Determination of the ratio of fluorophore/nanoparticle for fluorescence-labelled nanoparticles

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I. Synthesis and characterization of different types of nanoparticles

I.1. PbS nanoparticles

PbS nanoparticles (NPs), so-called quantum dots (QDs), were synthesized following the method described by Hines et al.1 with slight modifications. Briefly, 446 mg (2 mmol) of lead(II) oxide (PbO, Sigma-Aldrich, #15338) was dissolved in a mixture of 2 mL oleic acid (OA, Sigma-Aldrich, #75090) and 20 mL 1-octadecene (ODE, Sigma-Aldrich, #74738) in a three-necked flask. The mixture then was heated to 90 °C under inert gas atmosphere. The reaction was left to proceed until the PbO was completely decomposed to form lead oleate (indicated by a color change of the solution from yellow to clear), and then the temperature of the solution was raised to 120 °C. Afterwards 0.2 mL of bis(trimethylsilyl) sulfide (TMS, Sigma-Aldrich, #283134) dissolved in 10 mL 1-octadecene was rapidly injected into the lead oleate solution. The rapid injection of TMS solution into the reaction flask resulted in a change of the color of the reaction mixture from colorless to deep brown. The reaction was continued for 2 min allowing for growth of the QDs, and then was allowed to cool down to room temperature by removing the heating mantle. To isolate and purify the NPs, the reaction mixture was dissolved in 10 mL of anhydrous toluene (Sigma-Aldrich, #244511) and precipitated upon the addition of 5 mL methanol (Sigma-Aldrich, #34860) and 5 mL acetone (Sigma-Aldrich, #650501), followed by centrifugation, discarding of the supernatant and redispersion of the NP precipitate in toluene. This process of precipitation and redispersion was repeated 3 times. Finally, the precipitated NPs were redispersed in anhydrous chloroform (Sigma-Aldrich, #372978).

The oleic acid-capped PbS QDs were characterized by transmission electron microscopy (TEM) and UV/Vis absorption spectroscopy, cf. Figure SI-1. They had a core diameter of $d_c = 3.0 \pm 0.4$ nm, according to transmission electron microscopy (TEM) images.
Figure SI-1: a) TEM image of oleic acid-capped PbS QDs. b) Histogram of the size distribution of inorganic cores with diameter \(d_c = 3.0 \pm 0.4 \text{ nm}\). \(N(d_c)\) refers to the total counts and the scale bar corresponds to 50 nm. c) Absorption spectrum \(A(\lambda)\) of PbS QDs in chloroform.

I.2. Au nanoparticles

Au NPs were synthesized according to standard procedures based on the method described by Brust et al.\textsuperscript{2,3} Briefly, 300 mg of hydrogen tetrachloroaurate(III) hydrate (HAuCl\(_4\), Alpha Aesar, #42803) were dissolved in 20 mL Milli-Q water. 2.17 g of tetraoctylammonium bromide (TOAB, Sigma-Aldrich, #294136) were dissolved in 80 mL of toluene. Both solutions were transferred into a separator funnel and gently shaken for 5 minutes. The aqueous phase was drained when decolorized, showing the effective transfer of AuCl\(_4^-\) ions to the organic phase. The organic phase was transferred into a round flask and stirred with a magnetic stirrer. 334 mg of sodium borohydride (NaBH\(_4\), Sigma-Aldrich, #452882) were dissolved in 20 mL Milli-Q water and added dropwise into the organic phase accompanied by a color change from red to violet, indicating the nucleation of gold clusters mediated by sodium borohydride. The solution was stirred for one hour and afterwards transferred to a separator funnel. The aqueous phase was discarded and then 25 mL of 10 mM HCl were added to remove the excess of sodium borohydride. The solution was gently shaken for one minute and the aqueous phase was discarded. Next, 100 mL of 0.01 M NaOH were added, shaken gently for one minute and the aqueous phase was discarded in order to remove the excess of HCl. Finally, 100 mL of Milli-Q water were then added and the aqueous phase was removed after shaking for minutes in order to remove the excess
ions. This last step was repeated 3 times. The organic solution was transferred back into a round flask and stirred overnight, allowing for Ostwald ripening of the NPs. 10 mL of 1-dodecanethiol (Sigma-Aldrich, #471364) were added and the solution was then incubated at 65 °C for 3 hours. Afterwards the solution was transferred into several 40 mL glass vials which were centrifuged for 5 minutes at 2000 rpm. The supernatant containing the dispersed NPs was collected in 40 mL glass vials (10 mL each) and the precipitates (bigger NP agglomerates) at the bottom were discarded (note that the centrifugation speed is not enough to sediment single Au NPs due to the small size of the NPs). The glass vials were filled up with anhydrous methanol (Sigma-Aldrich, #322415), which caused flocculation of the NPs, and centrifuged for 5 minutes at 2000 rpm, leading to precipitation of Au NPs. The transparent liquid phase was discarded and the precipitated NPs were redispersed in anhydrous chloroform (Sigma-Aldrich, #372978). The resulting dodecanethiol-capped Au NPs were characterized by TEM and UV/Vis absorption spectroscopy, cf. Figure SI-2. From the analysis of the TEM images, the NPs had a core diameter of $d_c = 4.4 \pm 0.6$ nm. The absorbance spectrum of the Au NPs in chloroform showed a surface plasmon resonance (SPR) peak at approximately 530 nm.

