Non destructive and fast method for the detection of superparamagnetic iron oxide nanoparticles (SPION) biodistribution based on their magnetic properties

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Introduction

Superparamagnetic iron oxide nanoparticles (SPION) have become important for various *in vivo* and *in vitro* biomedical applications such as imaging, magnetic separation, biosensor devices and therapy.

Nowadays, several analytical techniques to detect SPION *in vivo* or *in vitro* exist. Magnetic resonance imaging (MRI) or magnetic resonance relaxometric methods can be used to detect magnetic particles, although MRI equipment is not available in many labs for routine use. The most widely used are chemical methods such as Induced Coupled Plasma (**ICP**) techniques or colorimetric assays by UV/VIS like Prussian Blue (**PB**). By dissolving the SPION into Fe^{III}, quantitative analyses are performed in comparison to iron calibration curves.
However, even if they are commonly used, these methods could be destructive and not selective because they will titrate the total iron species of the samples.

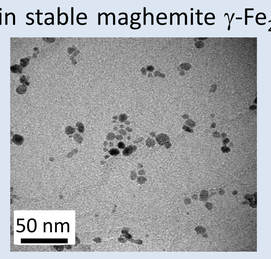
We propose an easier, faster and nondestructive method to detect SPION. We use their magnetic properties to measure their magnetic susceptibilities (**Mag S**). With adapted calibrations curves we were able to quantify iron from SPION in biological samples like sera and organs.

SPION preparation

Naked SPION

Iron oxide nanoparticles (SPION) are synthesized by co-precipitation method from mixed solution of $FeCl_2$ and $FeCl_3$ (molar ratio [1:2]) upon the addition of a base and resulting, after controlled oxidation step, in stable maghemite γ-Fe_2O_3 particles.[1]

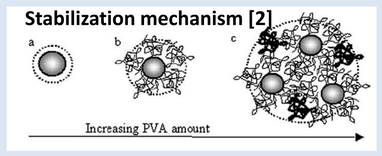
Stable acidic suspension of SPION (**10 mg_{Fe}/mL**)

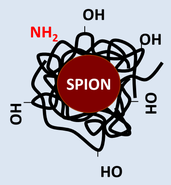


Properties of naked SPION [1]
Crystallites ≈ 9 nm
Aggregates ≈ 25 nm
Zeta Potential at pH 7 ≈ 0mV

Polymer coated SPION

SPION are coated with PVA to prevent their aggregation and sedimentation in high ionic strength medium [2]





PVA coated SPION
PVA-SPION
Zeta Potential at pH 7 ≈ +30 mV

Biological materials

Rats sera

Sera were purified from rats blood.
6 sera from male rats and 6 sera from female rats were taken.
For each sex, 2 samples were kept as control, 2 were incubated with 50 μg_{Fe}/mL of PVA-SPION and the 2 last one were incubated with 100 μg_{Fe}/mL of PVA-SPION.
Volume of sample: around 1 mL.

Rats organs

Female rats were injected either with NaCl solution at 0.15 M (4 control rats) or with 7 mg_{Fe} PVA-SPION (5 rats).
15 minutes after injections, the rats were sacrificed and organs were collected.
For this study, results were focused on livers (6 to 8 g) and spleens (0.35 to 0.55g).

Measurements methods

Prussian Blue (PB)

Solution analysis: **iron content** from dissolved SPION
UV analysis: Tecan plate reader compared to $FeCl_3$
$Fe^{III} + K_4Fe^{II}(CN)_6$, 3 H_2O → $[Fe^{III}_4(Fe^{II}(CN)_6]_3$
calibration: more Blue → more Iron in organs
Volume of analysis: 3 times 100 µL in acidic solution at 1.5 M

Induced Coupled Plasma Spectroscopy (ICP)

Solution analysis: **iron content** from dissolved SPION
Flame spectroscopy analysis of iron: ICP OES
Volume of analysis: 3 mL in acidic solution < 1M

Magnetic Susceptibility (Mag S)

