Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

PART I: MATERIALS AND METHODS

Materials

Rhodamine B (RhB, Aldrich, 99%), benzophenone (BP, Rhone Poulenc, 99%), acrylamide (AaM, Aldrich, 99%), N,N-methylenebis(acrylamide) (BIS, Aldrich, 99%), Acrylic acid (Aac, Aldrich, 99%), ammonium persulfate (APS, Aldrich, 99%), N,N,N′,N′-Tetramethylethylenediamine (TEMED, Aldrich, 99%), acryloxyethylthiocarbamoyl rhodamine B (Acr-RhB, Polyscience), ammonia (Calo Erba, 22.5%) and technical grade ethanol (EtOH, Aldrich) were used as received. Ferrous chloride, hydrochloric acid (37%), ferric chloride, ferric nitrate, acetone, diethyl ether and nitric acid (52.5%) were purchased from VWR and used as received. Spectrapore Dialysis membrane (SpectrumLabs, 12-14 kDa) was washed with distilled water prior use.

Instrumentation

UV irradiation was induced by a VL-215.L 2x15W – 365 nm tube UV lamb, Fischer Bioblock Scientific at a distance of 5 cm from the vials.

Transmission Electron Microscopy (TEM). Nanoparticles were observed using a Jeol-1011 TEM with a Gatan Camera (Orius). A droplet of diluted nanoparticles suspension in water was deposited on a carbon coated copper grid and the excess was drained using filter paper. Size analysis was achieved on TEM images using ImageJ software.

Thermogravimetric analysis was performed under nitrogen gas (50 mL/min) with a 10 °C/min temperature slope on a Texas Instrument TGA 550 in a platinum pan after drying samples at 70°C for 1 week.

Fourier Transform Infra-Red (FT-IR). Infrared spectra were obtained on a Bruker Tensor 27 spectrometer on pressed KBr pellets. Spectra were obtained at regular time intervals in the MIR Region of 4000 – 400 cm^{-1} at a resolution of 4 cm^{-1} and analysed using OPUS software.

Dynamic light scattering (DLS). Hydrodynamic diameter measurements were recorded using a Malvern Instruments Nanosizer ZS Ultra (red) in backscatter mode (173 °C, 633 nm laser) at 25 °C and a Cordouan Vasco KIN particle size analyser for in situ measurements during and after synthesis (170 °C, 632 nm) at 25 °C.

Fluorescence spectra were recorded using a MolecularDevices SpectraMax I3x plate reader in 300 µL black 96-wells plates with an excitation wavelength of 545 nm.

The total iron concentration was determined by atomic absorption spectroscopy with a PerkinElmer Analyst 100 apparatus after degradation of γ-Fe_2O_3 NPs in fuming HCl (35%).

Fluorescence microscopy images were taken on a Zeiss Axiovert 200 microscope equipped with a mercury vapor lamp and fluorescence filters and a Manta Allied vision camera.

Experimental

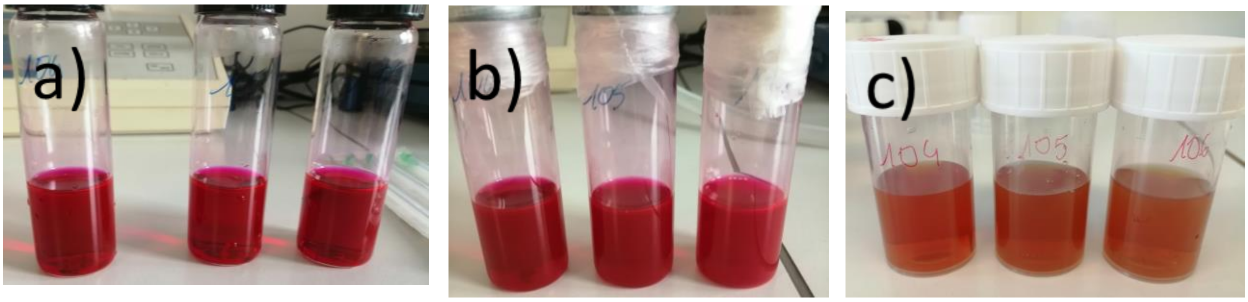
Synthesis of maghemite nanoparticles: 0.905 mol of ferrous chloride was dissolved in 600 mL of 6% aqueous hydrochloric acid solution. Next, 1.59 mol of ferric chloride and distilled water were added to obtain a total volume of 3 L. Then 1 L of ammonia (22.5%) was added to the mixture, under vigorous magnetic stirring at room temperature. Reaction was allowed to continue for 30 min and the obtained magnetite was oxidized using 0.80 mol of ferric nitrate. Then, the suspension was stirred by magnetic stirrer for 30 minutes at 100 °C, in order to speed up the oxidation of the particles. Maghemite nanoparticles were then washed three times with acetone and two times with diethyl ether, before being dispersed in water. To this mixture, 25 mL of nitric acid (52.5%) was added to partially destabilize the suspension and sedimented particles were collected using a magnet and re-dispersed in water. The same procedure was repeated with 9 mL of nitric acid (52.5%). After dispersing in water, the ferrofluid collected contained particles ranging between 13 and 16 nm with an iron concentration of 0.907 mol/L.

Surface modification of the maghemite nanoparticles: In a 25 mL glass container, 5 mL of previously synthesized maghemite nanoparticles (4,53 mmol of iron) and 150 µL of acrylic acid (2.175 mmol) were sonicated for 15 min and heated to 80 °C for 40 min. After cooling down, the particles were washed 3 times with 5 mL of acetone and once with diethyl ether before dispersing in water. The procedure described here is the synthesis with the mole ratio [Fe:AAc]=[1:0.48]. For other ratios ([1:0.16], [1:0.32] and [1:0.8]), the volume of acrylic acid should be adapted accordingly.

Poly(acrylamide) coating of acrylic acid functionalized maghemite nanoparticles: (The following protocol describes the synthesis with an iron to monomer mole ratio of [1:48], for other ratios, the amount of monomer should be adapted as described in Table 2)

In a 35 mL glass vial, 0.32 mmol of BIS (50 mg), 1.85 mmol of AaM (132 mg), 4.1 µmol of RhB (2 mg), and 22 µmol of BP (3 mg, previously dissolved in 600 µL of ethanol) were dissolved in 14.6 mL of deionized water. To this mixture, 50 µL of sonicated Aac functionalized maghemite nanoparticles (45 µmol of iron), which were prepared according to the abovementioned procedure, were added. Next, the mixture is sonicated for 10 min and purged with nitrogen gas for 15 min. The reaction vial was then sealed under nitrogen and put on an orbital stirrer (200 rpm) under the 2x15 W 365 nm UV light at ambient temperature (about 25 °C). After 3 h of UV irradiation, the reaction was stopped and the product was washed thoroughly with deionized water using a 12-14 KDa dialysis membrane to remove unreacted monomers and initiators (for a week, two bath changes per day) and half of the sample is dried in an oven at 60 °C, at ambient pressure until the weight is stable. The yield of polymerization is calculated by weighing the hydrogel at its dry state, and removing the known iron content. For this purpose, half of the sample, whose volume was known precisely, was dried and the dry weight was extrapolated for the whole sample to normalize between samples.

DLS sizes reported in Table 2 represent the maximum of main peak in number and the corresponding graphs are presented bellow both in number and intensity, recorded at the end of the synthesis on a Vascokin Particle analyzer. Results of DLS in number are close to those obtained by counting particles on TEM images.



Prior

After

After dialysis

In all images, samples from left to right: [1:48] [1:72] and [1:96]

Figure S1. Photographs of the samples prepared at [1:48] (left), [1:72] (middle), and [1:96] (right) iron to monomer mole ratio before (a) and after (b) polymerization and after dialysis (c) of the product.

