

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/359466616>

Characterization of commercial iron oxide clusters with high transverse relaxivity

Article · March 2022

DOI: 10.1016/j.jmro.2022.100054

CITATIONS

0

READS

41

7 authors, including:



Yves Gossuin

Université de Mons

76 PUBLICATIONS 2,274 CITATIONS

[SEE PROFILE](#)



Quoc Lam Vuong

Université de Mons

28 PUBLICATIONS 834 CITATIONS

[SEE PROFILE](#)



Jérôme Delroisse

Université de Mons

107 PUBLICATIONS 459 CITATIONS

[SEE PROFILE](#)



Sophie Laurent

Université de Mons

424 PUBLICATIONS 27,307 CITATIONS

[SEE PROFILE](#)

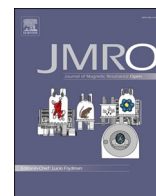
Some of the authors of this publication are also working on these related projects:



Gadolinium Nanohydrogels for Lymph Node Magnetic Resonance Imaging [View project](#)



MRI - Fluorinated Paramagnetic Contrast Agents [View project](#)



Research Article

Characterization of commercial iron oxide clusters with high transverse relaxivity

Yves Gossuin^{a,*}, Eléonore Martin^a, Quoc Lam Vuong^a, Jérôme Delroisse^b, Sophie Laurent^c, Dimitri Stanicki^c, Cédric Rousseau^a

^a Biomedical Physics Unit, UMONS, 25 avenue Maistriau, 7000 Mons, Belgium

^b Biology of Marine Organisms and Biomimetics Unit, Research Institute for Biosciences, UMONS, Place du Parc 23, 7000 Mons, Belgium.

^c Department of General, Organic and Biomedical Chemistry, NMR and Molecular Imaging Laboratory, UMONS, 19 Avenue Maistriau, 7000 Mons, Belgium

ARTICLE INFO

Keywords:

Iron oxide particles
Cluster
SPIO
Contrast agent
Relaxivity

ABSTRACT

Clusters of iron oxide particles are excellent contrast agents for molecular and cellular MRI because of their large effect on water proton transverse relaxation. Their efficiency depends on the magnetization of the particles, the size of the cluster and the intra-aggregate volume fraction occupied by the iron oxide particles inside the cluster. After optimization of these different parameters, a relaxivity of $\sim 750 \text{ s}^{-1}\text{mM}^{-1}$ can theoretically be achieved. PolymagTM clusters are initially intended for magnetofection but are here shown to present excellent relaxation properties. Magnetometry shows that the particles constituting the clusters have a high saturation magnetization ($M_v = 371000 \text{ A/m}$) and present a broad log-normal size distribution ($d_{0,c} = 4.9$ and $\sigma = 0.53$). The clusters have a hydrodynamic diameter of 180 nm and also present a large polydispersity index ($\text{PDI} = 0.15$). The transverse relaxivity of the clusters is remarkably high: $r_2 = 470 \text{ s}^{-1}\text{mM}^{-1}$ at 1.41 T and 37°C. As their relaxation properties are independent of temperature and interecho time, the clusters should be mainly in the Static Dephasing Regime (SDR), even if some large clusters are probably closer to the Partial Refocusing Model (PRM), as suggested by the large estimated $\Delta\omega\tau_D \sim 55$. Since they present a large magnetic moment, the PolymagTM clusters are sensitive to the magnetic field of the NMR device, in which they probably form linear chains: a reversible increase of the relaxation times is observed after the insertion in the NMR system. They could be good candidates for cellular imaging since they should be easily internalized in cells using the magnetofection protocol and then easily detected by MRI thanks to their high relaxivity.

Introduction

Magnetite Fe_3O_4 is a black ferrimagnetic iron oxide widespread in natural rocks. Maghemite $\gamma\text{-Fe}_2\text{O}_3$ is the oxidized form of magnetite and presents rather similar magnetic properties [1]. In the biomedical field, both compounds are often referred to as “iron oxide” even if there are many other members in the iron oxides family. Nanoparticles of magnetite/maghemite exhibit superparamagnetic behavior which means that their average magnetic moment is null in the absence of magnetic field [2, 3]. It also means that each particle, whose surface can be functionalized, behaves like a giant paramagnetic moment, which makes them attractive for biomedical applications [4,5]. Iron oxide nanoparticles have been used for 20 years, roughly from 1990 to 2010, as contrast agents for clinical Magnetic Resonance Imaging (MRI) especially for tumor imaging in liver, spleen and lymph nodes because

they are rapidly captured by the reticuloendothelial system [6]. Currently, iron oxide nanoparticles are still intensively used for molecular and cellular imaging in preclinical studies on small animals [7–9], but have almost disappeared from human clinical routine imaging, which is often performed without contrast agent or with good old gadolinium chelates. One reason for that is that the presence of gadolinium leads to an increase of MR signal while many iron oxide contrast agents cause a loss of signal [10]. Such a signal decrease can also be caused by artifacts, which complicates the interpretation of images obtained with iron oxide contrast agents. The contrast created by gadolinium complexes or magnetic particles is due to the change of water proton relaxation times T_1 and T_2 in the vicinity of the compound. Typically, large iron oxide particles – or clusters of small iron oxide cores – have a much larger effect on transverse relaxation (governed by T_2) than on longitudinal relaxation (governed by T_1). This is why they cause a loss of signal [11], contrarily to gadolinium chelates whose effects on T_1 and T_2

* Corresponding author,

E-mail address: yves.gossuin@umons.ac.be (Y. Gossuin).

<https://doi.org/10.1016/j.jmro.2022.100054>

Available online 24 March 2022

2666-4410/© 2022 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Abbreviations

NMR	Nuclear Magnetic Resonance
MRI	Magnetic Resonance Imaging
MAR	Motional Averaging Regime
SDR	Static Dephasing Regime
PRM	Partial Refocusing Model
PDI	Polydispersity Index
DLS	Dynamic Light Scattering
TEM	Transmission Electron Microscopy
NMRD	Nuclear Magnetic Relaxation Dispersion

are comparable. These large particles are often called SPIOs, for Superparamagnetic Particles of Iron Oxide. SPIOs have a hydrodynamic size larger than 50 nm, and are often constituted of several smaller iron oxide cores in a diamagnetic matrix [12]. The development of ultra-small iron oxide particles (USPIOs, with a size much smaller than 50 nm) has opened perspectives since their blood half-life is longer - which allows their use for angiography - and they can produce positive contrast in some conditions since the difference between T_1 and T_2 is smaller than for SPIOs [13–16]. Moreover, even if iron oxide nanoparticles are nowadays no more used in hospitals, the toxicity of gadolinium for patients with renal insufficiency, who sometimes develop nephrogenic systemic fibrosis [17], could maybe give them a new lease of life. For preclinical molecular and cellular imaging, SPIOs are still used - even if they create negative contrast - because they have two unquestionable advantages: first, each nanoparticle reaching the molecular target or internalized in a cell bears thousands of iron ions and second, the effect of SPIOs on T_2 can be huge - far larger than what can be achieved with gadolinium - if the compound is optimized. The efficiency of a contrast agent to quicken relaxation, and thus shorten T_1 and T_2 , is quantified by its longitudinal and transverse relaxivities r_1 and r_2 . The relaxivity r_1 is the increase in the relaxation rate $R_1 = 1/T_1$ brought by an increase of 1 mM in the magnetic ion concentration (Fe^{3+} and Fe^{2+} in this case):

