Microreactor Networks

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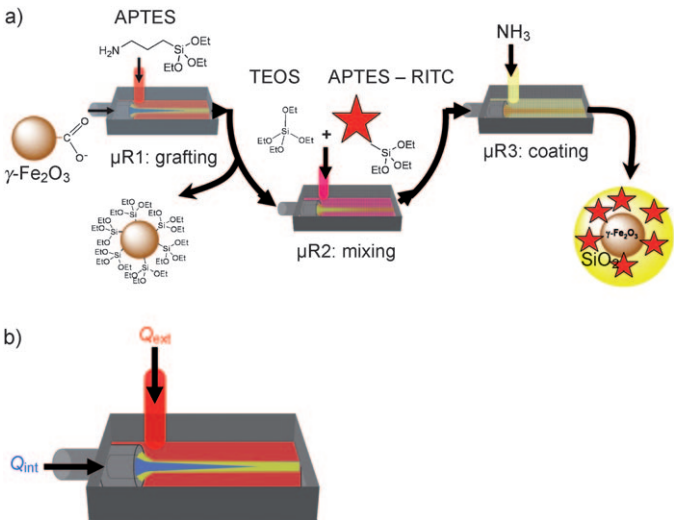
Multistep Continuous-Flow Microsynthesis of Magnetic and Fluorescent γ-Fe_2O_3@SiO_2 Core/Shell Nanoparticles**

Ali Abou-Hassan, Rana Bazzi, and Valérie Cabuil*

Microreactors are useful tools for optimizing and studying chemical reactions.[1–11] Compared to conventional reactors, microreactors improve chemical synthesis through advantages offered by their small length scales, such as reaction volumes, and enhanced heat and mass transfer.[1,2] These advantages can be very important for nanoparticle synthesis. Indeed, several kinds of inorganic particles have been synthesized in microreactors: quantum dots (as CdS[3] or CdSe/ZnS core/shell structures[4]), metallic nanoparticles (Pd, Co, Ag, Au[5]), and oxide nanoparticles (γ-Fe_2O_3,[6,7] α-FeOOH,[8] SiO_2,[9] TiO_2,[10] and SiO_2/TiO_2 core/shell nanoparticles[11]). Nanoparticles with tailored structural, magnetic, fluorescence, and chemical properties have a wide range of applications in the biomedical field, including imaging, targeting, and drug delivery.[12] Superparamagnetic γ-Fe_2O_3 nanoparticles (MNPs) are a good example of such multifunctional particles and are used for magnetic separation, drug delivery, magnetic resonance imaging (MRI), and hyperthermia cancer treatment.[13–16] Most of these applications require proven chemical stability of the nanoparticles, a narrow particle size distribution, and good dispersion of the nanoparticles in the liquid medium to avoid any unspecific aggregation. Encapsulating the MNPs in silica shells provides a protective, biocompatible, inert, and hydrophilic surface with excellent anchoring points for derivatizing molecules.[17,18] Moreover, incorporation of chromophores in the silica shell provides magnetic and luminescent core/shell nanocomposites with applications as contrast agents for molecular imaging.[19,20] Chromophores can be organic fluorescent dyes,[21] or luminescent inorganic particles such as quantum dots.[19] Methods reported for formation of MNP/silica nanocomposites in the bulk include the use of aerosol pyrolysis,[22,23] emulsions,[24] microemulsions,[25,26] and reactions performed under Stöber conditions.[27–29]

Looking for new methods in synthetic chemistry has led to the development of microreactors for the elaboration of lab-on-a-chip synthesis platforms.[30] Compared to droplets-based microreactors, continuous-flow microreactors appear to be easier to handle and more representative of bulk conditions with improved homogeneity leading to better control of nanoparticle characteristics.[31] In addition, the chemical composition of the mixture can be continuously varied, as different reagents can be added downstream without any synchronization, in contrast to droplets-based reactors.[32] An additional advantage of these synthesis platforms is that they allow linking of individual reactions into multistep sequences.[33] This enables one reaction to flow into another and thus to combine multiple synthetic steps into a continuous operation.[34] Multistep synthesis in continuous-flow microreactors involving multiple reactions and on-line or off-line separation (workup) were elegantly demonstrated for the synthesis of organic molecules.[2,34–36] In the field of inorganic chemistry, the use of microreactors for nanoparticle coating has been described but is limited to single-step modifications of the surface.[11,37] Herein we report a continuous multistep synthesis of magnetic and fluorescent nanoparticles dedicated to elaboration of a nanoparticle lab-on-a-chip platform.

