Fast and reproducible method to quantify magnetic nanoparticle biodistribution

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Electronic supplementary information

Experimental protocols of calibrations curves

Prussian Blue (PB) calibration curves: PB calibrations were prepared by dissolving $FeCl_3$, 6 H_2O solutions at 0, 0.5, 1, 2, 4, 6, 8, 10, 20 and 30 µg of iron / mL. 200 µL of each solution were diluted in 200 µL HCl 6M solution. Then 25 µL of these diluted solutions were distributed 3 times in well of 96 wells plate. 25 µL of HCl 6M and 50 µL of 5% ferrocyanide solution were then added in each well. The 96 wells plate was then shaking 15 minutes at room temperature and sonicated for 1 minute. The absorbance at 690 nm was then measured and a graph of absorbance (in arbitrary units: a.u.) as a function of iron concentration (μg_{Fe}/mL) was plotted (Fig. 5). The calibrations curves of PB measurements were done the same day of samples measurements.

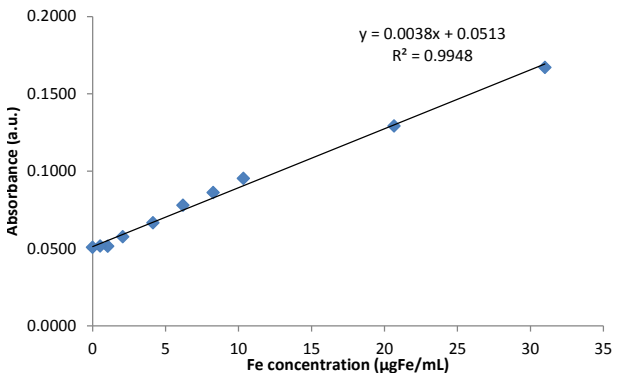

Fig. 1: Absorbance values (in arbitrary units: a.u.) measured in Prussian blue as a function of iron concentration (in μg_{Fe}/mL).

For ICP calibrations, standard iron (Fe^{3+}) solution was diluted in ultra-pure water to solutions at 0, 0.2, 0.5, 1, 2, 5, 10 and 20 ppm (μg_{Fe}/mL) and measured 3 times in ICP (Fig. 6).

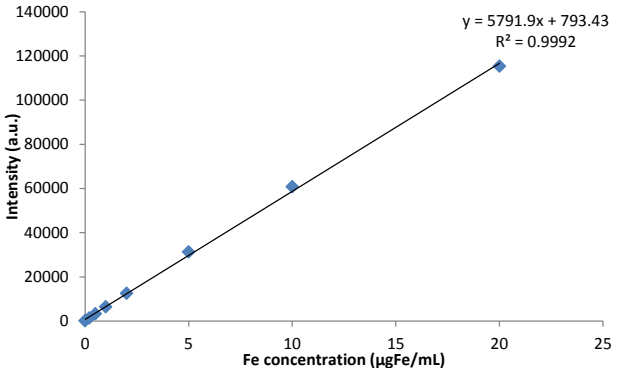

Fig. 2: Intensity values (in arbitrary units: a.u.) measured in ICP as a function of iron concentration (in μg_{Fe}/mL).

For MSM calibrations, in order to understand the coating and media effects on magnetic response, 3 different calibrations curves were done. First, naked-SPION suspensions with a known iron concentrations were diluted in HNO_3 10 mM to obtain suspensions at 0, 10, 20, 40, 80, 150, 300 and 600 μg_{Fe}/mL. Then PVA-SPION suspensions were diluted in HNO_3 10 mM and FBS at 0, 26.5, 53, 105, 210 and 425 μg_{Fe}/mL. 850 µL of each suspension were dropped in the 1 mL MS2G cells and their magnetic susceptibilities were measured in the MS2G sensor against 850 µL of control liquids which were respectively HNO_3 10 mM for Naked-SPION and HNO_3 10 mM and FBS for PVA-SPION. The Magnetic susceptibilities (SI) as a function of iron mass (μg_{Fe}) were plotted.

For organs SPION titrations, PB and ICP calibrations curves for SPION suspensions were used. For MSM calibration curves, specific volumes of PVA-SPION were added to a known mass of dry powder of a control liver and a control spleen stored in a 10 mL cell of MS2B sensor. Because the numbers of rat organs were not sufficient to prepare several calibrations standards, after a volume of PVA-SPION was added on the organ powder, the MS2B cell was measured against a MS2B cell with the same amount of control organ (dry powder for liver or dry for spleen). Then another volume of PVA-SPION was added again to the cell. These operations were repeated for 0, 10, 25, 50, 75, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 µL of PVA-SPION which gave final mass of iron in liver powder respectively at 0, 0.06, 0.15, 0.3, 0.45, 0.6, 1.2, 1.8, 2.4, 3, 3.6, 4.2, 4.8, 5.4 and 6 mg_{Fe}. These operations were repeated for 0, 10, 20, 30, 40, and 50 µL of PVA-SPION which gave final mass of iron in spleen respectively at 0, 0.06, 0.12, 0.18, 0.24, and 0.3 mg_{Fe}.