$$\frac{1}{T_1} = r_1[\text{Fe}] + \frac{1}{T_1^{\text{dia}}} \quad (1)$$

Where $[\text{Fe}]$ is the iron concentration and $1/T_1^{\text{dia}}$ is the relaxation rate of the diamagnetic solvent, water in the case of an aqueous suspension of particles. Relaxivities are indeed often measured in aqueous solutions of the iron oxide particles, even if their internalization in cells is known to affect their relaxation efficiency [18,19]. However, the value of relaxivity in water is a good first indication and it allows to compare the efficiency of different compounds. SPIOs with high r_2 are valuable: the higher the relaxivity, the lower the quantity of contrast agent to be used. But to achieve this optimization of relaxivity, the mechanism of water proton relaxation induced by magnetic particles first had to be thoroughly understood. In very brief, the time-modulated dipolar interaction between protons magnetic moment and particles magnetic moment is responsible for the relaxation. Even if a complete description of the relaxation models is beyond the scope of this paper and can be found elsewhere [20–24], some basic principles will be useful for the understanding of what follows, especially concerning the influence on relaxation of the particle size and saturation magnetization. There are three relaxation regimes with quite different characteristics. All of them provide a prediction of the transverse relaxation rate at high field, called the secular term. To know which one must be used, one must calculate the proton Larmor frequency shift at the equator of the particle, $\Delta\omega$, and the diffusion correlation time, τ_D :

$$\Delta\omega = \frac{\mu_0 \gamma M_v}{3} \quad (2)$$

and

$$\tau_D = \frac{R^2}{D} \quad (3)$$

μ_0 is the vacuum magnetic permeability, γ is the proton gyromagnetic ratio, M_v is the saturation magnetization of the particles expressed in A/m, R is the radius of the particle and D is the water diffusion coefficient. The choice of the model depends on the value of the product $\Delta\omega\tau_D$:

- If $\Delta\omega\tau_D \ll 1$, the relaxation can be described by the Motional Averaging Regime (MAR). The transverse relaxivity of the particles at high magnetic field is given by:

$$r_2^{\text{MAR}} = \frac{16 \gamma^2 \mu_0^2 v_{\text{mat}} M_v^2 R^2}{405 D} \quad (4)$$

- In this equation, v_{mat} allows to link f , the sample volume fraction occupied by particles - which is naturally appearing in the theory - to $[\text{Fe}]$ which is actually experimentally measured. Logically, f and $[\text{Fe}]$ are proportional and for magnetite $v_{\text{mat}} = f / [\text{Fe}] \approx 1.5 \times 10^{-5} \text{ m}^3 \cdot \text{mol}^{-1}$. The relaxation of small particles iron oxide particles, like USPIOs, is well described by the MAR.
- When $\Delta\omega\tau_D > 1$ the system moves toward the static dephasing regime (SDR) [25]. This model is in fact only valid for completely static protons and without the use of refocusing pulses for the measurement of T_2 , but it remains a good approximation even when using echoes if $5 < \Delta\omega\tau_D < 20$. This condition was established thanks to Monte Carlo relaxation simulations using an interecho time 2τ of 1 ms[26].

$$r_2^{\text{SDR}} \approx \frac{2\pi \gamma \mu_0 v_{\text{mat}} M_v}{9\sqrt{3}} \quad (5)$$

For this range of $\Delta\omega\tau_D$ values, the 180° refocusing pulses are not efficient and the echo time has almost no influence on transverse relaxation.

- Finally, when $\Delta\omega\tau_D \gg 20$, the Partial Refocusing Model (PRM) must be used. It takes the effect of the 180° refocusing pulses of the CPMG sequence into account. Those pulses decrease the efficiency of the particles since they decrease R_2 . In this model, the relaxation rate is no more proportional to f and $[\text{Fe}]$, which means that the relaxivity cannot be properly defined. For a given f , the transverse relaxation rate is given by:

$$R_2 = \frac{1}{T_2} = 2.25 f \frac{x^{1/3}}{\tau_D} (1.34 + fx)^{5/3} \quad \text{with} \quad x = \sqrt{\frac{4}{5}} (\Delta\omega) \tau \quad (6)$$

In this regime, an effect of the interecho time 2τ on transverse relaxation is expected (Eq. 6).

From a practical point of view, when considering single particles presenting a given saturation magnetization M_v , the maximum transverse relaxivity achievable is obtained when the particles are in the SDR, which means when $5 < \Delta\omega\tau_D < 20$. There is thus an optimal range of sizes that to fulfill this condition - through the τ_D value that is proportional to R^2 - and the maximum relaxivity, r_2^{max} , is thus given by Eq. 5. It is worth noting that this value is a real upper limit for r_2 except if other unknown relaxation mechanisms contribute, which has never been proven. For $M_v = 350000 \text{ A/m}$, the optimum size for single crystals is 55 nm and $r_2^{\text{max}} = 750 \text{ s}^{-1} \text{ mM}^{-1}$. Producing stable suspensions of such large monocrystals is really challenging, but highly efficient single particles have already been produced [27]. An alternative and easier

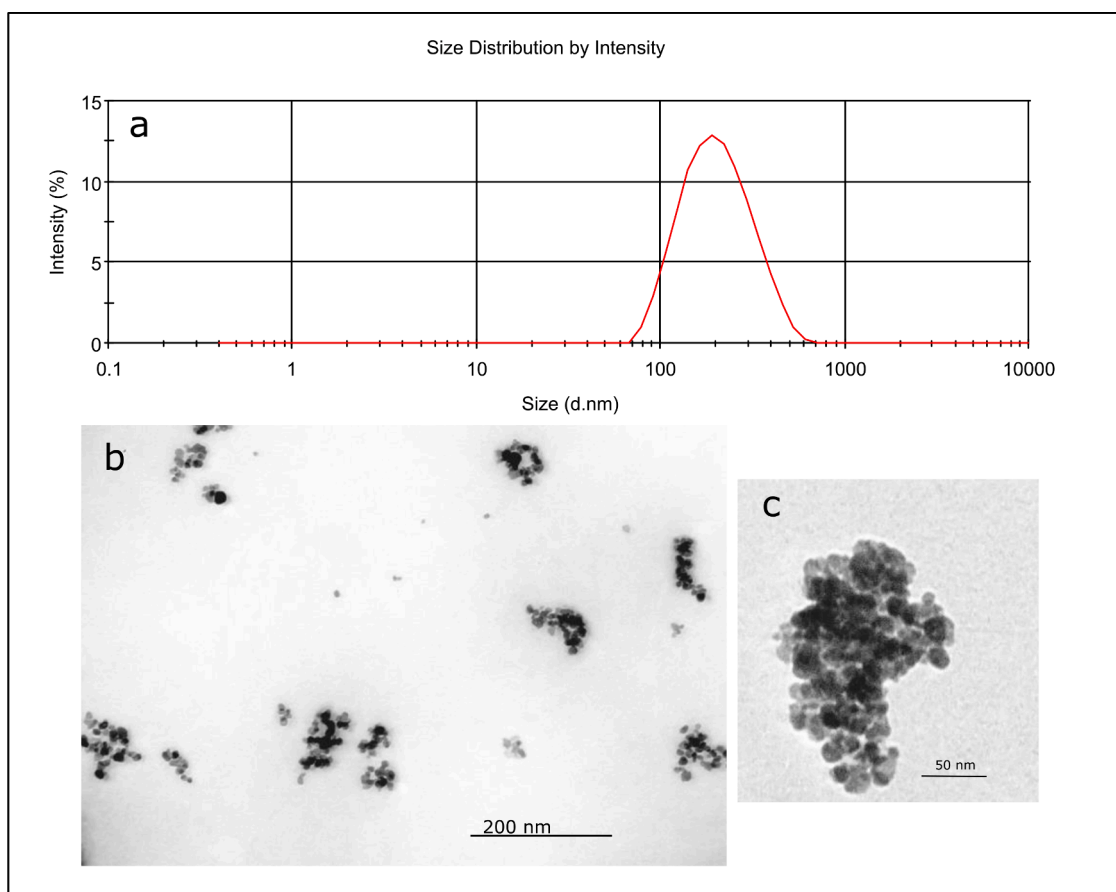


Figure 1. a) hydrodynamic size distribution obtained by DLS, b) TEM picture of several clusters, c) High magnification TEM image of a single cluster.

way of achieving large r_2 values is to produce clusters of iron oxide crystals, as first shown by computer simulations [26,28] and then achieved with real clusters [24,29–32]. If the magnetization of the iron cores contained in the clusters is also 350000 A/m, again $r_2^{\max} = 750 \text{ s}^{-1} \text{ mM}^{-1}$, but it will be reached for larger cluster sizes, depending on the intra-aggregate volume fraction ϕ_{intra} . This is the fraction of the whole volume cluster that is occupied by the magnetic materials, iron oxide crystals in our case. For $\phi_{\text{intra}} = 0.2$, the optimum size for the cluster is about 120 nm, independently of the iron oxide core size.