The basic concept of the proposed synthetic process is illustrated in Scheme 1 a. It is based on a continuous multistep



Scheme 1. a) Scheme for the continuous synthesis of fluorescent core/shell MNP/silica nanoparticles. μR1: microreactor for grafting APTES onto the citrated γ-Fe_2O_3 nanoparticles; μR2: microreactor for mixing of the sol–gel precursors TEOS and APTES-RITC; μR3: microreactor for coating of the γ-Fe_2O_3 nanoparticles with silica. b) Sketch of the coaxial-flow microreactor (μR) used in this study showing the mixing between the inner stream (Q_{in}) and the outer stream (Q_{out}).

[*] Dr. A. Abou-Hassan, Dr. R. Bazzi, Prof. V. Cabuil
UPMC Univ Paris 6, Laboratoire de Physicochimie des Electrolytes Colloïdes et Sciences Analytiques (PECSA)
UMR 7195, Equipe Colloïdes Inorganiques
Université Paris 6 (UPMC) Bat F(74), case 51
4 place Jussieu, 75252 Paris Cedex 05 (France)
Fax: (+33) 1-4427-3675
E-mail: ali.abou_hassan@upmc.fr
Homepage: http://www.pecsa.upmc.fr

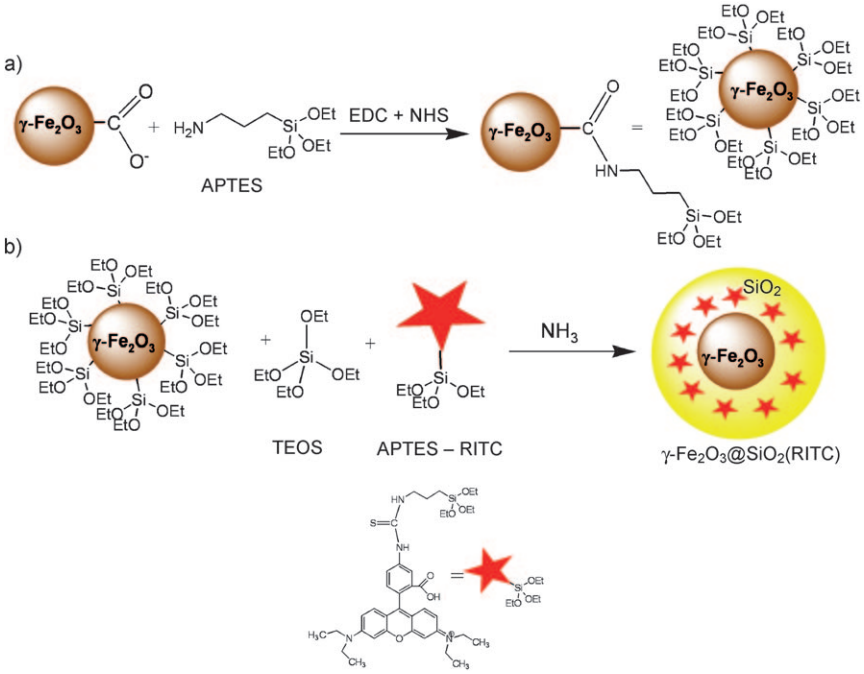
[**] We thank Sophie Neveu and Aude Michel from the PECSA lab for the TEM images. Abbreviations used: APTES = (3-Aminopropyl)-triethoxysilane, TEOS = tetraethyl orthosilicate, EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, NHS = *N*-hydroxysuccinimide, RITC = Rhodamine B isothiocyanate, PDMS = poly(dimethylsiloxane).