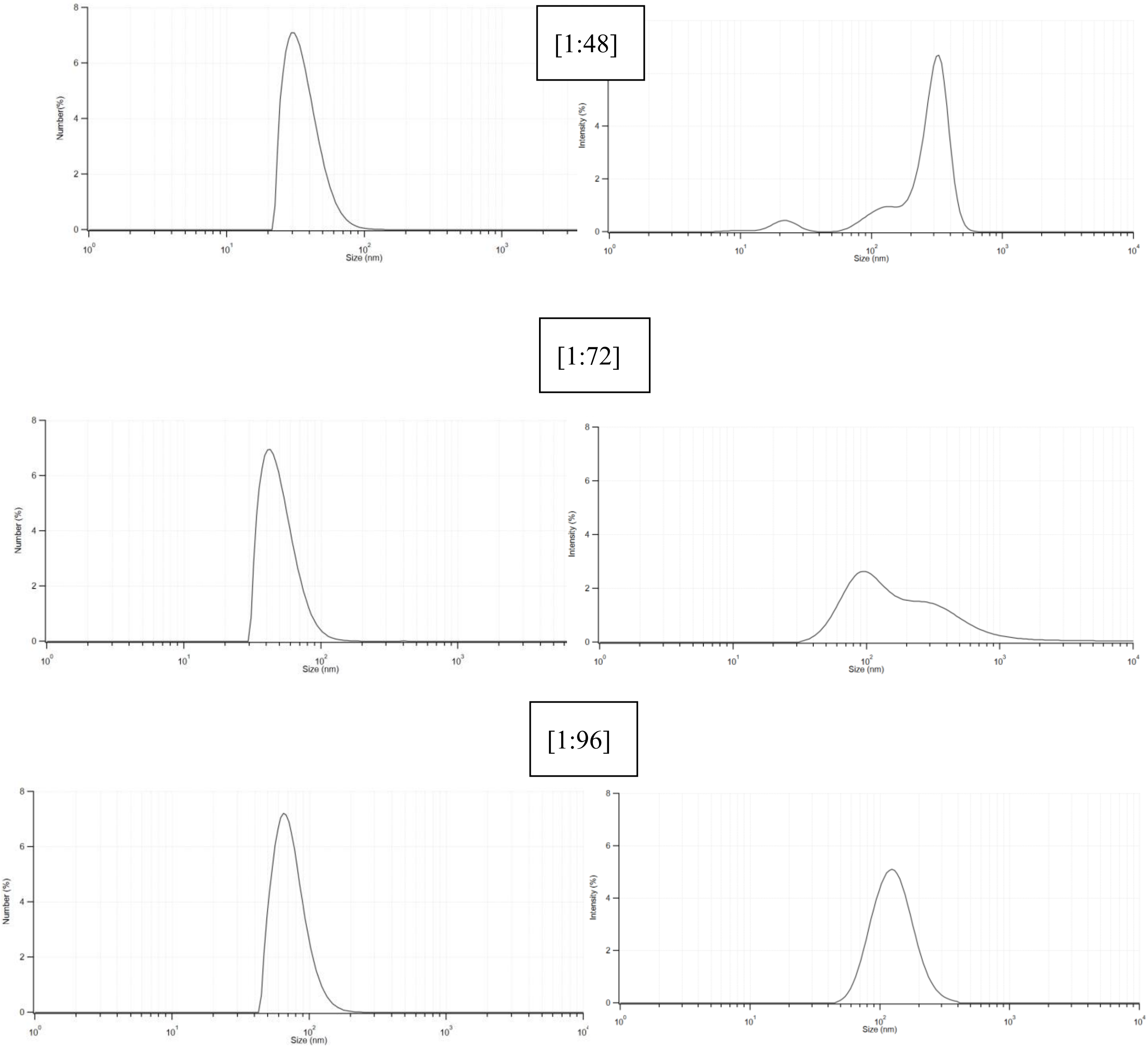
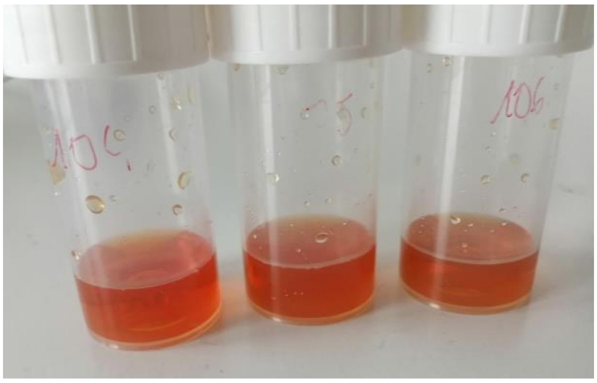


Figure S2. DLS measurement of the samples prepared at [1:48] (top), [1:72] (middle), and [1:96] (below) iron to monomer mole ratio

The particles in the samples, which were kept in the fridge for one month after dialysis, tend to aggregate at the bottom of the vials. The particles could be re-dispersed easily by shaking and ultrasonication for a few seconds. No noticeable aggregation of the particles was observed after 9 months by DLS



Samples after 9 months

Figure S3. Photograph of the samples prepared at [1:48] (left), [1:72] (middle), and [1:96] (right) iron to monomer mole ratio after 9 months

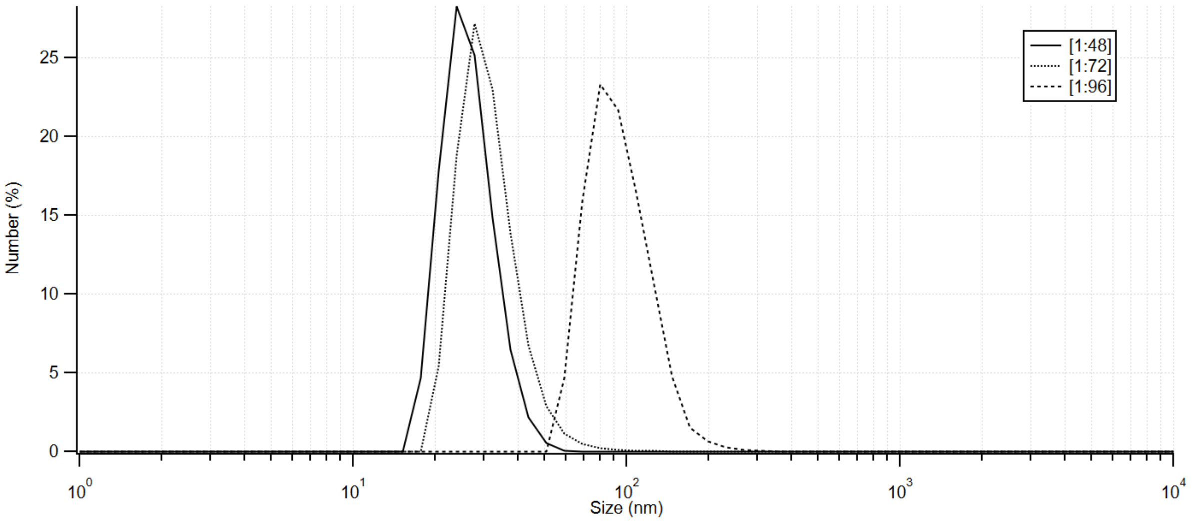


Figure S4. DLS measurement of the samples prepared at [1:48], [1:72], and [1:96] iron to monomer mole ratio after 9 months.