In this work, the morphological, magnetic and NMR properties of commercial Polymag™ clusters of iron oxide particles are studied. Interestingly those clusters are not initially intended to be used as MRI contrast agents but for magnetofection[33–35] – i.e. the introduction of nucleic acids inside cells using magnetic particles and a magnetic plate creating magnetic field gradients. They could thus be a good candidate of contrast agent for cellular imaging since they could be easily internalized in cells following the transfection protocol and then detected by MRI.

Materials and methods

Sample

Polymag™ particles (500 μl - lot PN160-317P) were purchased from OZ Biosciences (France). The particles are in fact clusters constituted of several magnetite nanocrystals and stabilized by polyethylene imine (PEI) polymers [36]. The iron concentration of the original sample was 2.4 mM as determined by atomic emission spectroscopy after microwave digestion of the sample. For the NMR measurements, six samples with different iron concentrations were prepared with deionized water. For the magnetic measurements, 200 μl of the original sample was

lyophilized and redispersed in 50 μl of water in order to increase the concentration by a factor of 4.

Dynamic light scattering (DLS)

DLS measurements were performed on a Malvern NanoZS apparatus operating at a 173° scattering angle, using a sample diluted with ultrapure water. The collective diffusion coefficient was determined from the second-order autocorrelation function of the scattered light. From the value of the coefficient, the hydrodynamic diameter of the clusters was calculated according to the Stokes-Einstein relation. The autocorrelation functions were interpreted using the 2nd order cumulants (Z-average diameter and polydispersity index) and the multimodal fit provided by the instrument software.

Transmission electron microscopy (TEM)

A 5 μl drop of diluted solution was deposited on a formvar coated copper grid. After drying, the grid was observed with a Zeiss Leo 906E transmission electron microscope.

Magnetic measurements

A mini high field system from Cryogenic Ltd (London, UK) with the Vibrating Sample Magnetometer (VSM) option was used for the magnetic measurements. The maximum field is 5 T and the temperature range is 2 K - 315 K. The magnetization versus magnetic field (M-B) experiments were carried out with 50 μl of liquid sample in order to be in the same conditions as the NMR experiments. In the liquid, the particles are free to rotate which ensures superparamagnetism even for larger particles. This allows the use of a Langevin function function weighted

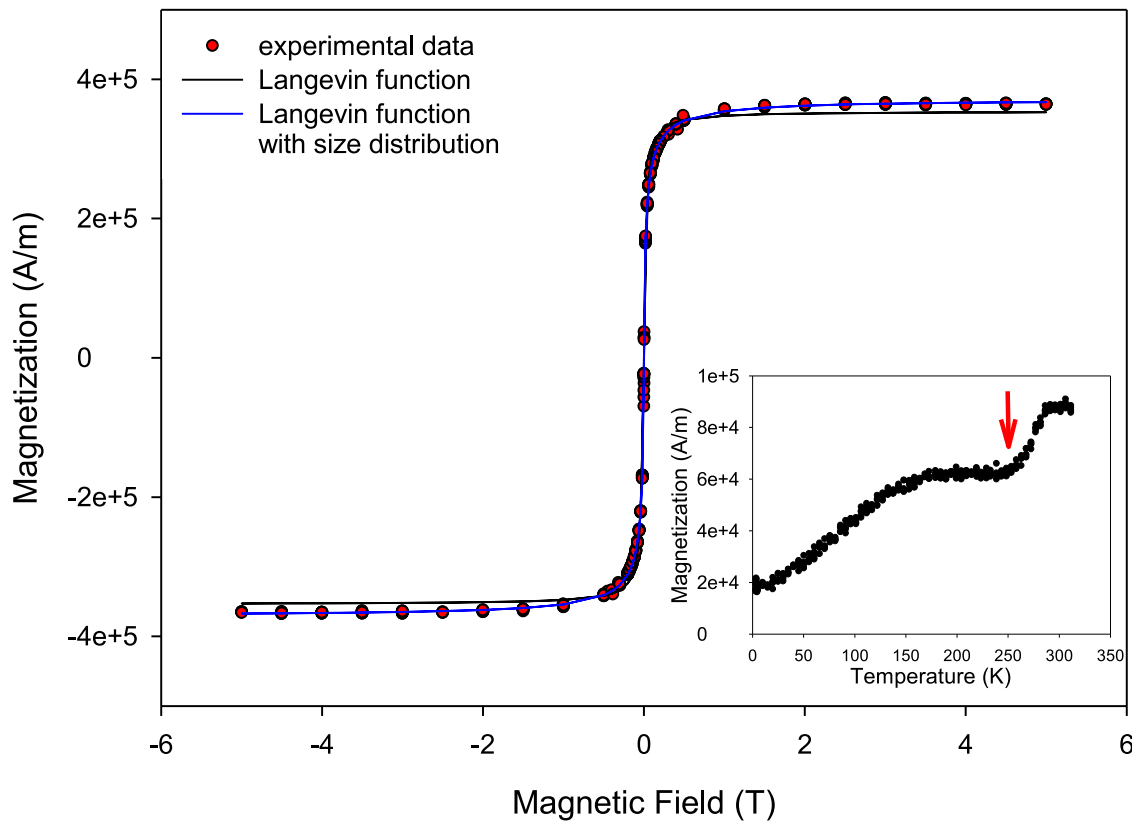


Figure 2. Effect of the field on the magnetization of the Polymag™ clusters. The data were fitted by a single Langevin function (black line) and by a Langevin function weighted by a log-normal core size distribution (blue line). The inset shows the ZFC curve of the clusters.

by a size distribution for the fitting of the M-B curves. The crystal diameter distribution was assumed to be lognormal with the following probability density:

$$p(d_c) = \frac{1}{\sqrt{2\pi}\sigma d_c} \exp\left(-\frac{\ln^2(d_c/d_{0,c})}{2\sigma^2}\right) \quad (7)$$

Where d_c is the diameter of the crystals, assumed to be spherical, $d_{0,c}$ is the median diameter and σ the variance of the distribution of logarithms.

For the Zero Field Cooling (ZFC) experiment, the sample in liquid form was cooled down from room temperature to 2K under zero magnetic field. At 2K the field was set to 5.9 mT and the magnetic moment of the sample was measured during the gradual heating up of the sample.

NMR relaxometry

Relaxation time measurements were performed on a Spintrack relaxometer (NMR Design, Germany) and a mq 60 (Bruker, Germany) instrument operating respectively at magnetic fields (B) of respectively 0.68 and 1.41 T. T_1 was measured with a saturation recovery sequence at 0.68 T and an inversion recovery sequence at 1.41 T. T_2 was measured with a CPMG sequence using an interecho time 2τ of 1 ms. A recycle delay of $5T_1$ was used for all the experiments which ensures a complete return to equilibrium between the measurements. T_1 Nuclear Magnetic Relaxation Dispersion (NMRD) profiles were recorded at 25°C and 37°C from 0.025 to 30 MHz on a Spinmaster fast field cycling relaxometer (STELAR, Mede, Italy). In the NMRD profile, the magnetic field is expressed in term of proton Larmor frequency: a field of 1 Tesla corresponds to a Larmor frequency of 42.6 MHz.