Analysis: **SPION content** from suspension or organ powder
Magnetic susceptibility is given in 10^{-5} SI units
Magneto-suceptometers from Bartington®
2 cells of analysis: 0.85 and 10 mL

Samples preparations

Sera

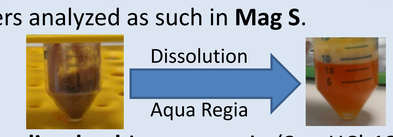
Serum suspensions were used as such for **Mag S** analyses.
Dissolution for **PB** and **ICP** (1 vol. of serum + 1 vol. of HCl 6M).
Solutions were **diluted** 4 times before **PB** analyses.
Solutions were **diluted** 6 times before **ICP** analyses.
Minimum volume of analysis: 0.1 mL for **PB**, 0.5 mL for **ICP** and from 0.25 to 0.85 mL for **Mag S**.

Sera samples preparation

	PB analyses	ICP analyses	Mag S analyses
Dissolution in HCl 6M?	Yes	Yes	No
Min. dilution	4 X	6 X	0 X
Min. vol. (mL)	0.1	0.5	0.85
Can it be re-used?	No ✘	No ✘	Yes ✓

Organs

Organs (livers and spleens) were freeze-dried at -50°C, 0.1 mbar for 24 hours and grounded to powder (to help dissolution step):
Livers (1.7 to 2.5g) ; Spleens (0.07 to 0.16 g)
Powders analyzed as such in **Mag S**.



Powder **dissolved** in aqua regia (3 v. HCl 12M : 1 v. HNO_3 15M), **diluted** 12 times and **filtered** at 0.2 µm before **PB** and **ICP** analyses.
Minimum mass of analysis (liver): 0.5 g for all
Minimum mass of analysis (spleen): whole sample for all

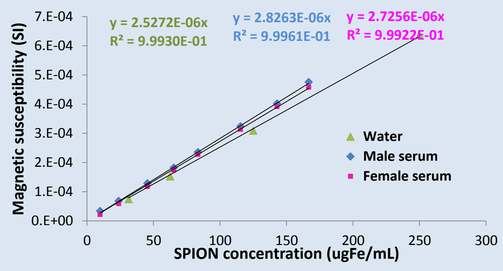
Organs samples preparation

	PB analyses	ICP analyses	Mag S analyses
Dissolution in Aqua Regia?	Yes	Yes	No
Min. dilution	12 X	12 X	0 X
Filtering?	Yes	Yes	No
Min. mass (g): liver	0.5	0.5	0.5
Min. mass (g): spleen	whole	whole	whole
Can it be re-used?	No ✘	No ✘	Yes ✓

Magnetic susceptibility calibrations

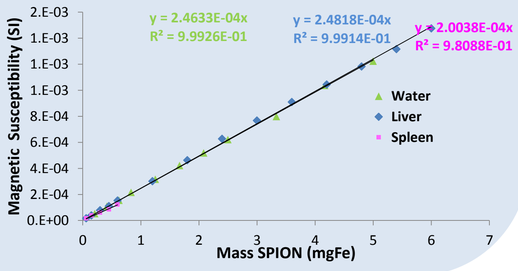
Sera

Water and sera of female and male rats (1 per sex) used as media spiked with controlled amount of SPION.
Mag S (in SI) were measured for each SPION amount with the 3 media.
Then the functions **Mag S** = f([Fe]) was plotted.
Conclusion: Linear model with $R^2 > 99.9\%$
No influence of the medium in measurements



Organs

Whole powder of control liver (around 2g) and control spleen (around 0.1g) were used as media.
Known amount of PVA-SPION were added and **Mag S** (in SI) was measured for each amount with the 2 powders and compared with water.
Then the functions **Mag S** = f(mass(Fe)) was plotted.
Conclusion: Linear model with $R^2 > 98\%$
No influence of the medium in measurements