**Figure SI-2:** a) TEM image of dodecanethiol-capped Au NPs. b) Histogram of the size distribution of inorganic cores with mean diameter $d_c = 4.4 \pm 0.6$ nm. $N(d_c)$ refers to the total counts and the scale bar corresponds to 50 nm. c) Absorption spectrum $A(\lambda)$ of Au NPs in chloroform showing the SPR peak at 530 nm.
I.3. FePt nanoparticles

The FePt NPs were synthesized following a previously published protocol by Moser et al. Briefly, platinum acetylacetonate (197 mg, 0.5 mmol, ABCR, #AB 121416), 1,2-hexadecanediol (390 mg, 1.5 mmol, Sigma Aldrich, #213748), and dioctylether (20 mL, Sigma Aldrich, #249599) were mixed and heated up to 100 °C under inert gas atmosphere. Oleic acid (0.16 mL, 0.5 mmol, Sigma Aldrich, #O1008), oleyl amine (0.17 mL, 0.5 mmol, Sigma Aldrich, #O7805), and Fe(CO)$_5$ (0.13 mL, 1 mmol, Sigma Aldrich, #481718) were added, and the mixture was heated to reflux (297 °C). The refluxing was continued for 30 min. The heating mantle used as heat source was then removed, and the reaction mixture was allowed to cool down to room temperature. The black product was precipitated by adding ethanol (∼40 mL, Carl Roth, #64-17-5) and separated by centrifugation. A yellow-brown supernatant was discarded. The remaining black precipitate was dispersed in hexane (∼25 mL) in the presence of oleic acid (∼0.05 mL) and oleyl amine (∼0.05 mL) and then precipitated by adding ethanol (∼20 mL) and centrifuging. The purified NP precipitate was dispersed in hexane (∼20 mL), centrifuged to remove any unsolved precipitation (almost no precipitation was found at this stage), and precipitated again by adding ethanol (∼15 mL) and centrifuging. The precipitated NPs were finally redispersed in anhydrous chloroform (Sigma-Aldrich, #372978). The resulting oleic acid/oleyl amine-capped FePt NPs were characterized by TEM and UV/Vis absorption spectroscopy, cf. Figure SI-3. From the analysis of the TEM images, the NPs showed a core diameter of $d_c = 3.2 \pm 0.4$ nm.