Results and discussion

Dynamic light scattering and electron microscopy

Fig. 1a shows the hydrodynamic size distribution (intensity weighted) obtained by DLS. The cumulants analysis provided a Z-average diameter $d_h = 180$ nm with a polydispersity index (PDI) of 0.15. This is in rather good agreement with the size of 230 nm previously reported[36]. Transmission electron microscopy confirms that the particles are indeed clusters of small iron cores, as shown in Fig. 1b. However, there seems to be a broad range of cluster sizes which is in agreement with the large PDI obtained by DLS. A typical cluster is shown in Fig. 1c. Concerning the iron cores constituting the clusters, they also present a broad crystal size distribution: cores with sizes from roughly 4 nm to 15 nm can be observed in the clusters. A more quantitative determination of core size distribution is impossible to achieve by TEM because of the overlap of the crystals in the clusters.

Magnetometry

The M-B curve of the Polymag™ clusters at 298 K is shown in Fig. 2. The data were first fitted with a single Langevin function corresponding to a single crystal size, providing a magnetization $M_v = 354000 \pm 1000$ A/m and a crystal diameter $d = 10.9 \pm 0.1$ nm. However, this first fitting is not satisfactory as shown in Fig. 2. A Langevin function weighted by a lognormal crystal size distribution was therefore used and the following parameters were obtained: $M_v = 370800 \pm 600$ A/m, median crystal diameter $d_{0,c} = 4.9 \pm 0.13$ nm and $\sigma = 0.53 \pm 0.01$. The M_v value is in good agreement with the saturation magnetization usually reported in the literature for magnetite/maghemite particles. The large σ value of the lognormal distribution confirms the broad range of crystal sizes observed in TEM images.

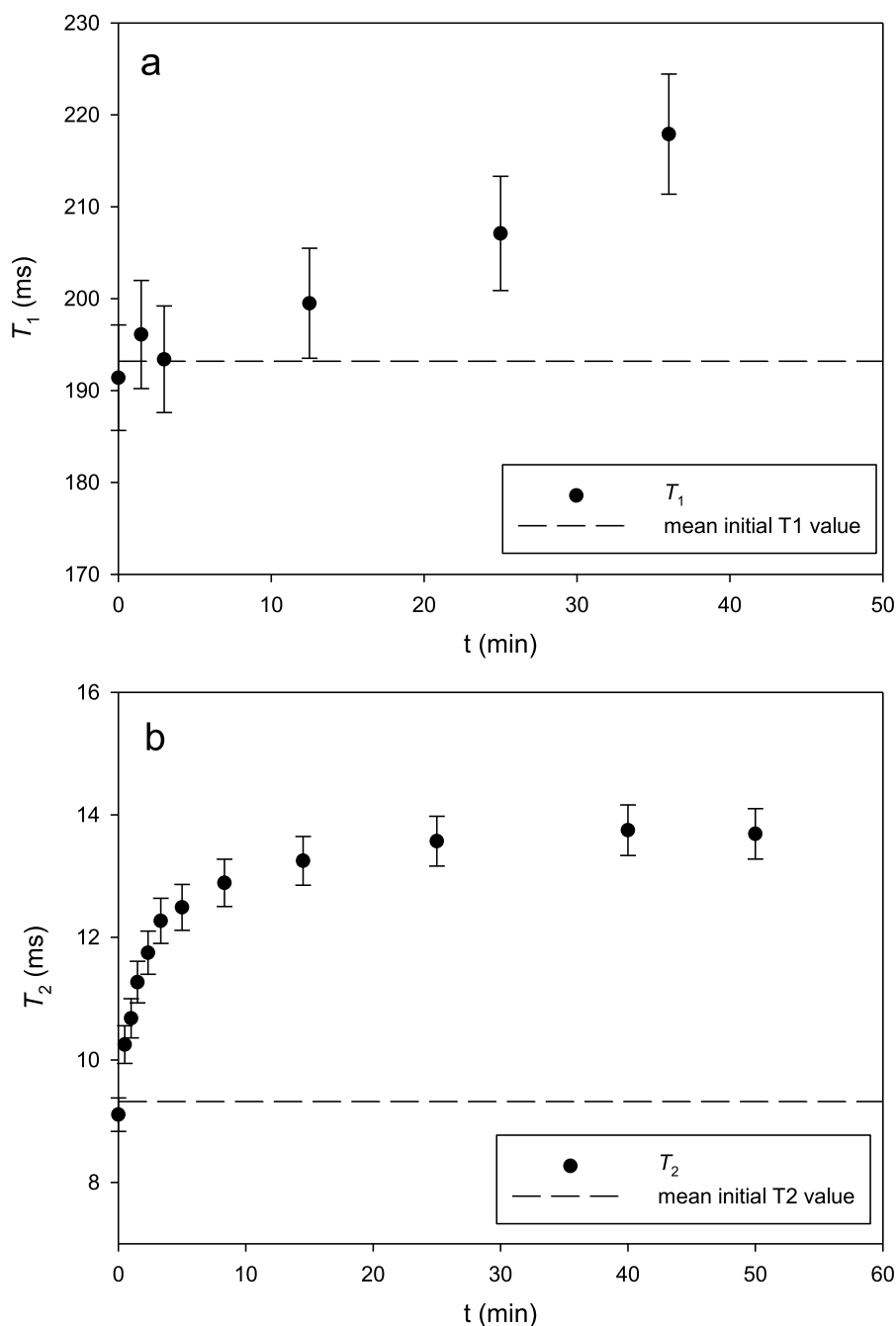


Figure 3. Evolution of T_1 (a) and T_2 (b) with time after vortexing and insertion in the magnetic field at 0.68 T and 25°C.

The ZFC curve of the Polymag™ clusters is shown in the inset of Fig. 2. The broad peak around 200 K is typical of either large crystals or dense clusters of smaller crystals. The sudden increase (marked by a red arrow) observed at high temperatures is due to the melting of the frozen aqueous sample. It clearly shows that, even at high temperature (~ 270 K), some clusters are still blocked because their Néel relaxation time is too long when compared to the characteristic experimental time (~ 1 s for a VSM). When the solution melts, the relaxation of the particles magnetic moment can occur through Brown relaxation: those clusters which were still blocked align themselves with the field thanks to the rotation of the cluster.

Relaxometry

Before the NMR measurements, the tube containing the solution was

shaken at 300 rpm for 5 s with a vortexer and then placed in the field of the magnet. Fig. 3 shows the evolution of T_1 and T_2 with time after the introduction in the field. Both relaxation times increase with time, but the effect is clearly more pronounced for T_2 , which increases by 50 % in one hour while T_1 only rises by 15 %. This effect is probably due to a reversible aggregation of the clusters within the field. Indeed, when the sample is removed from the field and shaken again with the vortexer, T_2 and T_1 return to their mean initial value, which is depicted by the dashed red lines in Fig. 3a and 3b. These mean values were calculated with three measurements carried out just after vortexing.