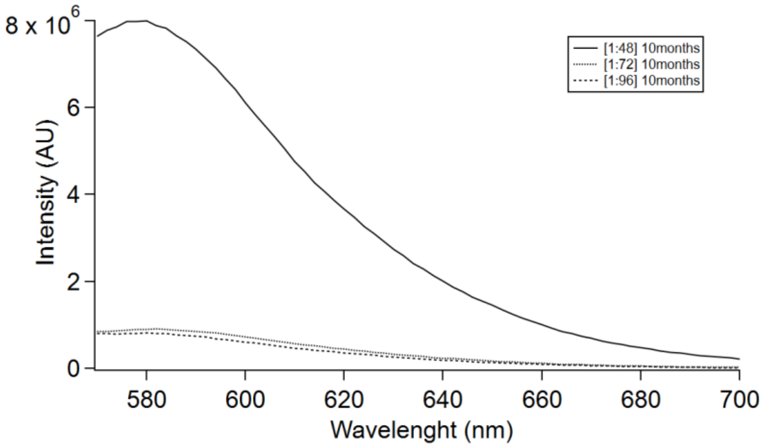


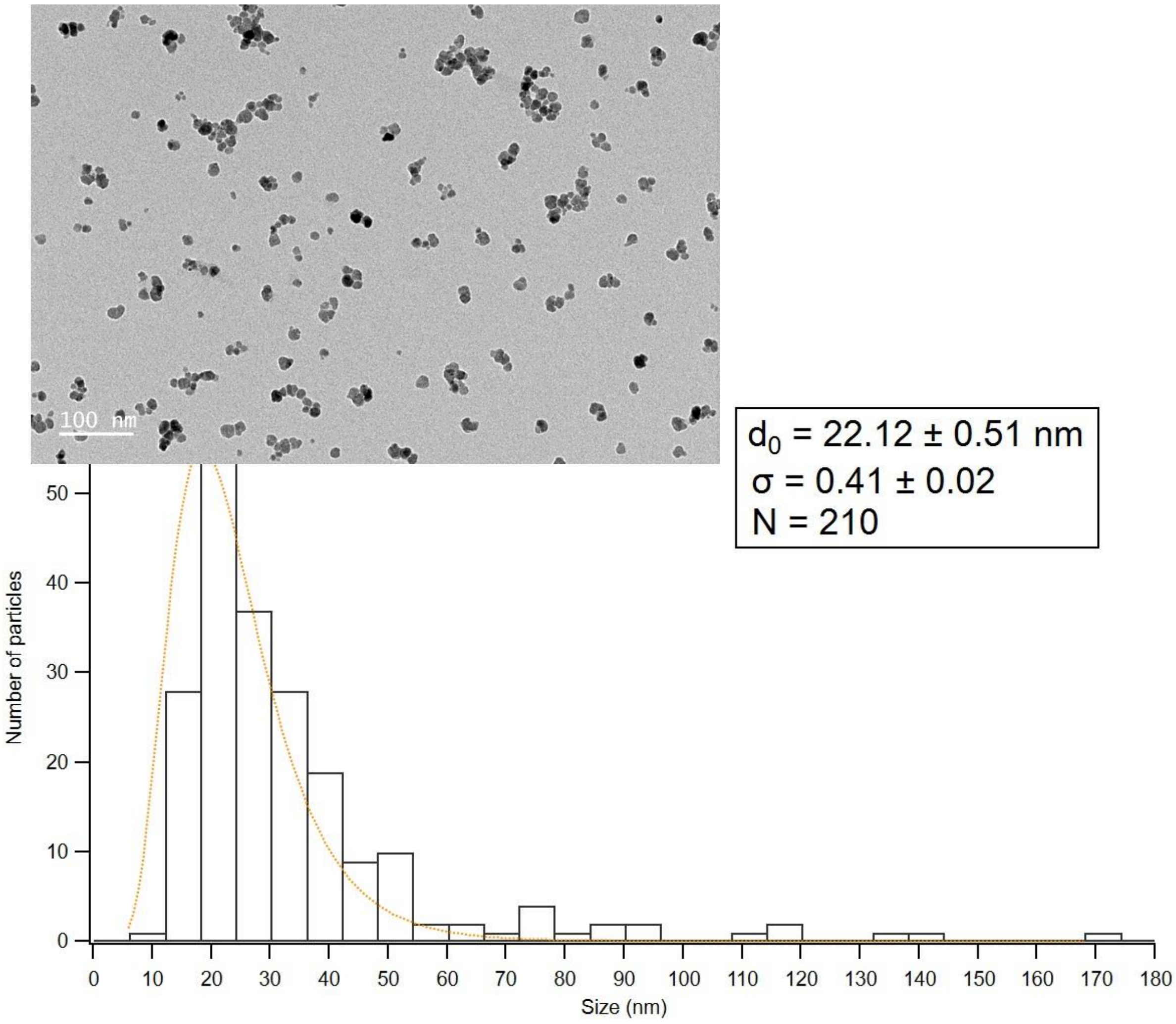
Figure S5. The fluorescence emission spectra of the samples after 9 months

PART II: TEM images

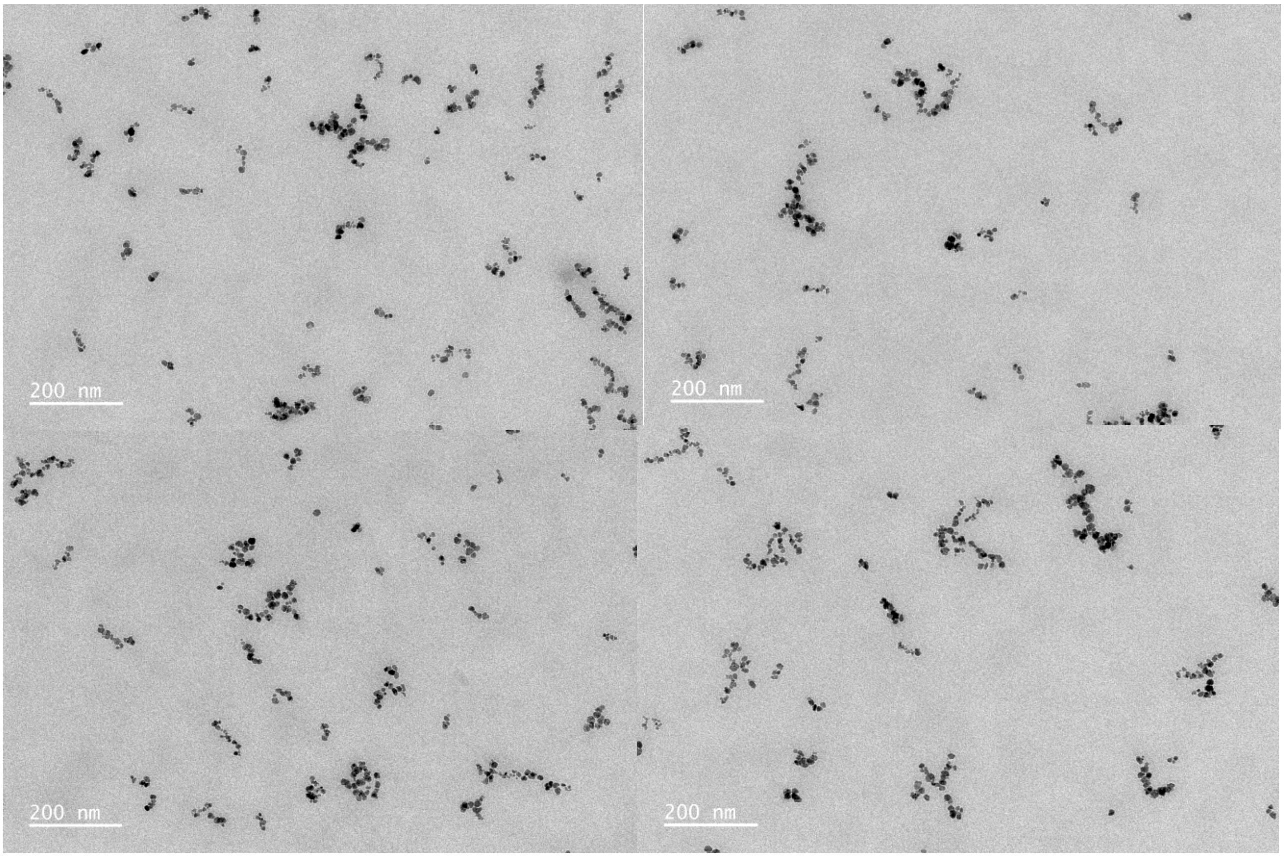
Additional images are presented below to represent the diversity of the samples. The dimensions of the aggregates were measured on ImageJ software. The histograms represent the distribution of the longest dimension for each aggregate, and bin width is statistically determined. N is the number of counted particles, other parameters are obtained by fitting the histogram to a normal law