![Figure SI-3: a) TEM image of oleic acid/oleyl amine-capped FePt NPs. b) Histogram of the size distribution of inorganic cores with diameter $d_c = 3.2 \pm 0.4$ nm. $N(d_c)$ refers to the total counts and the scale bar corresponds to 50 nm. c) Absorption spectrum $A(\lambda)$ of FePt NPs in chloroform.](image-url)
II. Synthesis of fluorophore-modified polymer

II.1. Synthesis of plain amphiphilic polymer

An amphiphilic polymer was used to transfer the hydrophobic NPs to aqueous solution and for the simultaneous incorporation of the fluorophore molecules. The amphiphilic polymer, synthesized according to previously published protocols, is based on a backbone of poly(isobutylene-alt-maleic anhydride), whereby 75% of the anhydride rings were conjugated with dodecylamine, yielding dodecylamine hydrophobic side chains through formation of amide bonds upon reaction with the maleic anhydride rings. 25% of the anhydride rings were left unreacted and can be used for attachment of further functionalities.

The synthesis of the dodecylamine-modified polyisobutylene-alt-maleic anhydride polymer (PMA) was as follows: 2.70 g (15 mmol) of dodecylamine (Sigma-Aldrich, #D22220-8) were dissolved in 100 mL of anhydrous tetrahydrofurane (THF, Sigma-Aldrich, #186562). This solution was poured into a 250 mL round bottom flask containing 3.084 g (20 mmol expressed in terms of polymer monomer units; as the polymer has a molecular weight of $M_w \sim 6000$ g/mol the molecular weight of one polymer unit is $M_w \sim 6000 \text{ g/mol} / 39 \approx 154 \text{ g/mol}$, as each polymer molecule comprises on average 39 monomer units) of poly(isobutylene-alt-maleic anhydride), average molecular weight (Sigma-Aldrich, #531278). After mixing, the mixture was sonicated and then heated to 60 $^\circ$C for three hours, whereby the amino-groups of dodecylamine reacted to the anhydride rings. Afterwards, the solution was concentrated to 30-40 mL by evaporation of THF, and the mixture was heated under reflux overnight. Finally, the solvent was completely evaporated and the obtained product was redissolved in 40 mL anhydrous chloroform obtaining a final polymer (monomer) concentration of $c_p = 0.5 \text{ M}$.

II.2. Modification of the polymer with different fluorophores

The fluorophores used were the following: Rhodamine 123 (Sigma-Aldrich, #R8004), Rhodamine 6G (Sigma-Aldrich, #R4127), Rhodamine B (Sigma-Aldrich, #R6626), Cresyl Violet (Sigma-Aldrich, #255246), OregonGreen cadaverine (Life Technologies, #O-
10465), amino-modified ATTO633 (ATTO-TEC, #AD 633-91), amino-modified ATTO590 (ATTO-TEC, #AD 590-91), Nile Red (Sigma-Aldrich, #19123), Crystal Violet (Sigma-Aldrich, #C6158), and amino-modified DY-636 (Dyomics, #636-02). Depending on the structure of the fluorophore, it can be incorporated in the polymer by different ways. In case of amino-group bearing fluorophores, they are supposed to react with the intact anhydride rings giving a covalent attachment, i.e. amide bonds. However, fluorophores without amine groups but with a hydrophobic structure are able to be embedded in the polymer through hydrophobic interactions. The modification of the polymer with the fluorophore molecules was performed according to the procedures already described in previously published protocols,\textsuperscript{5,7} and it was the same for all types of fluorophores. A stock solution of the fluorophore (dissolved in chloroform, methanol or dimethyl sulfoxide (DMSO)) was mixed with the PMA solution (dissolved in chloroform) in a ratio of 2\% (i.e. fluorophore/PMA monomer ratio of 2/100). The mixture was stirred at room temperature overnight and the chloroform was then evaporated. The resulting powder was redissolved in anhydrous chloroform to obtain a final (monomer) polymer concentration of $c_p = 0.05$ M.
**Table SI-1.** Structure of the different fluorophores used in this work, including their molecular formula and molecular weight $M_w$ (excluding counter ions). The structures of the ATTO633 dye and its amino-modified ATTO633 are not provided by the commercial manufacturer (ATTO-TEC GmbH).

