings, optical sensors, photonic crystals, separation media and optical codings systems [1–4]. Based on the well-known sol–gel process using either acid- or base-catalyzed hydrolysis of alkoxysilanes [3,5], hydrogels or xerogels can be easily obtained by a simple base addition. By manipulating different parameters such as composition of the alkoxysilanes, hydrolysis conditions and condensation procedures which were concisely reviewed in the past [1,3], products with excellent optical, chemical, and biocompatible characteristics can be obtained and exploited for the synthesis of a variety of innovative products.

In the past, silica sols were used to manufacture films, membranes, coatings, bulky materials [6–8] or spherically shaped particles [9,10]. The latter products are mainly based on the well-known

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Stöber process [11] resulting exclusively in nanoparticles. However, particles based on this method show unfavourable optical properties; they are opaque, and the encapsulation of biosubstances or the usage in DNA separation, which is one of the most prominent applications of silica carriers, has not been described so far. Encapsulation of magnetic colloids to render the particles magnetic results in particles with an uneven colloid coating [12]. If these particles are applied as fluorescence or dye-labelled tags e.g. for biomolecule detection, chemical coupling procedures using functionalized fluorescence dyes [13,14] or core-shell coating techniques [15,16] are required. All techniques necessitate time-consuming and cumbersome multistep preparations.

Apart from the Stöber process, there are very few suspension techniques described to produce spherical microsized silica beads as encapsulation matrix [17–19]. These techniques are very time-consuming requiring up to 24 h preparation.

The present study describes a novel inverse suspension method which not only enables an ultra-fast silica bead synthesis but also offers a broad platform for the encapsulation of magnetic colloids/ferrofluids, biosubstances, luminescent agents such as quantum dots and fluorescence dyes. The usage of these novel carriers as DNA separation media, photonic crystals and multiplexed optical code systems for bioanalysis is described.

2. Methods and materials

All chemicals used were of analytical grade supplied by Fluka, Germany, and without further purification. The preparation procedure is outlined elsewhere [20]. A typical silica sol is formed by an aqueous suspension consisting of 71 vol% alkoxysilane (e.g. tetraethoxysilane, tetramethoxysilane) and 29 vol% 0.05 M HCl. Hydrolysis leading to an homogeneous sol is assisted by a conventional ultrasonic treatment for 10 to 15 min. After sol formation, a water-based magnetic colloid/ ferrofluid and/or the substances to be encapsulated are added (concentration ranges: 5–20 vol%) and then mixed for a few seconds with the sol phase assisted by an ultrasonic treatment. The sol phase is subsequently added to a toluene liquid phase containing 3.5 vol% Prisorine 3700 and 0.8 vol% Span 85. Volume ratio organic phase to silica sol 7.5:1. After 5 s of stirring using either a conventional stirrer or an Ultra-Turrax mixer LR 1000 (IKA Werke, Germany), 15 vol% of a 1% ammonia solution is added. After 5 to 10 more seconds of mixing, this leads to stable solid silica beads, followed then by several washing steps with methanol and water. The quantum dots with sizes ranging from 3 to 10 nm and the magnetic colloids were synthesized according to the methods described elsewhere [21–23]. The iron oxide content can be adjusted up to maximal 15 wt% related to the SiO_2 content in the final polymer matrix.

The flow chart for the preparation of the silica spheres is shown in Fig. 1.

Apart from synthesizing the basic silica bead, the technology described above also enables a simple possibility to synthesize functionalized carriers to which bioligands such as DNA fragments, peptides or proteins can be covalently coupled. For this purpose 5 to 20 mol% functionalized silanes carrying either amino-, epoxy and/or mercapto groups such as 3-aminopropyltriethoxysilane, 3-glycidyloxypropyltrimethoxysilane, 3-mercaptopropyltrimethoxysilane are added to the original alkoxysilane mixture. The subsequent suspension procedure which leads to the final beads follows the same procedure as for