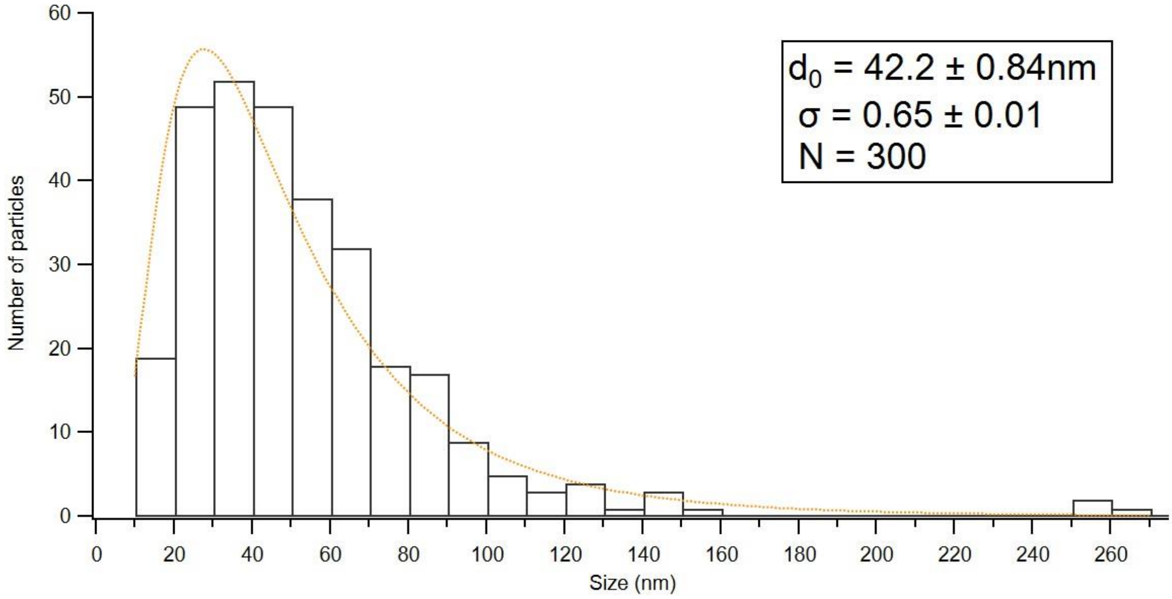
f(d)= (A/(2.506*d*s))*exp(-(ln(d/d0)^2)/(2*s*s))

Acrylic acid functionalized nanoparticles:

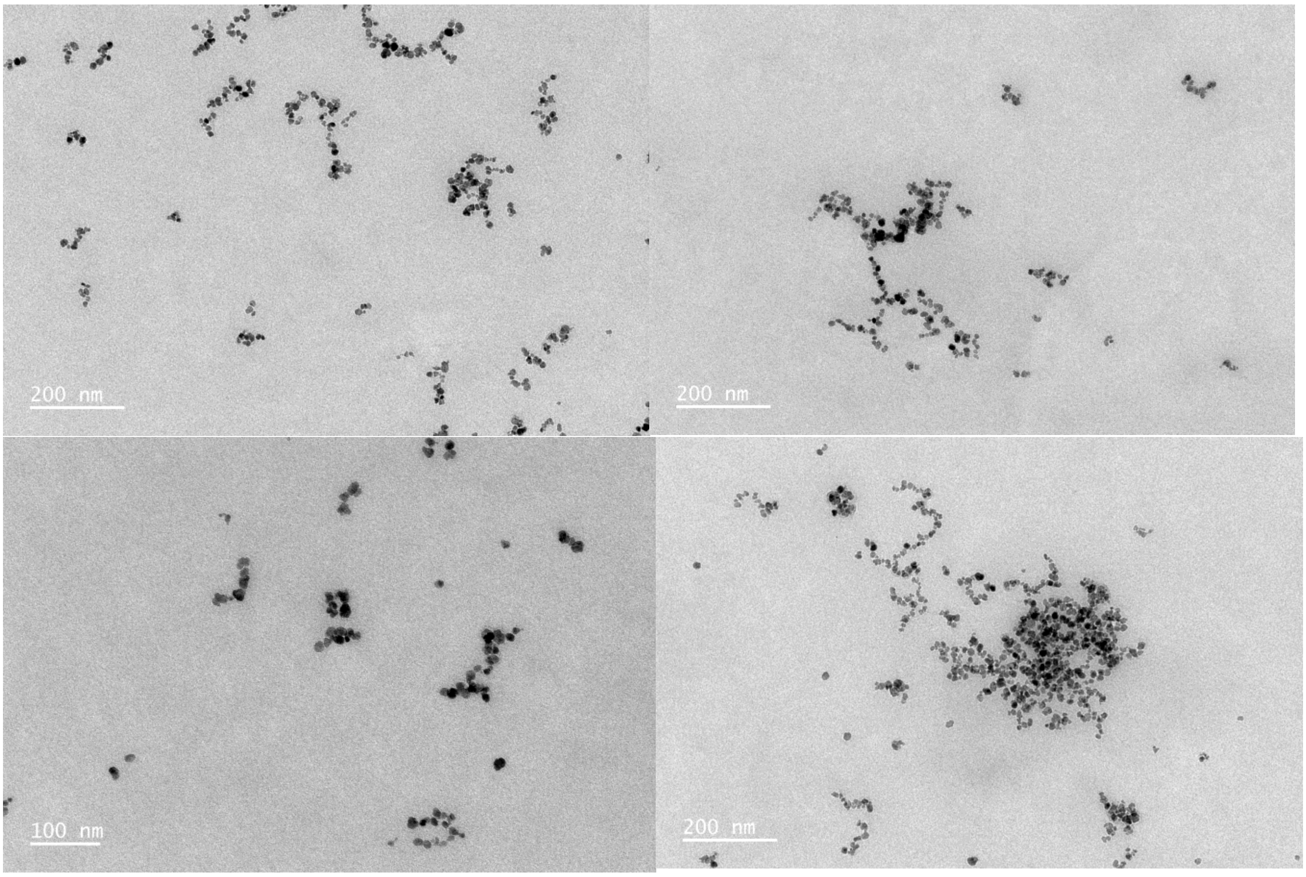


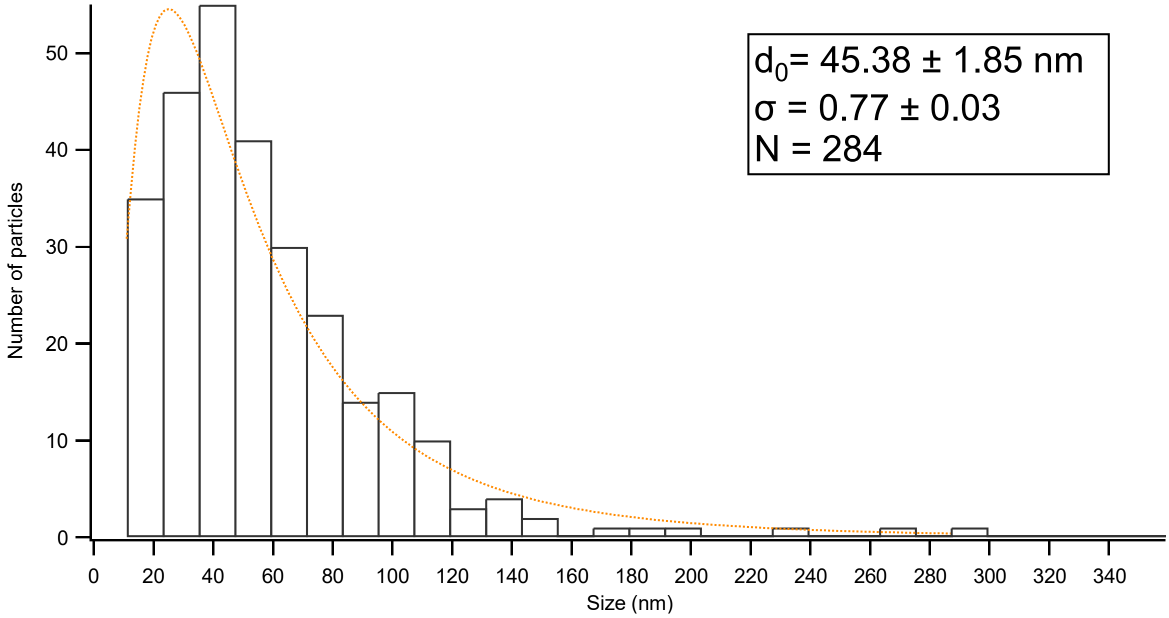
[1:48]