system that enables sequential reactions without leaving the microreactor environment. All the micro-operations (grafting, mixing, coating) were run inside an optimized coaxial flow microreactor (μR) that has been described in detail elsewhere.[6,8] In brief, the microreactor is based on a three-dimensional coaxial-flow device with two streaming reagents. Mixing occurs by diffusion at the point of confluence of the reagents. The 3D hydrodynamic geometry allows rapid homogenization of the reactants through flow focalization and avoids technical barriers such as channel clogging and precipitation of the chemical species along the reactor walls. To induce a reaction in the microreactor, the reagents are respectively injected into the inner and outer streams with flow rates Q_{in} and Q_{out} (Scheme 1b). The flow rate of the different species can be continuously adjusted to produce a stable inner stream and different mixing times. The best mixing time that can be achieved in the μR is about 80 ms for a Q_{out}/Q_{in} ratio of 400. At the outlet of each microreactor a polytetrafluoroethylene (PTFE) tube (500 μm inner diameter, Upchurch) leading to the next microreactor was connected to the reactor outlet. The length of the tubes was varied to provide additional residence time for the different micro-operations.

In the present work, the synthesis of magnetic and fluorescent core/shell γ-Fe_2O_3/silica nanoparticles serves as a model for demonstrating the practicability of the multistep microchemical synthesis. The choice of this system is motivated by the numerous applications of these core/shell nanoparticles in the biomedical field and the need for scaleup.[12] The latter is difficult to achieve in conventional batch reactors. Moreover, the protection from moisture and air provided by working in microreactors makes using reagents such as APTES easier.

The synthesis of fluorescent silica-coated magnetic nanoparticles includes two important steps: grafting of APTES on the surface of the magnetic nanoparticles and coating of the resulting nanoparticles by silica. The initial dispersion of nanoparticles used in this study was a stable suspension of citrate-coated γ-Fe_2O_3 nanoparticles (MNPs-Cit) that was prepared accordingly to previous works.[6,38] Prior to coating of these particles with silica, the MNPs-Cit solution was diluted in ethanol and mixed with EDC and NHS to activate the carbonyl groups of the citrate groups on the MNP surface. This technique, known in bioconjugation,[39] allows coupling of the surface of the MNPs to the amine group of APTES by formation of amide bonds with the activated carbonyl groups of the surface, and thus the APTES molecules are grafted onto the surface of the MNPs.

The stable suspension obtained in ethanol was then injected into the inner flow of microreactor μR1 and mixed with a solution of APTES in ethanol flowing in the outer stream. The reaction between the amine groups of APTES and the activated carbonyl groups to give amide bonds is illustrated in Scheme 2a. As the mixture is anhydrous, hydrolysis of the APTES precursor does not occur and only an "APTES monolayer" on the surface of the magnetic nanoparticles can be produced.



Scheme 2. a) Grafting of APTES onto the surface of the citrated magnetic nanoparticles in the presence of EDC and NHS. b) Polysiloxane coating of the citrated MNPs in the presence of NH_3 with TEOS and fluorescent APTES (APTES-RITC) leading to the formation of γ-Fe_2O_3@SiO_2(RITC) nanoparticles.

The output stream of μR1 containing MNPs-APTES was introduced into the central channel of microreactor μR2, and sol–gel precursors, for example, TEOS and APTES labeled with fluorescent dye Rhodamine B isothiocyanate (RITC), the isothiocyanate group of which can be coupled to the amino group of APTES in an addition reaction, was added in the outer stream of the channel. Then, the reaction flow from μR2 was passed into the center of microreactor μR3 and surrounded by an alkaline solution of ammonia. Hydrolysis and condensation reactions of the silica precursors on the APTES monolayer (Scheme 2b) led to encapsulation of the MNPs by a fluorescent polysiloxane layer. At the outlet of μR3 the reaction was quenched by fast solvent extraction in diethyl ether to avoid any further condensation of unconverted silica precursors. After optimization at room temperature, the optimal conditions for the different reactions running in μR1, μR2, and μR3 were about 3, 2, and 2 min, respectively. The total time of the process is thus 7 min, as opposed to the 24 h necessary for such a coating process under batch conditions.