To ensure the reproducibility of further measurements, it was decided to realize all the relaxometry experiments just after the insertion of the sample in the magnet, following the shaking with the vortexer. The relaxation rates at 0.68 T and 1.41 T of three solutions of different Polymag clusters concentrations are respectively presented in Fig. 4a

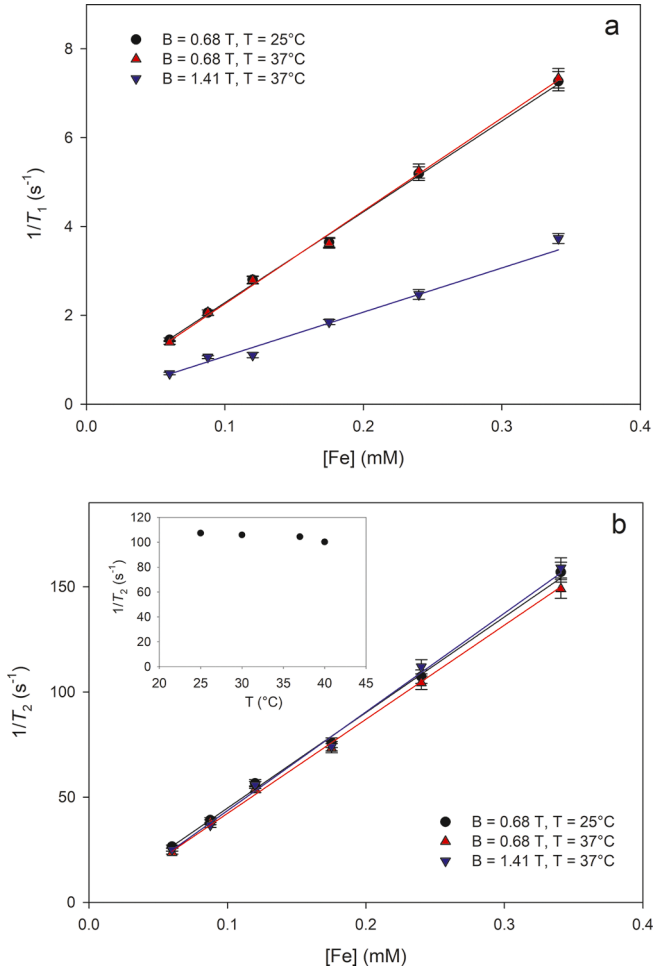


Figure 4. Evolution of $1/T_1$ (a) and $1/T_2$ (b) of aqueous solutions of the iron oxide Polymag™ clusters with the iron concentration, at 0.68 T (25°C and 37°C) and 1.41 T (37°C). The inset shows the effect of temperature on $1/T_2$ at 0.68 T for $[Fe] = 0.24$ mM.

Table 1

Relaxivities of the iron oxide Polymag™ clusters at 0.68 T and 1.41 T.

	r_1 ($s^{-1}mM^{-1}$)	r_2 ($s^{-1}mM^{-1}$)
0.68 T, 25°C	20.5 ± 0.6	454 ± 12
0.68 T, 37°C	20.9 ± 0.7	447 ± 13
1.41 T, 37°C	9 ± 0.9	469 ± 17

and 4b. As expected, there is a linear increase of the relaxation rate with the concentration, whose slope provides the corresponding relaxivity (Table 1). At 0.68 T, the transverse relaxivity is $r_2 \sim 450 s^{-1}mM^{-1}$ at 25 and 37°C and at 1.41 T and 37°C, $r_2 \sim 470 s^{-1}mM^{-1}$. The effect of the echo time τ on the transverse relaxation rate of the most concentrated solutions was also studied: T_2 only decreased by 4% when 2τ was increased from 0.5 ms to 2 ms.

The T_1 NMRD curve shows the typical shape usually observed for superparamagnetic particles (Fig. 5) with a marked decrease of $1/T_1$ at high fields, consistent with the change of r_1 observed between 0.68 and 1.41 T (Table 1). The r_2/r_1 ratio will thus continue to increase for increasing fields which amplifies the negative contrast effect. The curve was fitted with the theory of Roch et al [22], even though it is only valid for small iron oxide single particles rather than clusters. However, the parameters obtained thanks to this fitting (SI), namely a size and a magnetization, can bring interesting qualitative information about the system. The obtained saturation magnetization, $M_v^{relax} = 201000$ A/m, is

46% smaller than the magnetization of the crystals determined by magnetometry, and the diameter obtained by the fitting of the NMRD profile, $d_{relax} = 17.4$ nm, is clearly larger than the core diameter obtained by magnetometry. This is logical since from water protons point of view, relaxation is caused by large particles whose magnetization is diluted compared to the iron oxide core magnetization. Going further in the interpretation would be unwise, since the model of Roch is only valid in the MAR. Interestingly, the T_1 values of the NMRD profile, measured with the fast field cycling (FFC) method, showed no evolution with the time spent in the FFC device, contrarily to those measured with the benchtop NMR systems. During a FFC sequence, the field is only produced by the electromagnet during limited time intervals and it is turned off between the measurements, which is of course not the case with the permanent magnet of benchtop systems. This surely explains the stability of T_1 measured by FFC.

Comparison with theory and previous studies

As previously mentioned, the maximum relaxivity of iron oxide particles, given by Equation 4, corresponds to the SDR. For single crystals similar to those constituting the clusters (with $M_v = 371000$ A/m and thus $\Delta\omega = 4.16 \cdot 10^7$ Hz), SDR is reached for a size of about 50 nm, corresponding to $\Delta\omega\tau_D \sim 10$, and $r_2^{max} \approx r_2^{SDR} = 795 s^{-1}mM^{-1}$. Thapa et al [37] reported a transverse relaxivity of $791 s^{-1}mM^{-1}$ for single core truncated cubic iron oxide particles.

Once the crystals are structured in clusters, counter-intuitively, the maximum relaxivity is still $r_2^{max} \approx 795 s^{-1}mM^{-1}$ as shown in [24]. But it won't be reached for the same size: the condition to be fulfilled is now $\Delta\omega_{cluster}\tau_D^{cluster} \sim 10$ where $\Delta\omega_{cluster} = \phi_{intra}\Delta\omega$ is the frequency shift at equator of the cluster and $\tau_D^{cluster} = \frac{(R_{cluster})^2}{D}$ is the diffusion correlation time around the cluster. This allows to calculate the value of the cluster radius for which the maximum relaxivity is achieved:

$$R_{cluster}^{opt} = \sqrt{\frac{10D}{\phi_{intra}\Delta\omega}} \quad (8)$$

A previous study [36] reported the value of 0.6 g Fe/g dry weight for the iron content of Polymag™ clusters, measured by spectrophotometry. It corresponds to a magnetite content of 0.83 g Fe_3O_4 /g dry weight. Assuming that the density of magnetite is 5.1 and the PEI polymer density is 1, one obtains $\phi_{intra} = 0.49$. This is rather high, since the volume fraction of a dense cluster of spheres arranged in a cubic array in contact with each other is ~ 0.52 . Taking $\phi_{intra} = 0.49$ and $D = 3 \cdot 10^{-9}$ m²/s (for $T = 37^\circ C$), one obtains $R_{cluster}^{opt} \sim 40$ nm and thus an optimal diameter of 80 nm. The Polymag™ clusters are clearly larger (180 nm). This is confirmed by the value of $\Delta\omega_{cluster}\tau_D^{cluster} \sim 55$. The clusters are thus on the right side of the plateau of transverse relaxation represented in figure 1 of [21], closer to PRM than MAR.

Anyway, the relaxivity remains high even if it is smaller than r_2^{max} , with $r_2 \sim 470 s^{-1}mM^{-1}$ at 37°C. This value is among the highest values reported for clusters of iron oxide particles [29,30,38–44]. Zhang et al obtained almost the same relaxivity, but for smaller clusters with a smaller ϕ_{intra} . Tang et al synthesized 85 nm clusters (unknown ϕ_{intra}) with $r_2 = 175 s^{-1}mM^{-1}$. A r_2 of $262 s^{-1}mM^{-1}$ was measured for clusters of 92 nm with $\phi_{intra} = 0.21$. For dense clusters ($\phi_{intra} = 0.7$) with a diameter of 73 nm, Kostopoulou et al [39] measured $r_2 = 327 s^{-1}mM^{-1}$. A similar relaxivity ($r_2 = 364 s^{-1}mM^{-1}$) was observed [40] for 65 nm clusters (unknown ϕ_{intra}). Xu et al [29] produced clusters of 64 nm (unknown ϕ_{intra}) with a relaxivity of $650 s^{-1}mM^{-1}$. The highest r_2 value reported for iron oxide clusters is $835 s^{-1}mM^{-1}$ [30]. It was obtained with nanoconstructs incorporating magnetic cores whose $M_{sat} = 360000$ A/m. The authors explain this value, which is above r_2^{max} , by a modification of the diffusion coefficient inside the porous matrix of the nanosystem. If D clearly influences the cluster size intervals corresponding to the different relaxation regimes, it is unclear how it could