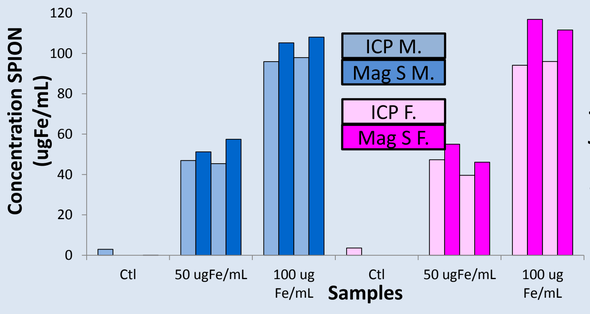
Quantification of SPION and bio-distribution study

Measurements

0.85 mL of serum were measured in **Mag S**.
→ SPION concentration (μg_{Fe}/mL) with the calibration curve.
These results were compared to **ICP** measurements.

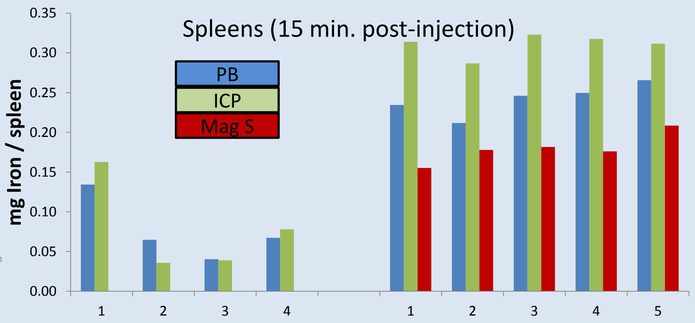
0.1 to 0.5 g of organs were measured in **Mag S**.
→ SPION mass in μg_{Fe} with the calibration curve.
The mass of SPION was corrected to the total mass of the organs.
These results were compared to **PB** and **ICP** measurements.

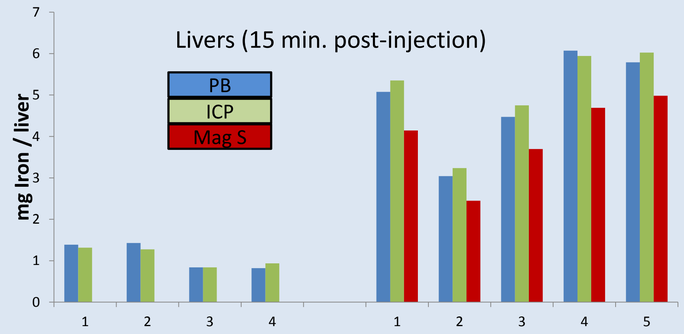
Sera



- **Mag S** and **ICP** gave same results in SPION detection, both close to the expected concentrations.
- No detection of residual iron content with **Mag S**.

Organs





- **Mag S** is able to detect SPION in the same conditions than **PB** and **ICP** (minimum detection around 0.1 mg of Fe).
- **PB** and **ICP** gave same results with an iron content not due to SPION. **Mag S** only detect the iron from SPION.
- **PB** and **ICP** seems to be too sensitive to the normal iron content in organs. **Mag S** more sensitive to low mass analyses (spleen).

Conclusion

In this work we proved that magnetic susceptibility measurements can be used to titrate magnetic materials, particularly SPION, in suspensions and biological media.
With this technique and compared to **PB** and **ICP** the samples are not destroyed and can so be saved for further analyses. Moreover, this **Mag S** measurement is more robust than **PB** or **ICP** to distinguish magnetic nanoparticle from tissue iron, which permit to decrease the number of control samples.
The magnetic susceptibility seems to be an ideal method requiring a minimal sample preparation, having high detection sensitivity and allowing to quantify low concentrations of particles with high reproducibility.

References and Acknowledgments

[1] Chastellain M. *et al.* Journal of colloid and interface science 278-2 (2004) 353-360; [2] Petri-Fink A. *et al.* European Journal of Pharmaceutics and Biopharmaceutics 68 (2008) 129–137

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