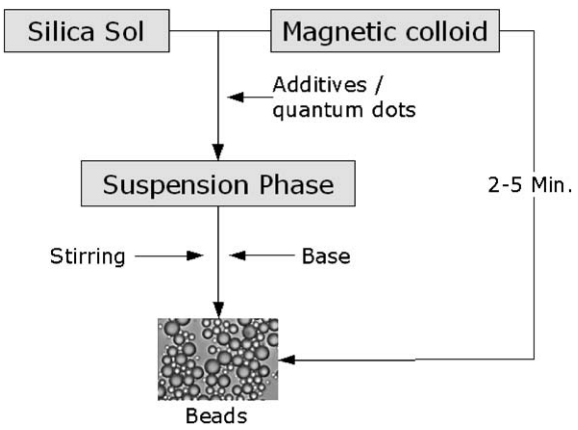

Fig. 1. Schematic flow diagram of the synthesis steps for silica-sphere preparation.

the basic silica carriers. Standard coupling protocols for the attachment of bioligands using e.g. carbodiimide, glutaraldehyde, BrCN can be applied [24].

Ion exchange particles carrying diethylaminoethyl groups for DNA binding were prepared using a standard procedure [20]: 100 mg vacuum dried silica beads were activated for 3 h under reflux in 4 ml distilled toluene (dried over sodium metal) to which 0.5 ml 3-glycidyloxypropyltrimethoxysilane was added. The ethanol- and water-washed carrier was subsequently incubated with 3 ml diethylamine overnight at room temperature, followed by several washing steps using ethanol and water.

3. Results and discussion

A novel inverse suspension technique that enables an ultra-fast synthesis was developed to obtain spherically shaped silica micro and nanoparticles platform for biosubstances and pigment/colloid encapsulation. The flow diagram outlined in Fig. 1 schematically shows the diverse preparation steps required to obtain the silica particles. The whole procedure starting with the hydrolysis of the alkoxysilanes to the final magnetic silica beads including diverse encapsulation steps, takes no more than 10 to 15 min. The actual silica bead formation after sol preparation does not necessitate more than 120 s including five washing steps. The viscosity of the silica sol preferably in the range of 5–30 mPa s and the continuous phase (0.7–2 mPa s) which contains carefully selected 1–5 wt% suspension stabilizers are the crucial parameters for obtaining ideally shaped particles. As the viscosity of the organic and the sol phase is low, the size range of the silica beads can be easily controlled by the stirring speed. Stirring speeds between 500 and 1500 rpm in general lead to microbeads covering a size range from 20 to 200 μm, whereas stirring speeds between 2000 and 10,000 rpm lead to sizes in the range of 10–20 μm. Particle sizes in the range of 5–10 μm, 2–5 μm, 1–3 μm and nanosized particles (500 to 1000 nm) can be prepared using a high-speed suspension tool (e.g. Ultra-Turrax mixer, IKA-Werke, Germany) adjusting the stirring speed to 11,000, 13,000, 16,000 and 22,000 rpm, respectively.

When compared to established procedures such as the Stöber process [11] or other previously described W/O suspension methods [12,14–18], the newly developed inverse suspension technique offers the following advantages and features: the particles are spherical and totally transparent, the sizes are adaptable in the range from 0.5 to 1000 μm, synthesis can be performed within minutes and encapsulation of both magnetic and luminescent substances is easily performed dispensing with any multistep coating or core-shell technique.

A selection of the substances used for encapsulation into the silica bead matrices are listed in Table 1. All products listed can be alternatively encapsulated in combination with up 10 wt% iron content. As can be seen from Table 1, the quantity of the magnetic colloid can be varied in a broad concentration range. This variation in the magnetic colloid content enables a precise control of the magnetic attraction properties so that an optimal adaptation to the appropriate bead application is easily possible. This particularly applies to an automated DNA separation where high separation speeds are required which can only be obtained using beads with a high magnetic portion resulting in strong magnetic attraction forces. The reverse is the case when using the magnetic particles in immuno assays where long suspension periods are required without mechanical mixing. For such purposes lower magnetic colloid contents are needed to adapt the specific density of the beads close to the density of the aqueous suspension phase. In Fig. 2. microscopic images of the basic magnetic micro and nanoparticles and their respective magnetic behaviour using a conventional hand magnet are shown.

As nucleic acid separation plays an ever-growing role in biotechnology and bioanalysis, high-performance separation media and especially magnetic ones which can fulfil the increasing demand for automatization in DNA separation are asked for. It is not surprizing that the development of magnetic adsorbents exhibiting high DNA binding capacities combined with distinct magnetic properties is of vital interest.