The output from the complete synthesis was collected at the outlet of μR3 and dialyzed against unconverted APTES-RITC. Transmission electron microscopy (TEM) images of MNPs@SiO_2(RITC) showed almost spherical core/shell structure with an average overall size of 50 nm with one or more magnetic cores of γ-Fe_2O_3 (Figure 1). These particles appear

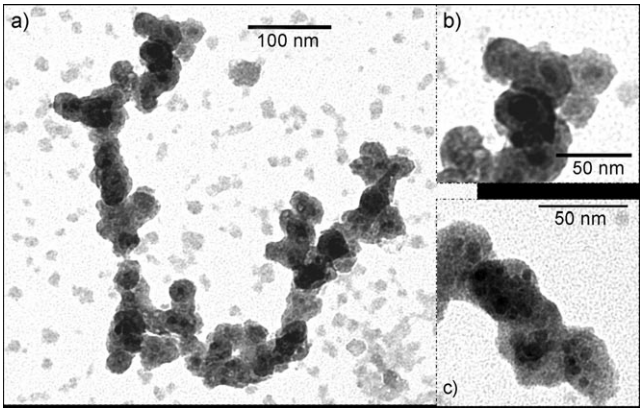


Figure 1. a) TEM images showing typical architectures of the core/shell MNPS@SiO_2(RITC) nanoparticles. b, c) Enlargements of image (a).

aggregated because the silica shell has not been functionalized to protect them against aggregation. They are thus very sensitive to aggregation, especially when deposited and dried on the microscope grid. Fluorescence optical microscopy images of the core/shell nanoparticles confirmed incorporation of the RITC dye in the silica shell (Figure 2). In the

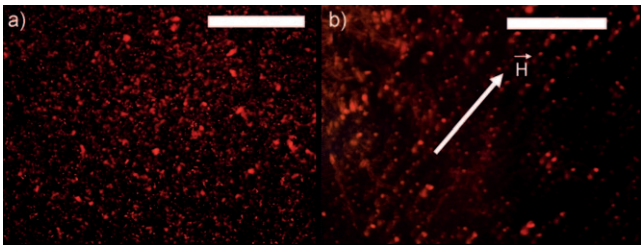


Figure 2. Fluorescence micrographs of silica-coated iron oxide nanoparticles a) in the absence of an external magnetic field and b) in the presence of an external magnetic field, showing the formation of chainlike structures. Scale bars: 50 μm.

presence of a magnetic field, the nanoparticles lined up along the magnetic field direction to form chainlike structures due to the attractive interaction between the magnetic moments, and thus the bifunctional character (magnetic and fluorescent) of the nanoparticles is evidenced. Grafting of APTES is crucial to obtain the final core/shell particles. Without such a layer, homogeneous nucleation of silica took place and core-free silica spheres were formed. This is not the case in the bulk: MNPs@SiO_2 core/shell nanoparticles, similar to those presented herein, are synthesized over 24 h, without any previous grafting step. This is related to the short contact time between the particles and the silica precursor in the lab-on-a-chip process, too short to allow dissolution of the silica and its recondensation on the ferric oxide nuclei.