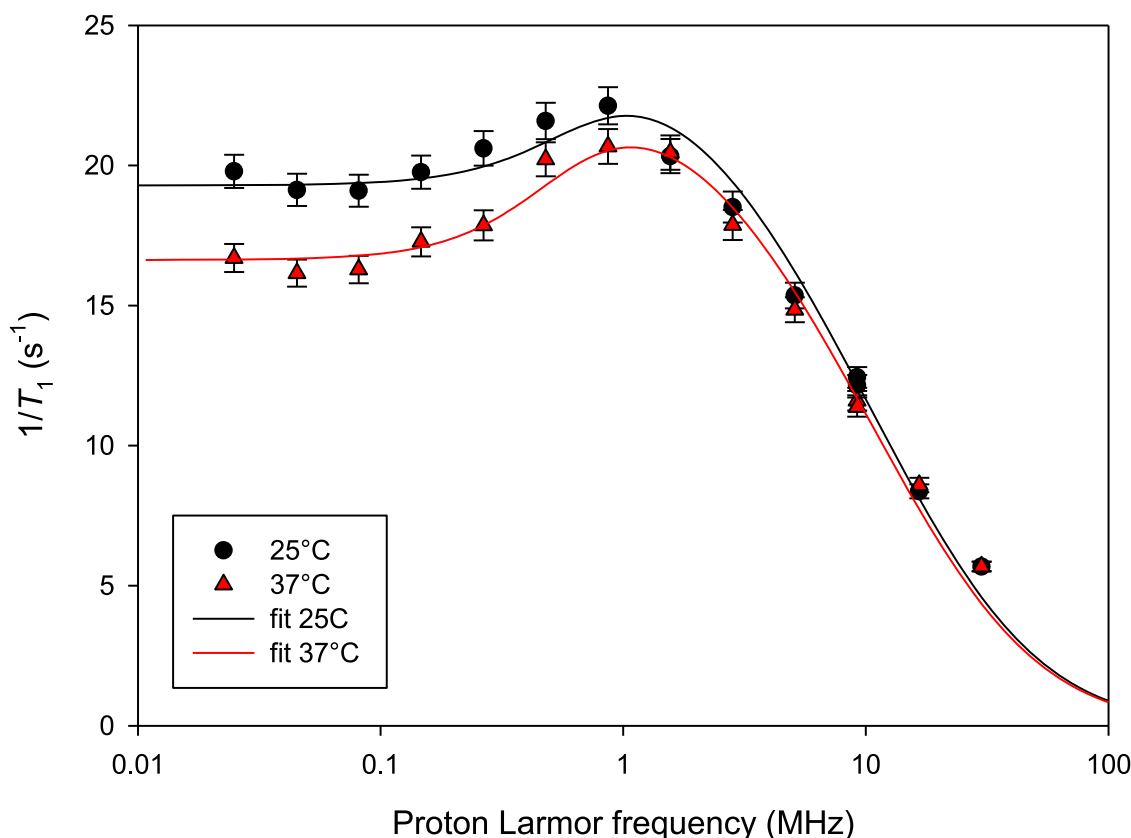


Figure 5. T_1 NMRD profiles of an aqueous solution of Polymag™ clusters at 25°C and 37°C, $[\text{Fe}] = 0.24 \text{ mM}$. Lines are best fit of the data with the model of Roch et al.

affect the value of r_2^{max} . Indeed, the maximum occurs in SDR, where diffusion has no influence on relaxation. In the light of all these studies, it seems that high relaxivities can be obtained for different combinations of size and intra-aggregate volume fraction. An accurate prediction of the optimal cluster characteristics remains difficult. Indeed, in addition to M_v , R_{cluster} and ϕ_{intra} , the size distribution of the clusters impacts the relaxivity value [23]. When it is broad, all the particles are not in the same relaxation regime. For the Polymag™ sample, the size distribution is quite large ($\text{PDI} = 0.15$) which explains why the relaxivity remains smaller than r_2^{max} since all clusters are not right in the middle of SDR. The large $\Delta\omega_{\text{cluster}}\tau_D^{\text{cluster}}$ value suggests that some clusters must be at the limit between SDR and PRM.

The value of r_2 of Polymag™ clusters is similar at 0.68 and 1.41 T because the magnetic moment of the particles already reaches saturation at 0.5 T. Temperature has almost no influence on the transverse relaxation rate at 0.68 T (inset of Fig. 4b), which is quite uncommon for magnetic particles whose relaxation process involves water diffusion. Indeed, D increases of more than 30% between 25°C and 37°C [45] which impacts the relaxation rate in the MAR (through the τ_D dependence) and PRM (through the $1/\tau_D$ dependence). Only the relaxation rate predicted by the SDR is diffusion independent and therefore not influenced by temperature. This is an indirect proof that the Polymag™ clusters are mainly in the SDR even if some clusters are close to PRM.

The high r_2 value has to be moderated by the instability of the Polymag™ clusters within the magnetic field. The r_2 values reported above were obtained when the relaxation rates were measured just after the insertion of the samples. Had the relaxation rates been measured 30 minutes after the insertion in the field, the r_2 value would have been 35% lower as shown in Fig. 3b. This evolution of the relaxation time with the time spent in the field was already reported in different studies [38,40], and is probably due to the reversible formation of linear chains in an homogeneous magnetic field. This aggregation seems inevitable

since the highest relaxivities are reached with large particles or clusters that are logically more sensitive to the magnetic field, because they present a large magnetic moment. To increase their stability, a second layer of polymer could be added to the first PEI coating, as described by Thünemann et al [46]. This would increase the steric repulsion of the particles [47]. However, the influence of magnetic field on the Polymag™ clusters is rather logical and maybe impossible to completely prevent: the clusters are precisely used to introduce nucleic acids inside cells thanks to a permanent magnet, which means that they migrate in magnetic field gradients. This property can in fact turn into an advantage: it could be used to internalize the superparamagnetic clusters into cells for a subsequent detection by MRI. In the perspective of developing iron oxide clusters that could be internalized in cells using the magnetofection protocol and then easily detected by MRI, a question remains: since the optimization of r_2 allows different combinations of cluster size and ϕ_{intra} values, which combination is best: small and dense clusters or diluted large clusters? As seen above, the r_2 optimization requires, for a given ϕ_{intra} value, a cluster radius $R_{\text{cluster}}^{\text{opt}} \propto (\phi_{\text{intra}})^{-1/2}$. The effect of magnetic field gradients on the cluster is proportional to its magnetic moment, which is given by: $\mu = \left(\frac{4}{3} \pi (R_{\text{opt}}^{\text{cluster}})^3 \right) \phi_{\text{intra}} M_v$. The moment is

thus finally proportional to $(\phi_{\text{intra}})^{-1/2}$. This means that for a dual use for internalization with magnetic gradients and MRI contrast agent, large and dilute clusters could be more efficient than small dense clusters. Eqn 1, 2, 3, 7, (8)

Conclusion

The magnetic and NMR properties of Polymag™ iron oxide clusters have been studied in this work. The clusters of 180 nm are constituted of small iron oxide crystals presenting a high saturation magnetization

(~371000 A/m). The transverse relaxivity of the clusters is remarkably high, close to the maximum value achievable. Transverse relaxation is independent of the echo time and of temperature, which, together with high relaxivity, indicates that the relaxation of the system is mainly governed by the SDR model even if large clusters are close to the PRM. The relaxation time evolves with the time spent within the magnetic field, which is a clear sign of aggregation. The phenomenon is reversible once the sample is removed from the field. The Polymag™ clusters could thus constitute a good candidate of contrast agent for cellular MR imaging, especially because they could be easily brought into cells using the existing magnetofection protocol. The potential performance of the Polymag™ clusters for cellular imaging still has to be confirmed by NMR relaxometry and MRI experiments with cells after internalization of the iron oxide clusters.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Acknowledgment

This work was partly supported by F.R.S.FNRS and UMONS

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.jmro.2022.100054](https://doi.org/10.1016/j.jmro.2022.100054).