Table 1
List of selected substances and bioproducts, respectively, successfully encapsulated into the silica matrix. Bead size range can be adjusted between 0.5–600 μm

Silica sample	Encapsulated product[a]	Max. concentration[b] (related to SiO_2 content)
A (Fig. 2)	Magnetic colloid [22]	45 wt% (35 vol%)
B (Fig. 4,5)	CdSe/Zn quantum dots	20 wt%
C (Fig. 4c)	Rhodamin B	5 wt%
D	Yeast cells	—[b]
E (Fig. 4d)	Methylene blue	8 wt%
F	Herring sperm DNA	18 wt%
G	Green fluorescent protein	—[c]
H	Fluorescein	10 wt%
I	Gold colloid (size 17–23 nm, Sigma)	20 vol%
K	ß-Cyclodextrin	25 wt%
L	Silica nanoparticles [12]	35 vol%
M (Fig. 6)	Polystyrene latex (200 nm)	35 vol%
N	Polyvinyl alcohol (Mw: 48 kDa)	20 wt%

[a]All listed products can be encapsulated simultaneously with maximal 20 wt% of the magnetic colloid.
[b]The amount of substance which can be encapsulated into the beads at >95%.
[c]Not determined.

In a first test series, anion exchanger magnetic silica particles were used to study the basic DNA adsorption properties. For this purpose, diethylaminoethyl groups were introduced into the silica by reacting the 3-glycidyloxypropyltrimethoxysilane activated matrix with diethylamine (see Section 2). The adsorption performance of the modified silica carrier was examined and compared with a commercial anion silica resin (Qiagen, Germany) (for details see legend of Fig. 3.). As can be concluded from test results using standard ethidium bromide staining, the modified silica carriers show a distinctly better adsorption performance of the magnetic silica beads than the commercial carrier under identical test conditions.

Among the encapsulated substances listed in Table 1, luminescent particles such as quantum dots and fluorescent dyes are certainly of particular interest from the bioanalytical or diagnostic point of view. In recent years semiconductor nanocrystallites, so-called quantum dots, mainly composed of elements belonging to II and VI group such as CdS, ZnS, CdSe, have gained much interest because of their exceptional luminescent properties and chemical stability [25]. Research in this field has focused primarily on the synthesis, quantum confinement effect and their size-tuneable optical features [26]. Interesting approaches have recently been made to use quantum dots for biosensing and biolabelling [27]. Apart from using the nanosized basic quantum dots for cell- and biomolecule labelling, efforts have been made to encapsulate the nanocrystals into a polymer carriers such as polystyrene and mesoporous silica particles [28,29]. Both matrices, however, exhibit unfavourable basic optical properties due to the autofluorescence (polystyrene) and opaque properties (silica beads), thereby substantially restricting the luminescent intensities of the carries [29]. The silica bead technology introduced here provides an exciting basis to circumvent both the optical impairments as well as the cumbersome preparation procedures of established methods. By direct encapsulation of the quantum dots or alternatively fluorescent dyes into the silica matrix, an almost unlimited amount of luminescent substances can be applied thus circumventing all particle- and pore size-related restrictions during the doping process of the polymers with the quantum dots. In Fig. 4. some real colour fluorescent images of CdSe/ZnS quantum dots, rhodamine B and methylene blue-doped silica particles are shown.

As demonstrated, by varying the type of the embedded fluorescent substance or dye, the whole spectral range can be covered and by varying the quantity of the encapsulated substances the colour