200 nm
200 nm
200 nm
200 nm

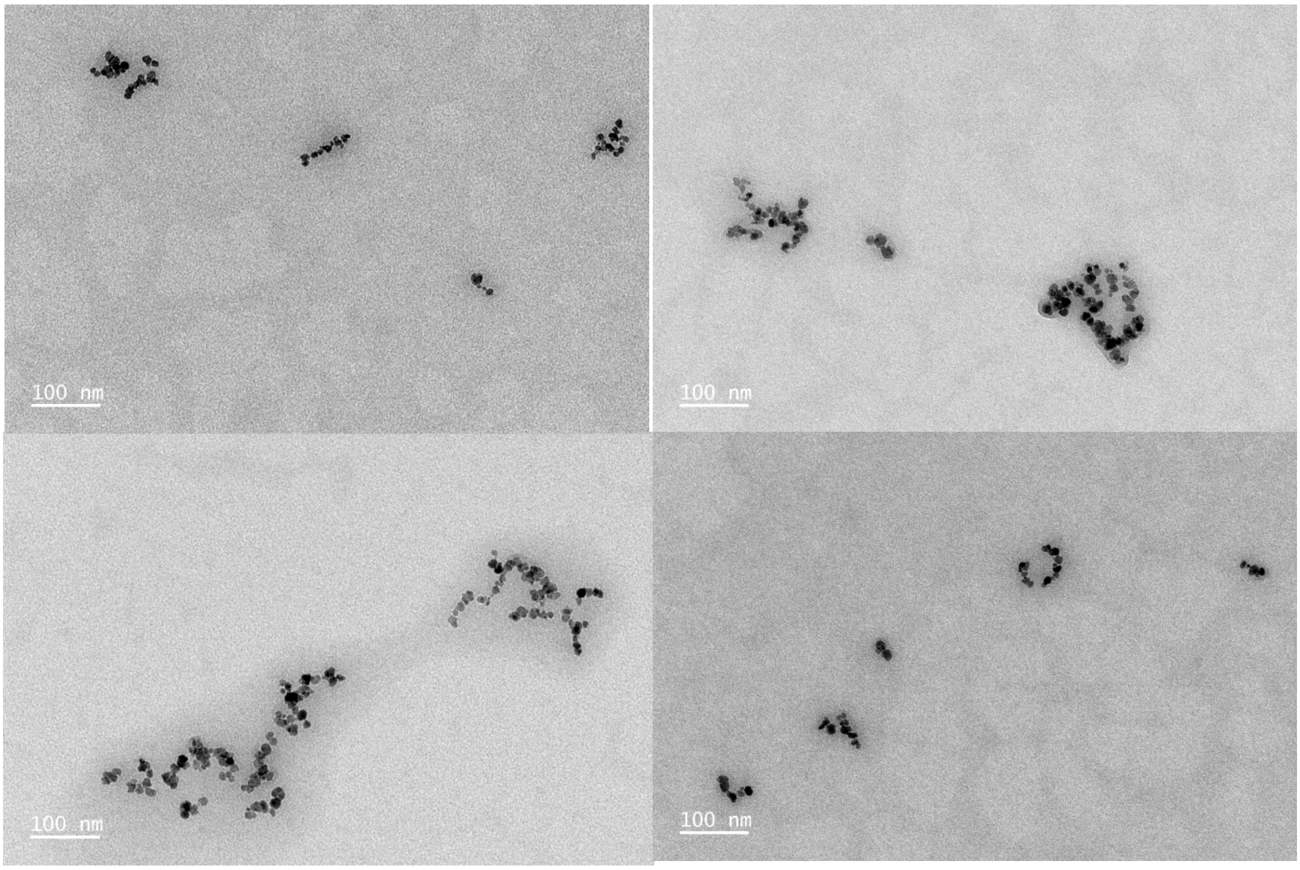

d0 = 42.2 ± 0.84nm
σ = 0.65 ± 0.01
N = 300
Number of particles
Size (nm)
0
10
20
30
40
50
60
0
20
40
60
80
100
120
140
160
180
200
220
240
260

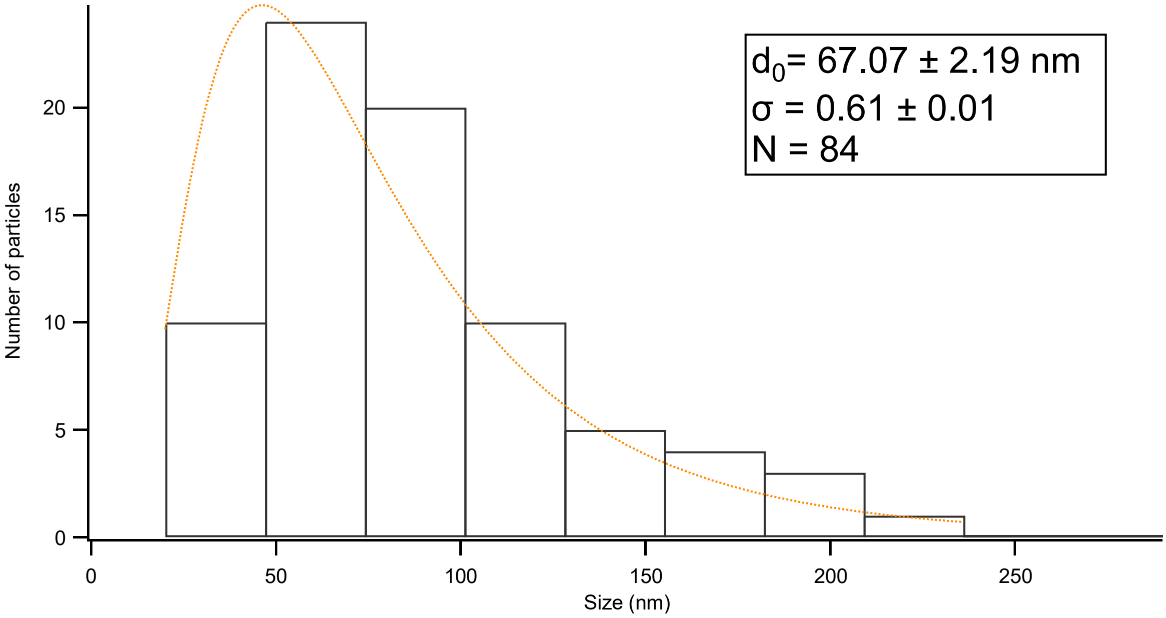
[1:72]


200 nm
200 nm
100 nm
200 nm


d₀= 45.38 ± 1.85 nm
σ = 0.77 ± 0.03
N = 284
Number of particles
0
10
20
30
40
50
Size (nm)
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340

[1:92]





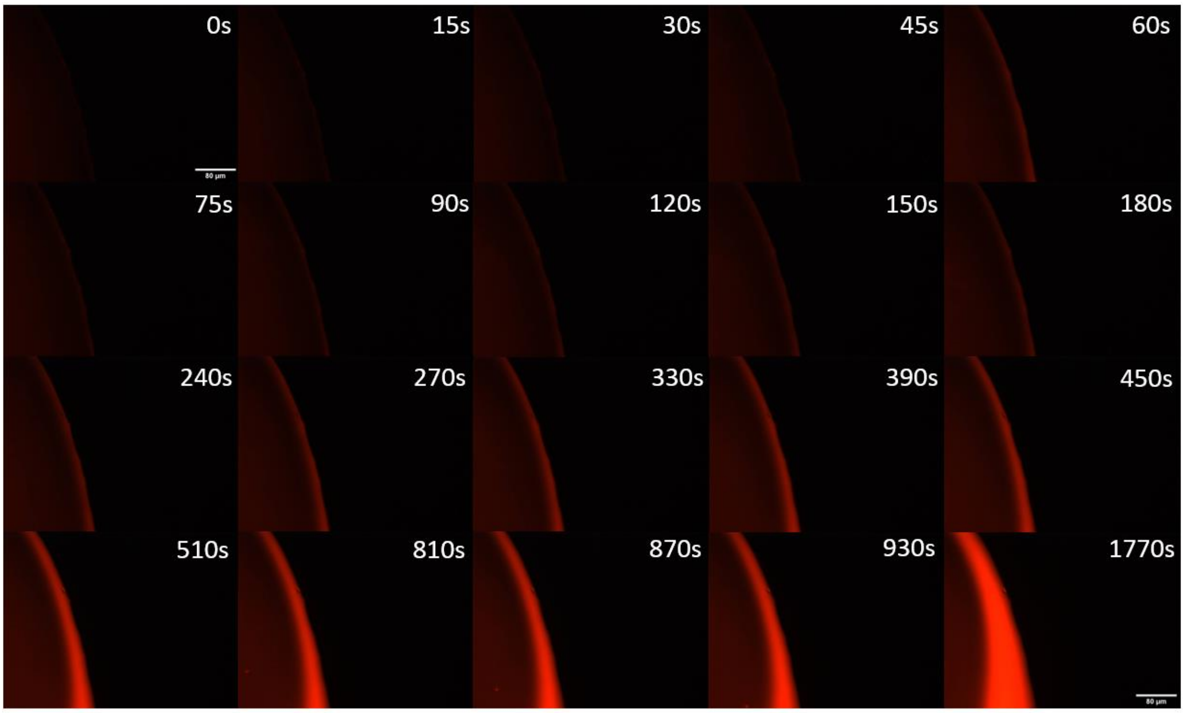
As less particles were present on the images for sample [1:96] (but bigger, so a similar number of magnetic nanoparticles were present), less particles have been counted for this sample, resulting in a less accurate histogram.