To compare the dry organs response to SPION in suspensions; 0, 0.2, 0.4, 0.6, 0.8, 1.3, 1.7, 2.1, 2.5, 3.3, 4.2 and 5 mg of iron were diluted in 10 mL of HNO_3 10mM. The Magnetic susceptibilities (SI) as a function of iron mass (mg_{Fe}) were plotted.

Samples preparation

For PB and ICP measurements, the samples were prepared in the same conditions as calibration curves. 80 µL of naked-SPION, PVA-SPION and sera injected with PVA-SPION were dissolved in 920 µL of HCl 6M overnight at room temperature. Then the solutions were diluted 6 times in distilled water before further analyses. Around 200 mg of liver and 100 to 200 mg of spleen were dissolved in respectively 2 and 1 mL of Aqua regia overnight at room temperature. Then the solutions were filtered at

0.45 µm with cellulose syringe filter and diluted 12 times in distilled water before further analyses.

For magnetic susceptibility measurements, the SPION suspensions and dry organs were analyzed as such.

Experimental protocols of samples measurements

For PB measurements, 3 times 25 µL of the solutions of dissolved SPION suspensions or organs were dropped in wells of 96 wells plate. 25 µL of HCl 6M and 50 µL of 5% ferrocyanide solution were added in each well. The absorbance of each well was measured at 690 nm. The average of the 3 absorbance measurements gave, using the appropriate PB standard curve, the concentration of iron ($[PB]_{dill}$: μg_{Fe}/mL) for the diluted SPION suspensions or the mass of iron (m^{dill}_{PB} : mg_{Fe}) for the diluted organs solutions.The concentration of whole iron in the suspensions of SPION was obtained by the multiplication of $[PB]_{dill}$ by the dilution factor 6. The concentration of whole iron in the organs ($PB\_m^{organ}_{Fe}$) was obtained by multiplication of m^{dill}_{PB} by the dilution factor 12, by the volume of aqua regia used to dissolve them (2 mL for the livers and 1 mL for the spleen: V^{diss}_{PB} in mL) and by the ratio total mass of organ / mass organ dissolved (R_{PB}).

$$PB\_m_{Fe}^{organ} = m_{PB}^{dill} * 12 * V_{PB}^{diss} * R_{PB}$$

For ICP measurements, 3 times 1 mL of the solutions of dissolved SPION suspensions or organs were analyzed 3 times each in ICP. The results given are the concentration of iron ($[ICP]_{dill}$: μg_{Fe}/mL) for the diluted SPION suspensions and the mass of iron (m^{dill}_{ICP}: mg_{Fe}) for the diluted organs solutions. The concentration of whole iron in the suspensions of SPION was obtained by multiplication of $[ICP]_{dill}$ by the dilution factor 6. The concentration of whole iron in the organs ($ICP\_m^{organ}_{Fe}$) was obtained by multiplication of m^{dill}_{ICP} by the dilution factor 12, by the volume of aqua regia used to dissolve them (2 mL for the livers and 1 mL for the spleen: V^{diss}_{ICP} in mL) and by the ratio total mass of organ / mass organ dissolved (R_{ICP}).

$$ICP\_m_{Fe}^{organ} = m_{ICP}^{dill} * 12 * V_{ICP}^{diss} * R_{ICP}$$

For MSM measurements, 0.85 mL of SPION suspensions without any further modification were put in the 1 mL MS2G cell and measured against the liquid used for the dilution of the SPION (HNO_3 10mM for naked-SPION and PVA-SPION and FBS for PVA-SPION). For the organs measurements, a measured mass of powder of liver or spleen was added in the 10 mL MS2B cell and measured against a control dry liver or spleen in a 10 mL MS2B cell.

Their magnetic susceptibilities (in SI) were measured 3 times for 1 second. By using the appropriate magnetic susceptibility standard curve, the average of the 3 results gave the concentration of iron per volume of measurement (μg_{Fe}/mL) for the SPION suspensions and the mass of iron ($m^{partial}_{MSM}$: mg_{Fe}) in the mass of organ analyzed. The whole iron concentration of the organs ($MSM\_m^{organ}_{Fe}$) was obtained by multiplication of $m^{partial}_{MSM}$ by the ratio total mass of organ / partial mass of organ analyzed (R_{MSM}).

$$MSM\_m_{Fe}^{organ} = m_{MSM}^{partial} * R_{MSM}$$

The averages of the 3 iron concentrations in suspension were plotted for PB, ICP and MSM measurements with standard deviation errors. For organs iron content, the 9 results were shown separately because of the incertitude of *in vivo* biodistribution with standard deviation for the 3 measurements per sample.