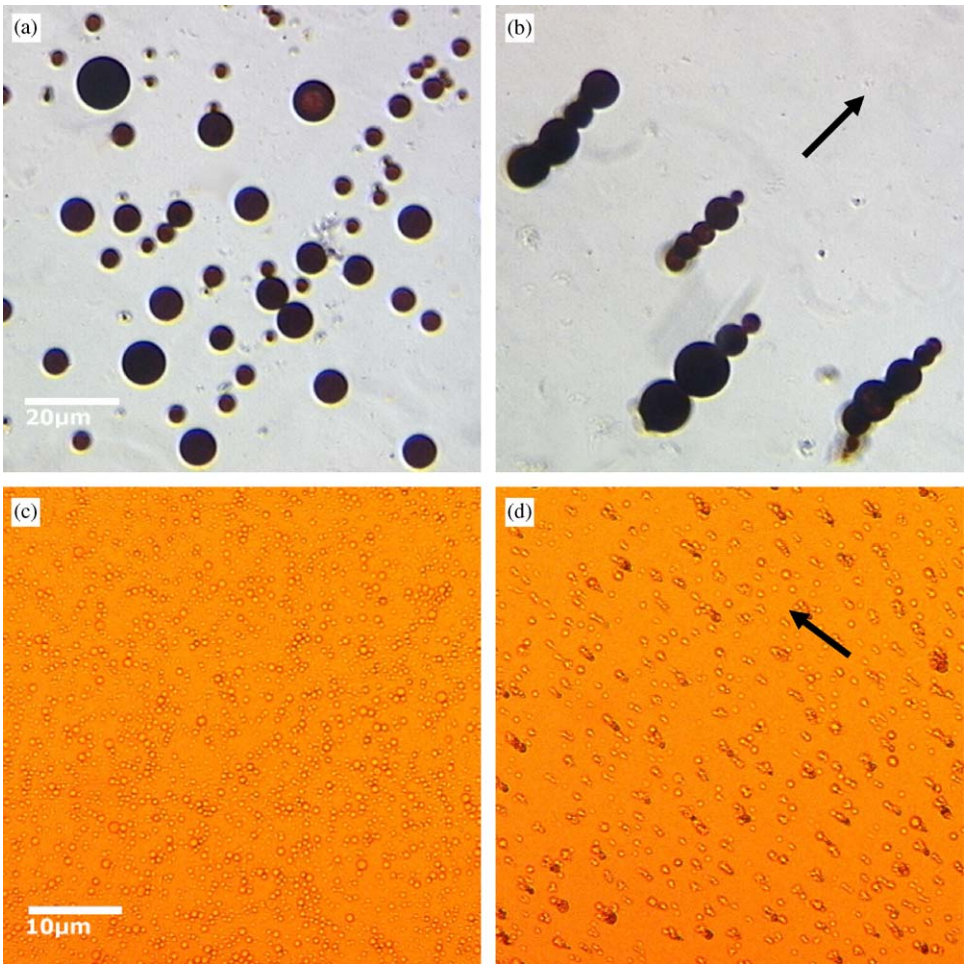

Fig. 2. Light microscopy images of typical magnetic silica micro (a,b) and nanoparticles (c,d) in response to an external magnetic field (b,d). Aligning occurs within 2 s after magnet application; magnet applied: neodymium–boron–iron. Arrows indicate the direction of the applied magnetic field. All images were taken with a confocal laser scanning microscope Zeiss Axioplan 2, Carl Zeiss, Germany, using a transmission mode. Scale bar: µm.

brightness adjusts accordingly. In addition to the monochromatic carriers, the silica technology also offers a simple means to synthesize multicoloured beads by simultaneously encapsulating either quantum dots having different sizes or mixtures of different fluorescent dyes. This combinatorial encapsulation approach using different luminescent agents with different concentration amounts results in silica beads which can be favourably used as addressable optical systems for the individual encoding of biomolecules such as nucleic acids or proteins. Han et al. [28] have described a theoretical model for such multiplexed optical coding using quantum dots-doped polystyrene particles. On the basis of n colour intensities and m different colours n^m-1, individual codes can be generated whose large numbers are, however, limited in practise by spectral overlapping.

Apart from the widely tuneable parameters used to control the optical properties of the luminescent silica particles, the present technology offers an intriguing approach to simultaneously encapsulate a magnetic colloid and a fluorescent substance.