PART III: Fluorescence microscopy images

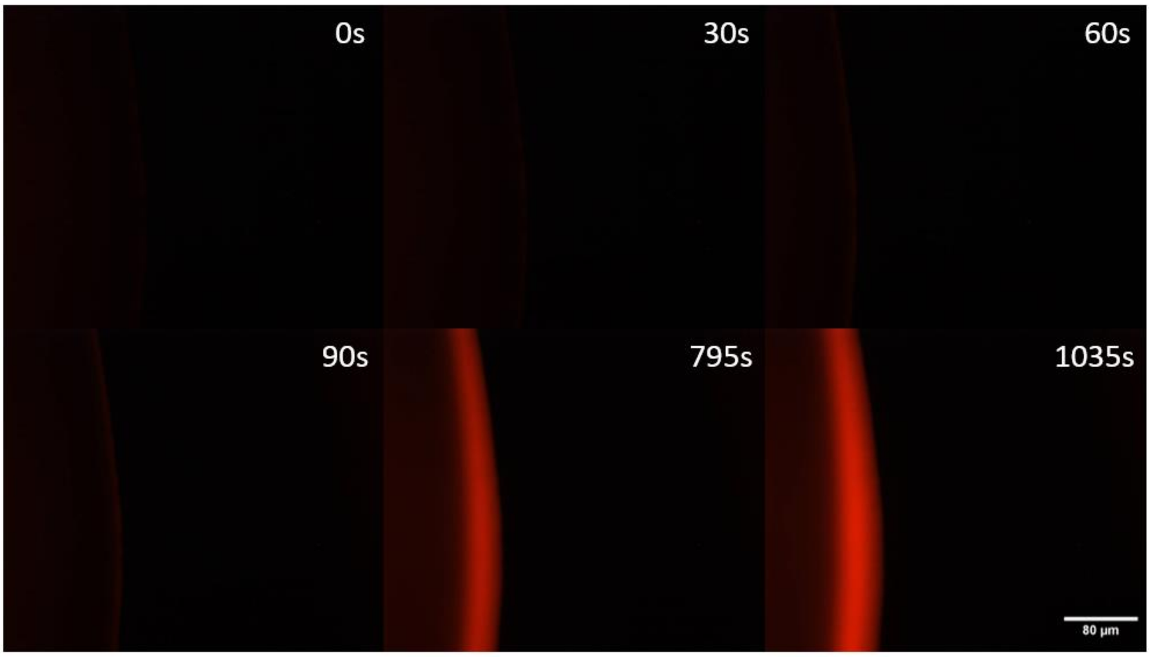
A drop of sample was deposited on a glass slide and microscope settings were done on the edge of the drop. After taking a picture, a NdFeB was placed out of the field, south of the image and pictures were taken at known intervals to illustrate particles accumulation at the edge of the drop.

More of those images are presented below, scale bar corresponds to 80 µm. While at time 0 s fluorescence could be seen inside the drop using the binocular, it does not appear strongly enough to be seen on the unmodified images as displayed below. However, this can be corrected by increasing the contrast.

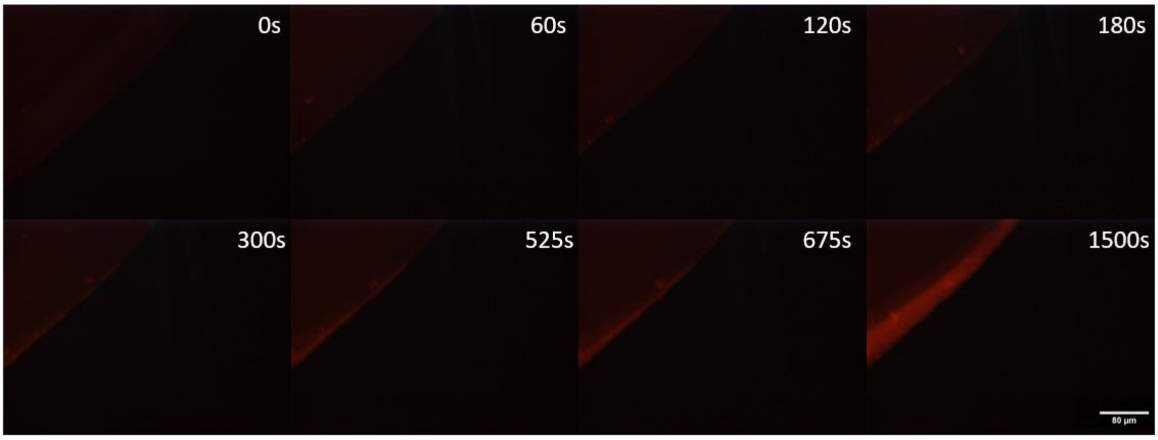
[1:48]



[1:72]


0s
30s
60s
90s
795s
1035s
80 µm

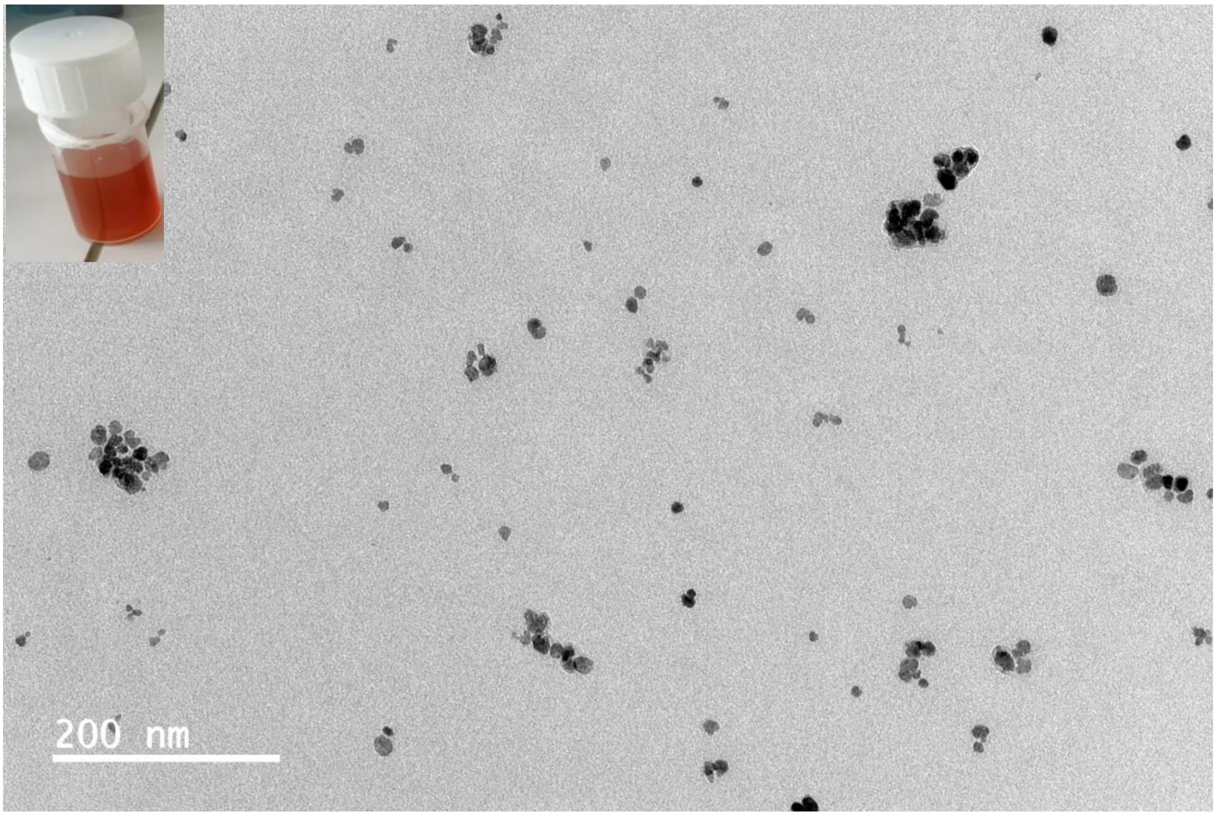
[1:96]