<table>
<thead>
<tr>
<th>Fluorophores with amino-groups</th>
<th>Fluorophores without amino-groups</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rhodamine 123</strong></td>
<td><strong>Rhodamine 6G</strong></td>
</tr>
<tr>
<td><img src="image" alt="Rhodamine 123" /></td>
<td><img src="image" alt="Rhodamine 6G" /></td>
</tr>
<tr>
<td>$C_{22}H_{16}N_2O_3$ ; $M_w=344.4$</td>
<td>$C_{28}H_{30}N_2O_3$ ; $M_w=442.5$</td>
</tr>
<tr>
<td><strong>Oregon Green cadaverine</strong></td>
<td><strong>Cresyl Violet</strong></td>
</tr>
<tr>
<td><img src="image" alt="Oregon Green cadaverine" /></td>
<td><img src="image" alt="Cresyl Violet" /></td>
</tr>
<tr>
<td>$C_{28}H_{23}F_2N_2O_6$ ; $M_w=496.5$</td>
<td>$C_{16}H_{11}N_3O$ ; $M_w=261.3$</td>
</tr>
<tr>
<td><strong>amino-ATTO590</strong></td>
<td><strong>amino-DY636</strong></td>
</tr>
<tr>
<td><img src="image" alt="amino-ATTO590" /></td>
<td><img src="image" alt="amino-DY636" /></td>
</tr>
<tr>
<td>$C_{39}H_{45}N_4O_4$ ; $M_w=633.0$</td>
<td>$C_{20}H_{52}N_2O_6S_2$ ; $M_w=781.0$</td>
</tr>
<tr>
<td><strong>amino-ATTO633</strong></td>
<td><strong>Nile Red</strong></td>
</tr>
<tr>
<td><img src="image" alt="amino-ATTO633" /></td>
<td><img src="image" alt="Nile Red" /></td>
</tr>
<tr>
<td><em>Not available</em></td>
<td>$C_{20}H_{18}N_2O_2$ ; $M_w=318.4$</td>
</tr>
</tbody>
</table>


III. Polymer coating of nanoparticles with fluorophore-modified polymer

Polymer coating of all the hydrophobic NPs (i.e. PbS QDs, Au NPs, and FePt NPs) was carried out following previously published protocols. The fluorophore-modified PMA as dissolved in chloroform was added to the hydrophobic NPs as dissolved in chloroform. The amount of polymer was calculated so that 100 monomers units per \( \text{nm}^2 \) of effective NP surface were used. The effective surface area \( A_{\text{NP,eff}} = 4\pi \cdot (d_{\text{eff}}/2)^2 \) of each NP was calculated. The effective diameter \( d_{\text{eff}} = d_c + 2 \cdot l_{\text{surfactant}} \) comprises the diameter of the inorganic part of the NP \( (d_c) \) as determined by TEM, as the thickness of the organic surfactant shell \( l_{\text{surfactant}} \). In the present work, \( d_c \) was 3.0 nm, 4.4 nm, and 3.2 nm for PbS NPs, Au NPs, and FePt NPs respectively, and \( l_{\text{surfactant}} = 1.2 \) nm was assumed in all the cases. Then, \( d_{\text{eff}} \) is 5.4 nm, 6.8 nm, and 5.6 nm for PbS NPs, Au NPs, and FePt NPs respectively. In order to have 100 polymer units per \( \text{nm}^2 \) of effective surface area \( (R_{p/\text{area}} = 100 \text{ nm}^{-2}) \), the total volume \( V_p \) of PMA solution of monomer concentration \( c_p = 0.05 \text{ M} \) which needed to be added to a volume \( V_{\text{NP}} \) of NP solution with concentration \( c_{\text{NP}} \) was calculated as:

\[
V_p = c_{\text{NP}} \cdot V_{\text{NP}} \cdot R_{p/\text{area}} \cdot A_{\text{NP,eff}} / c_p
\]

After adding the appropriate amounts of PMA and NP solutions to a round flask, the mixture was stirred in a rotavap system for \( \sim 10 \text{ min} \) at 40 °C in order to give more flexibility to the polymer chain, and to allow for a better coating. Then, the solvent was slowly evaporated until the sample was completely dried and the solid thin film formed that was then dissolved in 50 mM sodium borate buffer at pH = 12 (SBB 12).