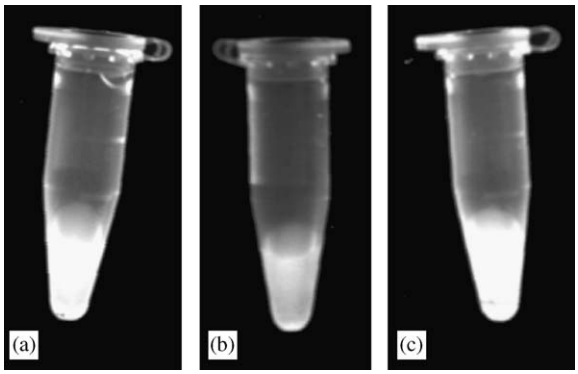

Fig. 3. DNA adsorption capacities of cationic silica particles. (a): Magnetic silica beads (mean diameter 26 μm) (b): DEAE-silica, Qiagen, (c): Ethidium bromide blind test with 200 μl starting DNA concentration. 30-μl settled carrier volume were incubated for 10 min with 200 μl 0.01 phosphate buffer, pH 5.5, containing 300 ng phage λ DNA (48502 base pairs, MBI Fermentas, Germany). Elution was conducted with 200 μl 10 mM Tris–HCl/1 M NaCl/ 1 mM EDTA buffer, pH 8.0; elution time 2 min.Visualization using ethidium bromide follows standard protocols.

This provides an additional parameter to extend the application of such particles for multiplexed optical coding of biomolecules [29].

In Fig. 5 CdSe/ZnS quantum dots embedded magnetic particles are exemplarily depicted. Application of a neodymium–boron–iron magnet leads to a pronounced magnetic response within a fraction of a second showing the typical aligning process (Fig. 5b). The pictures unambiguously show that when compared with the non-magnetic spheres (Fig. 4a) the luminescent properties are practically not impaired by the encapsulated magnetic colloid. Similar applies to rhodamine B, fluorescein and green fluorescence protein (GFP) encapsulated beads (not shown) which exhibit similar magnetic and luminescent properties.

Exploiting the combinatorial principles of luminescence and magnetism, the newly developed silica particles as 'optical signature' provide versatile encoding possibilities which may open up broad perspectives for multiplexed assays, biochemistry sensing, protein library and/or pathogen (e.g. tumor cell) screening and drug delivery systems.

A further outstanding feature of the silica carriers in comparison to conventional synthetic polymer beads is the ultimate heat stability. Heat treatments at 500 °C and above are feasible without destroying the bead structure. This special properties can now be exploited to manufacture pore size-selective silica beads. By encapsulating biomolecules/-substances such as nucleic acids, proteins, cells or viruses possessing a defined spacial structure and subsequent calcination of these embedded particles at temperatures $>500\,^{\circ}C$, silica replica with defined mesopore structures are formed. Figs. 6a and b exemplarily show a polystyrene latex encapsulated silica carrier whose pores were created by calcination of the sample at 800 °C (Fig. 6b). Using this combined encapsulation and calcination technique, a variety of differently structured porous silica matrices can be designed which may serve as affinity separation, tetoxification medium or decontamination agent for heavy metals.

Apart from the bioanalytical application there is a great interest in manufacturing novel photonic crystals for light modulation, optical switches or mirrors. Xu et al. [30] recently described a novel approach to synthesise magnetically controllable photonic crystals using ferromagnetic polystyrene nanoparticles incorporated into a acrylamide gel matrix. The silica bead technology described here with its simple encapsulation of nanoparticles, magnetic colloid and/or quantum dots provides a favourable platform to produce such photonic crystals. The quantum dots encapsulated magnetic silica beads depicted in Fig. 5 can serve as such a basic photonic crystal. Using a rotating neodymium–boron–iron magnet exerting a magnetic field of ≈ 0.8 T, a maximal induced oscillation of approx. 120 Hz can be achieved. This represents an almost 60% higher frequence than recently achieved with a polystyrene-doped crystalline colloidal array [30]. When applying photonic crystal sizes <10 μm, the respective response rates can be even increased up to approx. 300 Hz (not shown). Hence the silica technology with its variability regarding size, selection and concentration of embeddable agents as well as optical properties, offers an excellent basis for the development of tuneable photonic crystals which could be used as magneto-optical device whose diffraction and transmission can be externally controlled by a magnetic field.