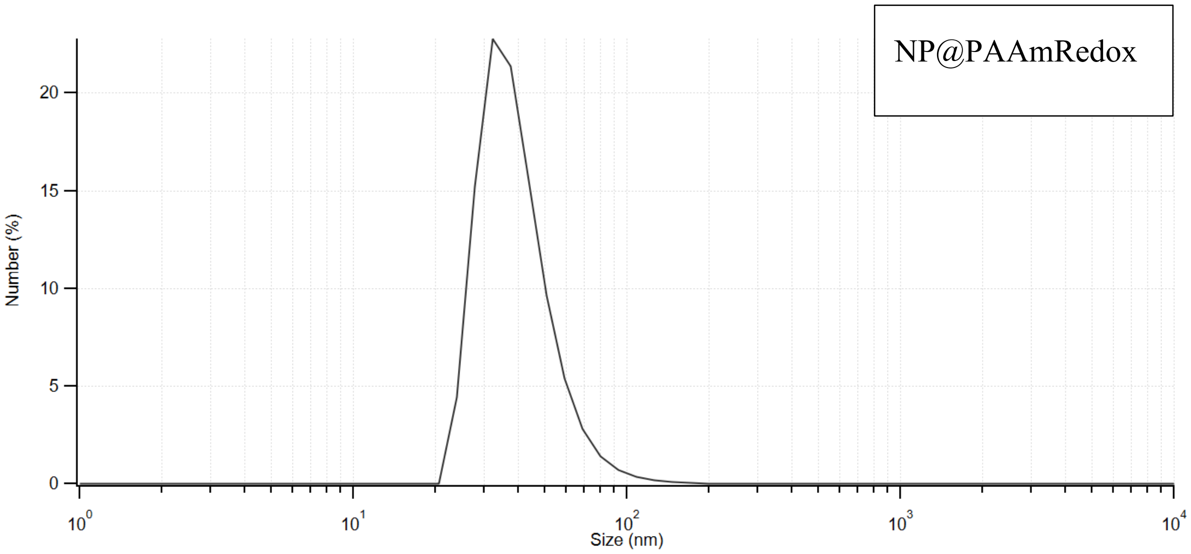

0s
60s
120s
180s
300s
525s
675s
1500s
80 µm

PART IV: RedOx synthesis

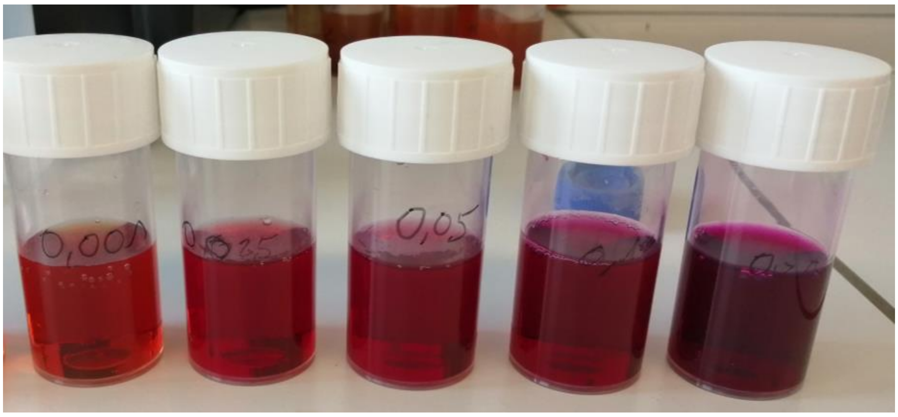
The RedOx synthesis of core-shell particles using APS and TEMED as initiation system and acrylic acid coating were performed as described below.

In a glass vial, 50 µL of maghemite nanoparticles functionalized with a ratio [Fe:AAc]=[1:0,48] as reported above, 0.32 mmol of BIS (50 mg), 1.85 mmol of AaM (132 mg) were mixed in 15 mL of water. After sonication and 15 min of purge with nitrogen gas, 135 µL of a freshly prepared 10% (w/w) solution of APS in water and 27 µL of TEMED were added. The solution was agitated at 200 rpm on an orbital stirrer for 1 h and washed by dialysis as described above. The obtained TEM image for the particles, which were stable, non-fluorescent, and core-shell like, is shown below.

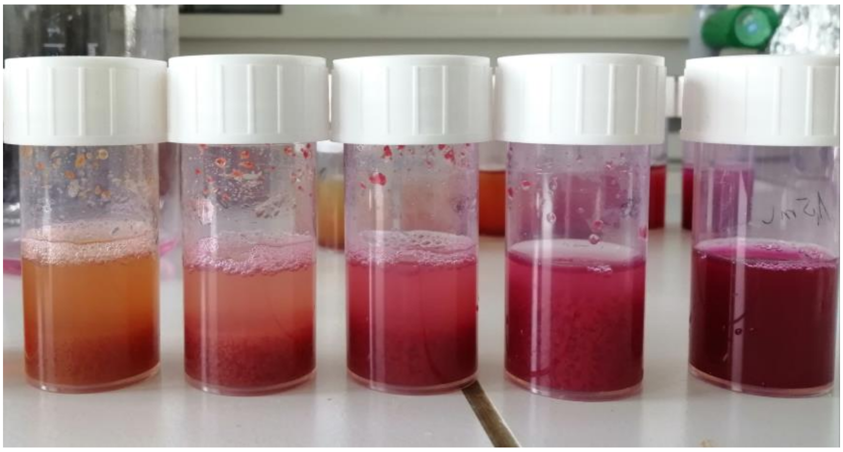




To produce fluorescent core-shell particles, the synthetic procedure was repeated as described above but with different amounts Acr-RhB (652 g/mol) were added :0.023 µmol, 0.56 µmol, 1.15 µmol, 2.3 µmol and 5.75 µmol. Pictures taken before and after polymerization are presented below.



Before polymerisation

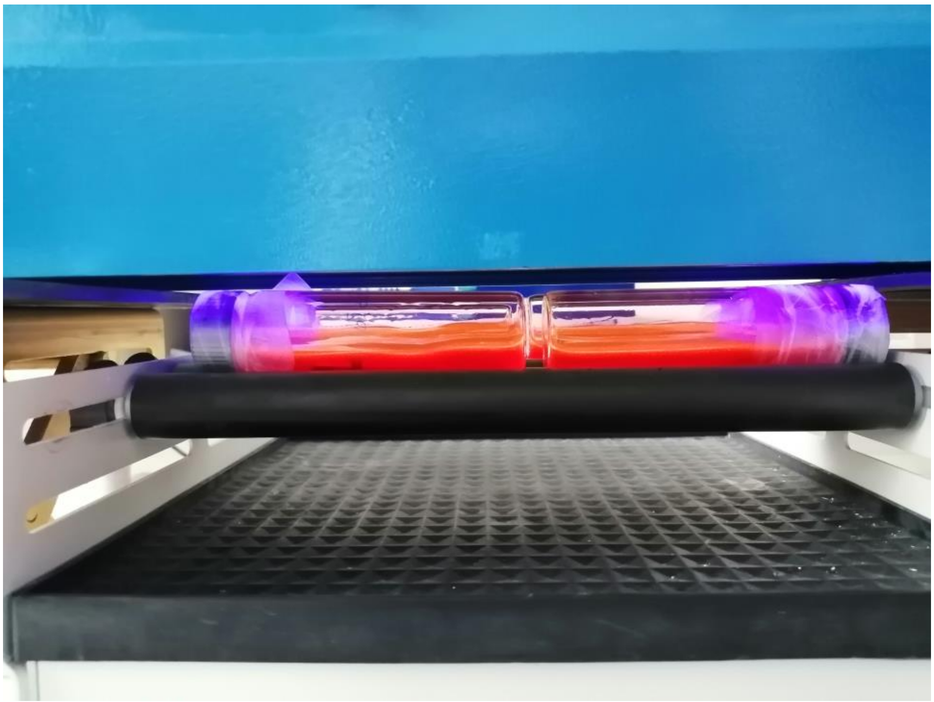


After polymerisation

Even at very low concentration of fluorescent monomer, polymerization lead to the formation of big unstable magnetic clusters.