IV. Purification of fluorophore-modified polymer-coated nanoparticles

After the polymer coating of the NPs with the fluorophore-modified PMA polymer several purifications were carried out. First, the samples were passed through syringe filters (0.22 µm) to remove eventual NP aggregates generated during the NPs solubilization process. Second, the buffer was exchanged for MilliQ-water by two rounds of dilution and preconcentration through a centrifuge filter (molecular weight cut-off (MWCO) = 100 kDa, SatoriusStedim, 3000 rpm centrifugation speed). Then, gel electrophoresis was performed in 2 % agarose (Invitrogen #16500-500), in 0.5x Tris-
Borate-EDTA buffer (TBE, Sigma-Aldrich, #T3913) to remove the empty fluorophore-modified polymer micelles. The concentrated samples mixed with 20 % of loading buffer (stock solution of 35 mL TBE + 25 mL glycerol + 130 mg orange G), were loaded into the well of the prepared gel. Upon application of an electric field of 10 V·cm⁻¹ the negatively charged NPs (electrical charge is provided due to the COO⁻ groups in the polymer shell) migrated towards the plus pole. After applying the voltage for the time of one hour the fluorophore-NP conjugates formed a visible band in the gel, which was separated from the band of the empty micelles. Images of the gels were recorded with a digital camera (Canon Digital Ixus IIs) under the irradiation of UV light and the indications of the position of the bands corresponding to the fluorophore-NP conjugates are shown in Figure SI-4. Note that often there is no clear separation between the band of the free polymer micelles and the band of the polymer coated NPs. The resulting bands were cut out from the gels and transferred into a dialysis membrane (50 kDa MWCO) filled with TBE buffer. The dialysis pack was put into the gel trough and again an electric field of 12 V·cm⁻¹ was applied for ca. 20 min. This caused the NPs to migrate out of the gel staying trapped inside the surrounding dialysis membrane. Afterwards the NP solution was taken out of the dialysis membrane and were washed and concentrated using a centrifuge filter (MWCO = 100 kDa, SatoriusStedim, 3000 rpm centrifugation speed). Finally the buffer was exchanged to MilliQ water by adding water 3 times and concentrating again with centrifuge filters until to get a final volume of ~0.5 mL. Again, due to the overlap of bands of empty micelles and polymer coated NPs some small residue of empty polymer micelles in the purified NP solution cannot be excluded.
Figure SI-4. Images upon UV light illumination of 2 % agarose gels taken after running the different prepared fluorophore-NP conjugates. “+” and “-” indicate the poles of the electric field. The arrows indicate the direction in which the NPs migrate. The red sections correspond to the positions of the fluorophore-NP conjugates.
V. Determination of fluorophore/nanoparticle ratio

V.1. Calculation of fluorophore concentration

The molar concentration of each fluorophore in the fluorophore-NP conjugates was determined by UV/Vis absorption spectroscopy using the Lambert-Beer law. UV/Vis absorption measurements of the fluorophore-NP conjugates were carried out with an Agilent Technologies 8453 UV/Vis spectrometer (see Figure SI-5). The extinction coefficient and absorption maximum wavelength for each assayed fluorophore are reported in Table SI-1.
Figure SI-5. Absorption spectra $A(\lambda)$ of the different prepared fluorophore-NP conjugates after purification, as recorded in water.

Table SI-1. Extinction coefficients ($\varepsilon$) at the absorption maximum and absorption maximum wavelengths ($\lambda_{\text{Abs}}$) of the different fluorophores.