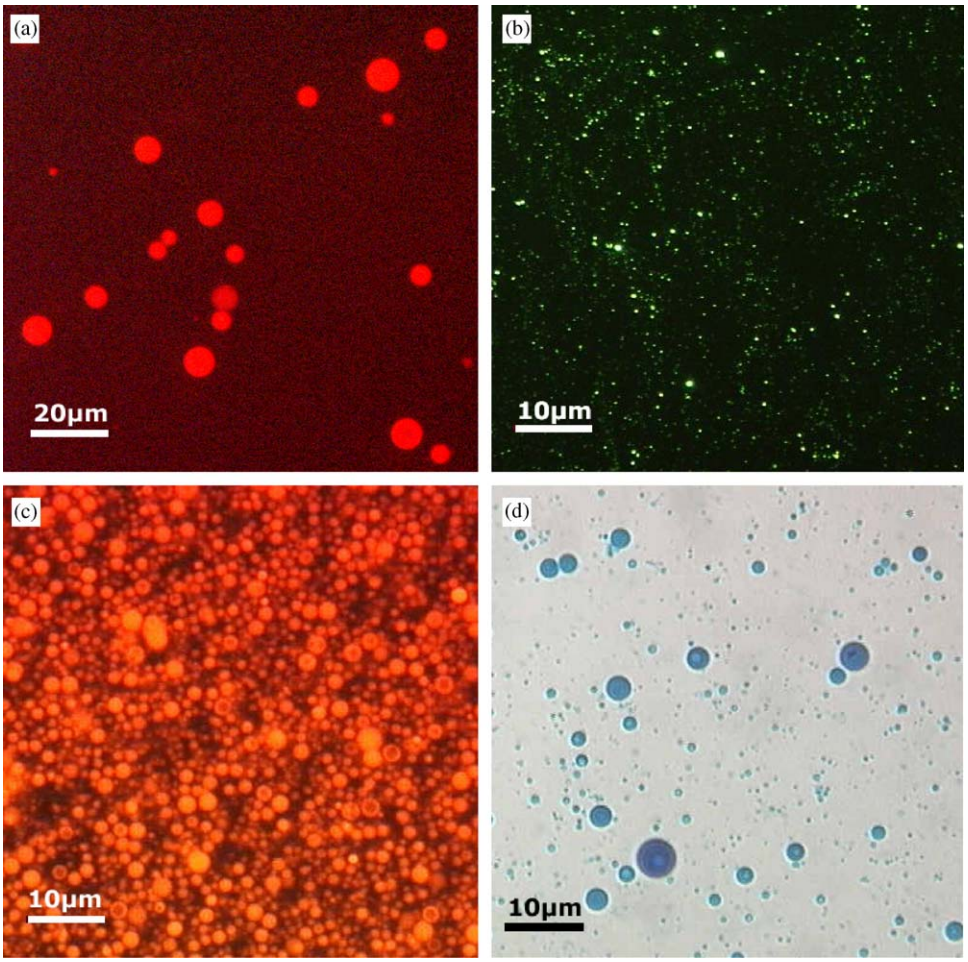

Fig. 4. Fluorescence microscope image of differently doped silica beads; (a): CdSe/ZnS quantum dot (size 7 nm, λ_{ex}: 470 nm, λ_{max} 610 nm), (b): CdSe/ZnS quantum dot (size 3 nm, λ_{max} 522 nm), (c): rhodamine B (λ_{max} 595 nm; (d): methylene blue. Fluorescence images were taken with a confocal laser scanning microscope Zeiss Axioplan 2 operating in a luminescent (a,b,c) and transmission mode (d).

4. Conclusion

Nano and microsized silica particles can be manufactured on the basis of a novel inverse suspension technology. By controlling the viscosity of the sol phase in combination with low-viscosity organic suspension phases, a broad variety of magnetic and non-magnetic beads can be synthesized within a short time frame requiring just a few minutes. Due to the high suspension stability of the silica sol when mixed with a broad range of diverse biological and fluorescent substances and pigments e.g. proteins, quantum dots, cells and fluorescent dyes, stable silica beads in which these substances can be encapsulated are able to be formed with the aid of an extremely simple process.

The special features of the technology and particles are: (i) ultra-fast synthesis of spherically shaped particles, (ii) adjustable bead size ranges, (iii) total transparency (iv) an almost unlimited encapsulation potential for bio and fluorescent substances. Exploiting the combinatorial encapsulation potential the silica carries can offer