References

- [1] The Iron Oxides: Structure, Properties, Reactions, Occurrences and Uses, 2nd, Completely Revised and Extended Edition, Wiley, Wiley.Com. (n.d.). <https://www.wiley.com/en-us/The+Iron+Oxides%3A+Structure%2C+Properties%2C+Reactions%2C+Occurrences+and+Uses%2C+2nd%2C+Completely+Revised+and+Extended+Edition-p-9783527606443> (accessed September 6, 2021).
- [2] J.L. Dormann, Le phénomène de superparamagnétisme, *Revue de Physique Appliquée*. 16 (1981) 275–301. <https://doi.org/10.1051/rphysap:01981001606027500>.
- [3] N.A. Spaldin, *Magnetic Materials: Fundamentals and Applications*, 2 edition, Cambridge University Press, Cambridge; New York, 2010.
- [4] S.M. Dadfar, K. Roemhild, N.I. Drude, S. von Stillfried, R. Knüchel, F. Kiessling, T. Lammers, Iron oxide nanoparticles: Diagnostic, therapeutic and theranostic applications, *Advanced Drug Delivery Reviews* 138 (2019) 302–325, <https://doi.org/10.1016/j.addr.2019.01.005>.
- [5] J. Dulińska-Litewka, A. Łazarczyk, P. Hałubiec, O. Szafranski, K. Karnas, A. Karewicz, Superparamagnetic Iron Oxide Nanoparticles—Current and Prospective Medical Applications, *Materials* 12 (2019) 617, <https://doi.org/10.3390/ma12040617>.
- [6] H.E. Daldrop-Link, Ten Things You Might Not Know about Iron Oxide Nanoparticles, *Radiology* 284 (2017) 616–629, <https://doi.org/10.1148/radiol.2017162759>.
- [7] J.W.M. Bulte, D.L. Kraitchman, Iron oxide MR contrast agents for molecular and cellular imaging, *NMR Biomed* 17 (2004) 484–499, <https://doi.org/10.1002/nbm.924>.
- [8] S. Sharifi, H. Seyednejad, S. Laurent, F. Atyabi, A.A. Saei, M. Mahmoudi, Superparamagnetic iron oxide nanoparticles for in vivo molecular and cellular imaging, *Contrast Media Mol. Imaging*. 10 (2015) 329–355, <https://doi.org/10.1002/cmmi.1638>.
- [9] D.J. Korchinski, M. Taha, R. Yang, N. Nathoo, J.F. Dunn, Iron Oxide as an MRI Contrast Agent for Cell Tracking, *Magn Reson Insights* 8 (2015) 15–29, <https://doi.org/10.4137/MRI.S23557>.
- [10] Y. Gossuin, A. Hocq, P. Gillis, Q.L. Vuong, Physics of magnetic resonance imaging: from spin to pixel, *Journal of Physics D: Applied Physics* 43 (2010), 213001, <https://doi.org/10.1088/0022-3727/43/21/213001>.
- [11] Y. Gossuin, P. Gillis, A. Hocq, Q.L. Vuong, A. Roch, Magnetic resonance relaxation properties of superparamagnetic particles, *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology* 1 (2009) 299–310.
- [12] S. Laurent, D. Forge, M. Port, A. Roch, C. Robic, L. Vander Elst, R.N. Muller, Magnetic Iron Oxide Nanoparticles: Synthesis, Stabilization, Vectorization, Physicochemical Characterizations, and Biological Applications, *Chemical Reviews* 108 (2008) 2064–2110, <https://doi.org/10.1021/cr068445e>.
- [13] M.R. Bashir, L. Bhatti, D. Marin, R.C. Nelson, Emerging applications for ferumoxytol as a contrast agent in MRI, *Journal of Magnetic Resonance Imaging* 41 (2015) 884–898, <https://doi.org/10.1002/jmri.24691>.
- [14] X. Ma, S. Wang, L. Hu, S. Feng, Z. Wu, S. Liu, S. Duan, Z. Chen, C. Zhou, X. Zhao, Imaging Characteristics of USPIO Nanoparticles (<5 nm) as MR Contrast Agent In Vitro and in the Liver of Rats, *Contrast Media & Molecular Imaging* 2019 (2019) 1–9, <https://doi.org/10.1155/2019/3687537>.
- [15] E.A. Neuwelt, B.E. Hamilton, C.G. Varallyay, W.R. Rooney, R.D. Edelman, P. M. Jacobs, S.G. Watnick, Ultrasmall superparamagnetic iron oxides (USPIOs): A future alternative magnetic resonance (MR) contrast agent for patients at risk for nephrogenic systemic fibrosis (NSF)? *Kidney International* 75 (2009) 465–474, <https://doi.org/10.1038/ki.2008.496>.
- [16] M. Jeon, M.V. Halbert, Z.R. Stephen, M. Zhang, Iron Oxide Nanoparticles as T1 Contrast Agents for Magnetic Resonance Imaging: Fundamentals, Challenges, Applications, and Prospectives, *Adv. Mater* 33 (2021), 1906539, <https://doi.org/10.1002/adma.201906539>.
- [17] T. Grobner, Gadolinium - A specific trigger for the development of nephrogenic fibrosis dermopathy and nephrogenic systemic fibrosis? *Nephrology Dialysis Transplantation* 21 (2006) 1104–1108, <https://doi.org/10.1093/ndt/gfk062>.
- [18] G. Klug, T. Kampf, S. Bloemer, J. Bremicker, C.H. Ziemer, A. Heymer, U. Gbureck, E. Rommel, U. Nöth, W.A. Schenk, P.M. Jakob, W.R. Bauer, Intracellular and extracellular T1 and T2 relaxivities of magneto-optical nanoparticles at experimental high fields, *Magnetic Resonance in Medicine* 64 (2010) 1607–1615, <https://doi.org/10.1002/mrm.22557>.
- [19] M. Lévy, C. Wilhelm, M. Devaud, P. Levitz, F. Gazeau, How cellular processing of superparamagnetic nanoparticles affects their magnetic behavior and NMR relaxivity: MAGNETIC AND NMR BEHAVIORS OF CELL-PROCESSED USPIO, *Contrast Media & Molecular Imaging* 7 (2012) 373–383, <https://doi.org/10.1002/cmmi.504>.
- [20] Q.L. Vuong, P. Gillis, A. Roch, Y. Gossuin, Magnetic resonance relaxation induced by superparamagnetic particles used as contrast agents in magnetic resonance imaging: a theoretical review, *WIREs Nanomedicine and Nanobiotechnology* 9 (2017) e1468, <https://doi.org/10.1002/wnan.1468>.
- [21] P. Gillis, F. Moyné, R.A. Brooks, OnT2-shortening by strongly magnetized spheres: A partial refocusing model, *Magnetic Resonance in Medicine*. 47 (2002) 257–263. <https://doi.org/10.1002/mrm.10059>.
- [22] A. Roch, R.N. Muller, P. Gillis, Theory of proton relaxation induced by superparamagnetic particles, *The Journal of Chemical Physics* 110 (1999) 5403–5411.
- [23] M.R.J. Carroll, R.C. Woodward, M.J. House, W.Y. Teoh, R. Amal, T.L. Hanley, T. G. St Pierre, Experimental validation of proton transverse relaxivity models for superparamagnetic nanoparticle MRI contrast agents, *Nanotechnology* 21 (2010), 035103, <https://doi.org/10.1088/0957-4484/21/3/035103>.
- [24] Q.L. Vuong, J.-F. Berret, J. Fresnais, Y. Gossuin, O. Sandre, A Universal Scaling Law to Predict the Efficiency of Magnetic Nanoparticles as MRI T2-Contrast Agents, *Advanced Healthcare Materials* 1 (2012) 502–512, <https://doi.org/10.1002/adhm.201200078>.
- [25] D.A. Yablonskiy, E.M. Haacke, Theory of NMR signal behavior in magnetically inhomogeneous tissues: the static dephasing regime, *Magn Reson Med* 32 (1994) 749–763.
- [26] Q.L. Vuong, P. Gillis, Y. Gossuin, Monte Carlo simulation and theory of proton NMR transverse relaxation induced by aggregation of magnetic particles used as MRI contrast agents, *Journal of Magnetic Resonance* 212 (2011) 139–148, <https://doi.org/10.1016/j.jmr.2011.06.024>.
- [27] Z. Zhao, Z. Zhou, J. Bao, Z. Wang, J. Hu, X. Chi, K. Ni, R. Wang, X. Chen, Z. Chen, J. Gao, Octapod iron oxide nanoparticles as high-performance T₂ contrast agents for magnetic resonance imaging, *Nat Commun* 4 (2013) 2266, <https://doi.org/10.1038/ncomms3266>.
- [28] Y. Matsumoto, A. Jasanoff, T2 relaxation induced by clusters of superparamagnetic nanoparticles: Monte Carlo simulations, *Magnetic Resonance Imaging* 26 (2008) 994–998, <https://doi.org/10.1016/j.mri.2008.01.039>.
- [29] F. Xu, C. Cheng, D.-X. Chen, H. Gu, Magnetite Nanocrystal Clusters with Ultra-High Sensitivity in Magnetic Resonance Imaging, *ChemPhysChem* 13 (2012) 336–341, <https://doi.org/10.1002/cphc.201100548>.
- [30] A. Gizzatov, J. Key, S. Aryal, J. Ananta, A. Cervadoro, A.L. Palange, M. Fasano, C. Stigliano, M. Zhong, D. Di Mascio, A. Guven, E. Chivavazzo, P. Asinari, X. Liu, M. Ferrari, L.J. Wilson, P. Decuzzi, Hierarchically Structured Magnetic Nanoconstructs with Enhanced Relaxivity and Cooperative Tumor Accumulation, *Adv. Funct. Mater.* 24 (2014) 4584–4594, <https://doi.org/10.1002/adfm.201400653>.
- [31] X. Xie, C. Zhang, Controllable Assembly of Hydrophobic Superparamagnetic Iron Oxide Nanoparticle with mPEG-PLA Copolymer and Its Effect on MR Transverse Relaxation Rate, *Journal of Nanomaterials* 2011 (2011) 1–7, <https://doi.org/10.1155/2011/152524>.
- [32] C. Paquet, H.W. De Haan, D.M. Leek, H.-Y. Lin, B. Xiang, G. Tian, A. Kell, B. Simard, Clusters of superparamagnetic iron oxide nanoparticles encapsulated in a hydrogel: A particle architecture generating a synergistic enhancement of the T2 relaxation, *ACS Nano* 5 (2011) 3104–3112, <https://doi.org/10.1021/nn2002272>.
- [33] C.H. Lee, J.-H. Kim, H.J. Lee, K. Jeon, H. Lim, H. yeon Choi, E.-R. Lee, S.H. Park, J.-Y. Park, S. Hong, S. Kim, S.-G. Cho, The generation of iPS cells using non-viral magnetic nanoparticle-based transfection, *Biomaterials* 32 (2011) 6683–6691, <https://doi.org/10.1016/j.biomaterials.2011.05.070>.
- [34] C. Plank, O. Zelpati, O. Mykhaylyk, Magnetically enhanced nucleic acid delivery. Ten years of magnetofection—Progress and prospects, *Advanced Drug Delivery Reviews* 63 (2011) 1300–1331, <https://doi.org/10.1016/j.addr.2011.08.002>.
- [35] Y. Sanchez-Antequera, O. Mykhaylyk, N.P. van Til, A. Cengizoglu, J.H. de Jong, M.W. Huston, M. Anton, I.C.D. Johnston, Z. Pojda, G. Wagemaker, C. Plank, Magselectofection: an integrated method of nanomagnetic separation and genetic