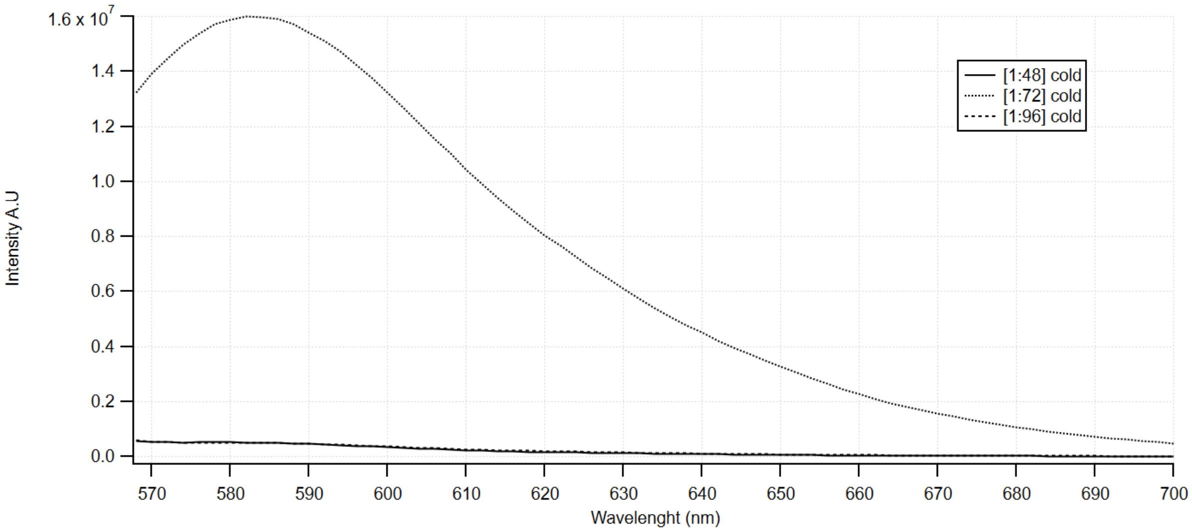
PART V: Sensitivity to external parameters

The synthesis presented in this article were performed in a room at 25 °C with 40 mL borosilicate glass vials, which were laid horizontally on an orbital stirrer, as displayed below. As the lamp heats the area, the temperature under the lamp was between 30 and 34 °C during the polymerization.



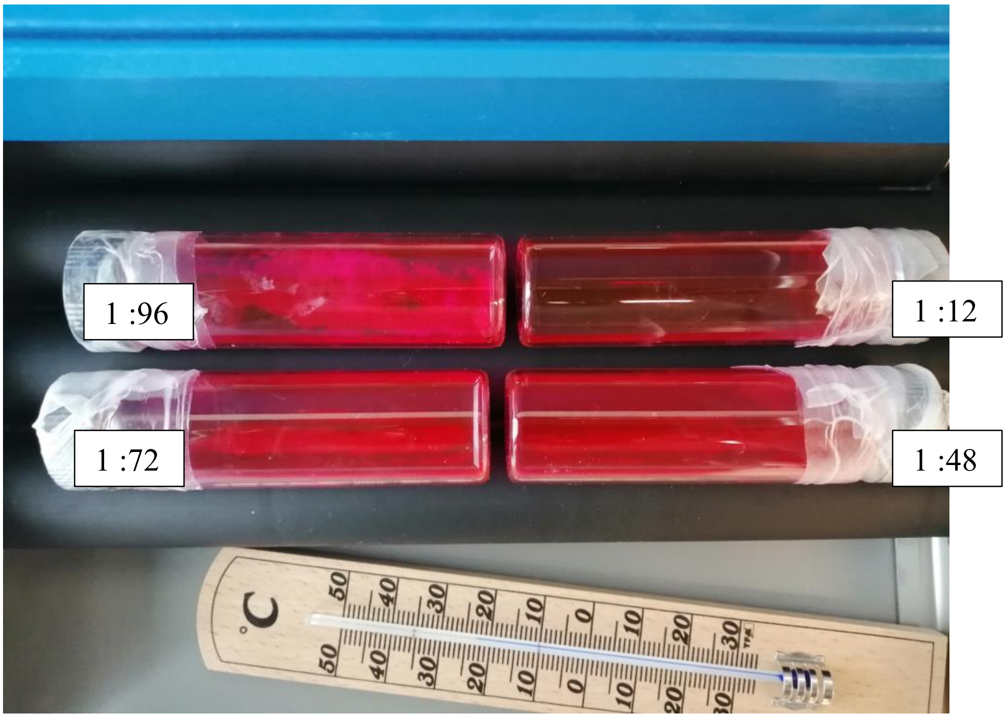
However, the authors performed this synthesis in other circumstances and noticed differences in the obtained samples mainly caused by two factors: temperature and stirring.

First, carrying out the exact same synthesis in a colder room (15 °C) with the same stirring setup lead to very low yield compared to those previously obtained (1.6, 17.1, and 2.7% for particles prepared at [1:48], [1:72], and [1:96] mole ratios, respectively). Fluorescence spectra obtained for those materials supported the yield measurements.

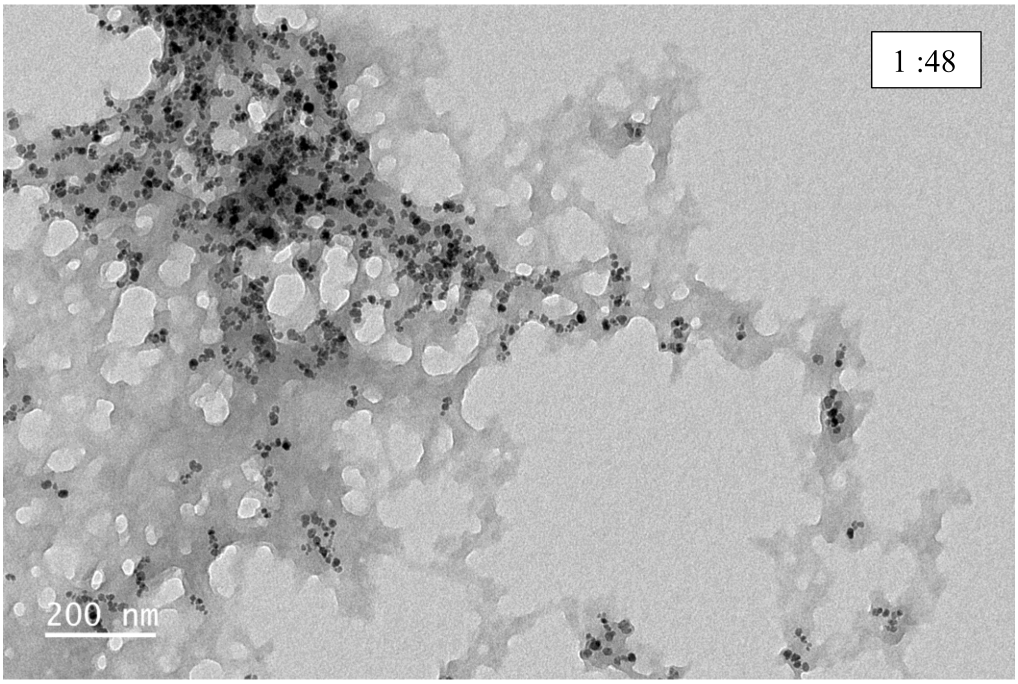


The results indicate a strong temperature dependency of the kinetic of the reaction.

Other parameters that influence the reaction product are stirring method and speed. Since roller stirrers have the reputation to allow better polydispersity of polymerized nanoparticles, the same synthesis was performed as before with this method of shaking on an IBI scientific low-profile roller. The lamp was placed above, at a distance of 5 cm. After one hour at the minimum rolling rate, phase separation of polymer chunks was observed.



By increasing the speed of the roller, more homogeneous solutions could be obtained; however, TEM images showed large polymer films on the grid.



In this context, the authors recommend to work in a temperature-controlled space and to evaluate different stirring conditions to obtain core-shell particles.