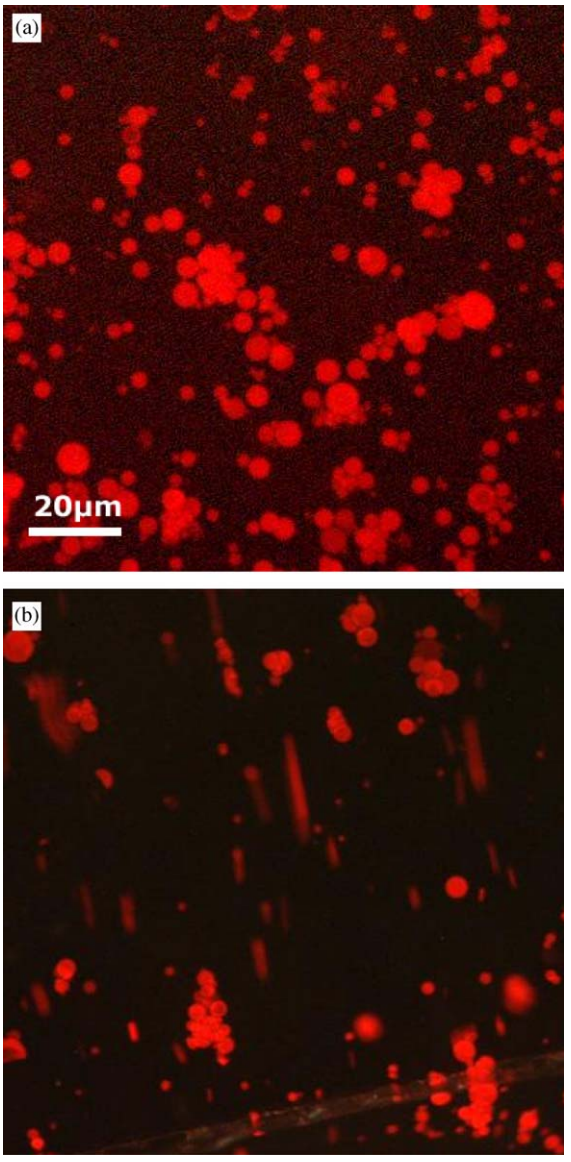

Fig. 5. Fluorescence microscopy image of CdSe/ZnS quantum dot (size 6 nm) and magnetic colloid encapsulated silica beads without (a) and with (b) external magnetic field (neodymium–boron–iron magnet). Magnetic colloid content 6 wt%.

intriguing perspectives for the design of multiplexed optical coding for biomolecule or pathogen, tuneable photonic devices, nano and micromotors and/or drug releasing systems.

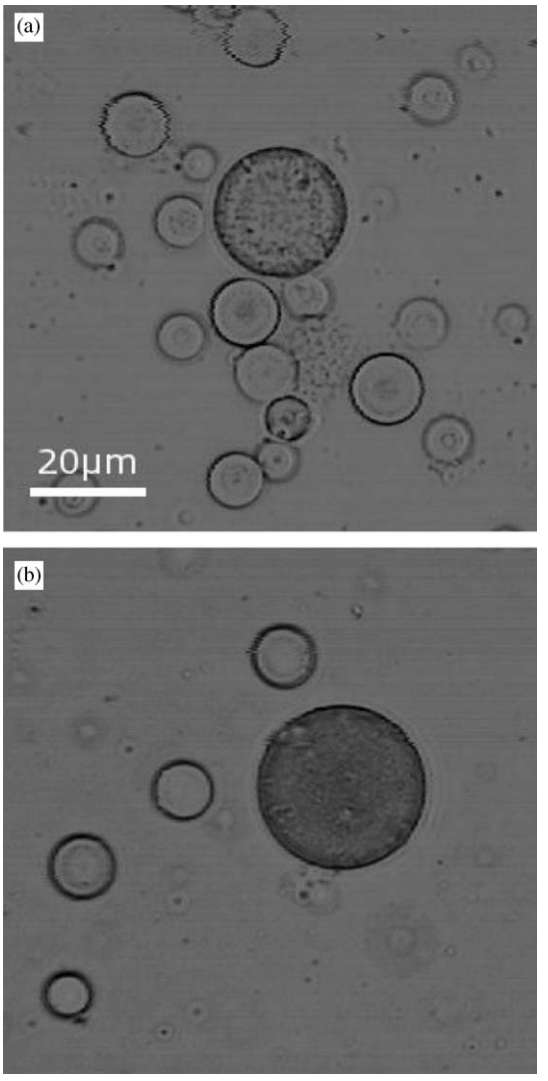

Fig. 6. Microscopic image (× 400) of silica particles doped with polystyrene latex (particle size 200 nm) (a) and after calcination for 30 min at 700 °C (b).

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