<table>
<thead>
<tr>
<th>Fluorophore</th>
<th>$\varepsilon$ [cm$^{-1}$ M$^{-1}$]</th>
<th>$\lambda_{\text{Abs}}$ [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oregon Green</td>
<td>80000</td>
<td>493</td>
</tr>
<tr>
<td>Rhodamine 123</td>
<td>85200</td>
<td>505</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>116000</td>
<td>530</td>
</tr>
<tr>
<td>Rhodamine B</td>
<td>107000</td>
<td>555</td>
</tr>
<tr>
<td>Nile Red</td>
<td>38000</td>
<td>555</td>
</tr>
<tr>
<td>Crystal Violet</td>
<td>87000</td>
<td>590</td>
</tr>
<tr>
<td>ATTO590</td>
<td>120000</td>
<td>595</td>
</tr>
<tr>
<td>Cresyl Violet</td>
<td>83000</td>
<td>600</td>
</tr>
<tr>
<td>ATTO633</td>
<td>130000</td>
<td>630</td>
</tr>
<tr>
<td>DY636</td>
<td>200000</td>
<td>645</td>
</tr>
</tbody>
</table>
V.2. Calculation of nanoparticle concentration

Concentration of the fluorophore-NP conjugates was determined by ICP-MS (Agilent 7700 Series) after acidic digestion of the NP sample with aqua regia in an ultrasonic bath. After the digestion, the samples were appropriately diluted in ultrapure water. External calibration was applied to quantify the amount of elemental lead, platinum and gold. External standards were prepared by diluting ICP-MS standard lead, platinum and gold solutions, respectively (all the standards were obtained from Agilent) in the same background solution as the samples. All samples were measured by triplicate and the external calibration curves obtained were:

\[ I = 6469.9 \, \text{L/} \mu \text{g} \cdot C_{\text{Pb}} + 11656; \quad r^2 = 0.9996 \quad \text{for Pb} \]

\[ I = 8018.6 \, \text{L/} \mu \text{g} \cdot C_{\text{Au}} + 8518.8; \quad r^2 = 0.9998 \quad \text{for Au} \]

\[ I = 6426.9 \, \text{L/} \mu \text{g} \cdot C_{\text{Pt}} + 7606.6; \quad r^2 = 0.9988 \quad \text{for Pt} \]

where \( I \) is the intensity of the ICP-MS signal in counts per second (cps) of the isotopes \(^{208}\text{Pb}, \, ^{197}\text{Au}, \, ^{195}\text{Pt} \), respectively; \( C_{\text{Pb}}, \, C_{\text{Au}}, \, C_{\text{Pt}} \) are the concentrations of each elemental in parts per billion (ppb or \( \mu \text{g/L} \)); \( r^2 \) is the coefficient of determination of the linear regression analysis of the calibration curve.

V.2.1. Determination of PbS QD concentration

To determine the concentration \( c_{\text{NP}} \) of PbS NPs, the elemental concentration of lead obtained by ICP-MS measurements was converted to NP concentration, considering an average particle diameter of \( d_c = 3.0 \, \text{nm} \) obtained by TEM and taking into account the spherical shape of the NPs. In this way, the volume of one NP (\( V_{\text{NP}} \)) is:

\[ V_{\text{NP}} = \frac{4}{3} \pi (d_c/2)^3 \]

Then, it is necessary to calculate the mass of NPs in the solution of volume \( V \), as the mass of elemental lead is determined by ICP-MS (\( m_{\text{Pb}} \)). Taking into account that the molar stoichiometry Pb:S is assumed to be 1:1 in the NP, the mass of elemental sulfur \( m_s \) in solution can be calculated as:

\[ m_s = (m_{\text{Pb}} \cdot M_s)/M_{\text{Pb}} \]

Hereby \( M_s \) and \( M_{\text{Pb}} \) are the atomic weight of sulfur and lead, respectively. Thus the mass of NPs all NPs in solution is \( m_{\text{NP}} = m_s + m_{\text{Pb}} \) (neglecting the mass of the surface
coating). The lead sulfide density is \( \rho_{\text{PbS}} = 7.6 \, \text{g/cm}^3 \) and as indicated above, the total volume of NPs (\( V_{t,\text{NP}} \)) in solution can be calculated as:

\[
V_{t,\text{NP}} = \frac{m_{\text{NP}}}{\rho_{\text{PbS}}}
\]