In conclusion we have shown that it is possible to use a microfluidic platform for multistep synthesis of magnetic and fluorescent core/shell γ-Fe_2O_3@SiO_2 nanoparticles by using a network of continuous-flow microreactors. The novelty of this approach lies in the multistep continuous coupling of several chemical reactions in different microreactors, each of which acts as a micro unit operation. The synthesized nanoparticles have approximately the same shape and dimensions as particles obtained in bulk synthesis, but were produced in a few minutes (7 min) compared to several hours in the bulk. The very short time required for this multistep synthesis originates from the small dimensions of the microreactors and the direct coupling of the different operations, which reduces the time for the process and speeds up the coating of the magnetic nanoparticles. Further studies will determine the effects of precursor concentrations and residence time on the final coating (number of magnetic cores, silica shell thickness, etc.). Moreover, it is possible to carry out further on-line modifications of the silica surface and on-line detection and structural characterization of the nanoparticles. Surface modifications include functionalization of the silica shell with hydrophilic molecules (polyethylene glycol, dextran, etc.) to increase dispersibility of the nanoparticles in biological fluids, and immobilization of biomolecules such as enzymes and proteins. We have shown previously[6] that MNPs can be synthesized in a continuous-flow microreactor. It is thus also possible now to couple the synthesis of MNPs and their functionalization with integration of on-line workup steps such as magnetic separation, dilution, and dialysis. This would allow the production of functionalized nanoparticles to be accelerated from a few days to several minutes with the potential for scaling up to larger amounts through parallel operations.

Experimental Section

APTES, TEOS, EDC, NHS, and RITC were purchased from Sigma Aldrich. The coaxial-flow microreactors (μR) were obtained by molding in PDMS and are described in detail elsewhere.[6] The length of the tubes was varied to provide additional residence time for the different micro-operations. After optimization, the tubing connecting the outlet of μR1 to μR2 was 50 cm long, that connecting the outlet of μR2 to μR3 was 50 cm long, and that connecting the outlet of μR3 to the sample vial was 150 cm long. The flow rates were 50 (μR1), 100 (μR2), and 200 $\mu L\,min^{-1}$ (μR3) with a 1:1 ratio of reagents. Three syringe pumps, one of which can be loaded with two syringes, were connected independently to the three microreactors. The organic and aqueous phases were loaded in separate 3 mL syringes (Becton Dickinson) and connected to the reactors.

Synthesis of maghemite nanoparticles: 1) According to a procedure already described, superparamagnetic γ-Fe_2O_3 nanocrystals were prepared by alkaline coprecipitation of $FeCl_3$ (27%, VWR) and $FeCl_2{\cdot}4\,H_2O$ (VWR) by alkaline tetramethylammonium hydroxide (97%, Sigma Aldrich).[6] Polydisperse γ-Fe_2O_3 nanoparticles were synthesized by oxidizing magnetite (1.3 mol) in nitric acid (2 N, 1 L) containing iron nitrate (1.3 mol) with boiling. 2) Citrated nanoparticles: after decantation/sieving: The maghemite particles were heated at 80 °C for 30 min in water, and then supplemented with sodium citrate (70 g) before precipitation in acetone at 25 °C. The

final iron content was checked by flame spectrometry. The colloidal suspension was ready to use and stable at room temperature for years.

APTES grafting: 1) Activation of the carbonyl groups: 750 μL of the solution of citrated MNPs was diluted in 15 mL of ethanol (2.25×10^{-4} mol Fe). 1 mL of ethanol containing 2×10^{-5} mol of EDC and 4×10^{-5} mol of NHS was then added to the diluted suspension of MNPs, and the resulting suspension was mixed for 30 min. 2) 5 mL of APTES was diluted in 15 mL of ethanol. C) Functionalization of APTES with RITC: RITC (24 mg, 4.4×10^{-5} mol) was dissolved in 2 mL of ethanol, and APTES (2 μL, 8.5×10^{-5} mol) was then added. The mixture was agitated for 12 h in the dark.

MNP coating: 75 μL of the TEOS solution was mixed with 0.5 mL of the APTES-RITC solution and then allowed to react with MNPS-APTES in μR3 in the presence of 0.1 mol L^{-1} ammonia solution.

The γ-Fe_2O_3@SiO_2(RITC) was dialyzed against water for 48 h by using a Spectra/Pore dialysis membrane (molecular weight cutoff, 12000–14000; flat width 45 mm).

All TEM images were obtained by using a JEOL 10 CX instrument (100 kV). Fluorescence microscopy images were observed by using a Zeiss Axiovert 200 microscope (×40, NA 0.65, HBO 100) and rhodamine filter set with pictures taken by a CCD camera and digitalized on a computer.

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