Once, the \( V_{t,\text{NP}} \) and \( V_{\text{NP}} \) are known, the total number \( N_{\text{NP}} \) of NPs in solution can be calculated as:

\[
N_{\text{NP}} = \frac{V_{t,\text{NP}}}{V_{\text{NP}}}
\]

The molar concentration \( c_{\text{NP}} \) of PbS QDs thus can be calculated as:

\[
c_{\text{NP}} = \frac{N_{\text{NP}}}{N_A/V}
\]

Hereby \( N_A \) is the Avogadro constant.

### V.2.2. Determination of FePt NP concentration

To determine the concentration of FePt NPs, the elemental concentration of platinum obtained by ICP-MS measurements was converted to a NP concentration, considering an average NP diameter of \( d_c = 3.2 \, \text{nm} \) as obtained by TEM and taking into account the spherical shape of the NPs. In this way, the volume of one NP (\( V_{\text{NP}} \)) is:

\[
V_{\text{NP}} = \frac{4}{3}\pi\left(\frac{d_c}{2}\right)^3
\]

The mass of elemental lead in a NP solution of volume \( V \) was determined by ICP-MS (\( m_{\text{Pb}} \)). Taking into account that the molar stoichiometry Fe:Pt is assumed to be 1:1 in the NP, the mass of elemental platinum in solution can be calculated as:

\[
m_{\text{Fe}} = \frac{m_{\text{Pt}} \cdot M_{\text{Fe}}}{M_{\text{Pt}}}
\]

Hereby \( M_{\text{Fe}} \) and \( M_{\text{Pt}} \) are the atomic weight of iron and platinum, respectively. Thus, the mass of NP cores in solution is

\[
m_{\text{NP}} = m_{\text{Pt}} + m_{\text{Fe}}
\]

Using the iron platinium density of \( \rho_{\text{FePt}} = 14 \, \text{g/cm}^3 \) and the mass of NPs in solution, the total volume of NPs (\( V_{t,\text{NP}} \)) in solution can be calculated as:

\[
V_{t,\text{NP}} = \frac{m_{\text{NP}}}{\rho_{\text{FePt}}}
\]

Knowing \( V_{t,\text{NP}} \) and \( V_{\text{NP}} \), the total number \( N_{\text{NP}} \) of NPs in solution can be calculated as:

\[
N_{\text{NP}} = \frac{V_{t,\text{NP}}}{V_{\text{NP}}}
\]
The molar concentration $c_{NP}$ of FePt NPs thus is calculated as:

$$c_{NP} = \frac{N_{NP}}{N_A/V}$$

Hereby $N_A$ is the Avogadro constant.

**V.2.3. Determination of Au NP concentration**

To determine the concentration $c_{NP}$ of Au NPs, the elemental concentration of gold $m_{Au}/V$ of an NP solution with volume $V$, as obtained by ICP-MS measurements was converted to a NP concentration, considering an average particle diameter of $d_c = 4.4$ nm as obtained by TEM and taking into account the spherical shape of the NPs.

In this way, the volume of one NP ($V_{NP}$) is:

$$V_{NP} = \frac{4}{3}\pi\left(\frac{d_c}{2}\right)^3$$

The atomic gold density is $\rho_{Au} = 19.3$ g/cm$^3$. The mass $m_{Au}$ of the NPs is equal to the mass $m_{Au}$ of elemental Au, which was determined by ICP-MS ($m_{AuNP}$). The total volume of NPs ($V_{t,NP}$) in solution can be calculated as:

$$V_{t,NP} = \frac{m_{NP}}{\rho_{Au}}$$

The total number $N_{NP}$ of NPs in solution therefore can be calculated as:

$$N_{NP} = \frac{V_{t,NP}}{V_{NP}}$$

The molar concentration $c_{NP}$ of Au NPs then is

$$c_{NP} = \frac{N_{NP}}{N_A/V}.$$

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VI. References