- modification of target cells, *Blood* 117 (2011), <https://doi.org/10.1182/blood-2010-08-302646> e171–e181.
- [36] O. Mykhaylyk, D. Vaskou, N. Tresilwised, P. Pithayanukul, W. Möller, C. Plank, Magnetic nanoparticle formulations for DNA and siRNA delivery, *Journal of Magnetism and Magnetic Materials* 311 (2007) 275–281, <https://doi.org/10.1016/j.jmmm.2006.10.1178>.
- [37] B. Thapa, D. Diaz-Diestra, C. Santiago-Medina, N. Kumar, K. Tu, J. Beltran-Huarac, W.M. Jadwisieniczak, G.B.R. Weiner, Morell, T 1 - and T 2 -weighted Magnetic Resonance Dual Contrast by Single Core Truncated Cubic Iron Oxide Nanoparticles with Abrupt Cellular Internalization and Immune Evasion, *ACS Appl. Bio Mater.* 1 (2018) 79–89, <https://doi.org/10.1021/acsabm.8b00016>.
- [38] S. Balasubramaniam, S. Kayandan, Y.-N. Lin, D.F. Kelly, M.J. House, R. C. Woodward, T.G. St. Pierre, J.S. Riffle, R.M. Davis, Toward Design of Magnetic Nanoparticle Clusters Stabilized by Biocompatible Diblock Copolymers for T 2 -Weighted MRI Contrast, *Langmuir* 30 (2014) 1580–1587, <https://doi.org/10.1021/la403591z>.
- [39] A. Kostopoulou, K. Brintakis, E. Fragogeorgi, A. Anthousi, L. Manna, S. Begin-Colin, C. Billotey, A. Ranella, G. Loudos, I. Athanassakis, A. Lappas, Iron Oxide Colloidal Nanoclusters as Theranostic Vehicles and Their Interactions at the Cellular Level, *Nanomaterials* 8 (2018) 315, <https://doi.org/10.3390/nano8050315>.
- [40] Z. Xiao, Q. Zhang, X. Guo, J. Villanova, Y. Hu, I. Külaots, D. Garcia-Rojas, W. Guo, V.L. Colvin, Libraries of Uniform Magnetic Multicore Nanoparticles with Tunable Dimensions for Biomedical and Photonic Applications, *ACS Appl. Mater. Interfaces* 12 (2020) 41932–41941, <https://doi.org/10.1021/acsami.0c09778>.
- [41] H. Groult, J. Ruiz-Cabello, A.V. Lechuga-Vieco, J. Mateo, M. Benito, I. Bilbao, M. P. Martínez-Alcázar, Juan.A. Lopez, J. Vázquez, F.F. Herranz, Phosphatidylcholine-Coated Iron Oxide Nanomicelles for In Vivo Prolonged Circulation Time with an Antibiofouling Protein Corona, *Chem. Eur. J.* 20 (2014) 16662–16671, <https://doi.org/10.1002/chem.201404221>.
- [42] Y. Tang, Y. Liu, W. Li, Y. Xie, Y. Li, J. Wu, S. Wang, Y. Tian, W. Tian, Z. Teng, G. Lu, Synthesis of sub-100 nm biocompatible superparamagnetic Fe 3 O 4 colloidal nanocrystal clusters as contrast agents for magnetic resonance imaging, *RSC Adv* 6 (2016) 62550–62555, <https://doi.org/10.1039/C6RA09344B>.
- [43] C. Zhang, X. Xie, S. Liang, M. Li, Y. Liu, H. Gu, Mono-dispersed high magnetic resonance sensitive magnetite nanocluster probe for detection of nascent tumors by magnetic resonance molecular imaging, *Nanomedicine: Nanotechnology, Biology and Medicine* 8 (2012) 996–1006, <https://doi.org/10.1016/j.nano.2011.11.013>.
- [44] B. Garnier, S. Tan, S. Miraux, E. Bled, A.R. Brisson, Optimized synthesis of 100 nm diameter magnetoliposomes with high content of maghemite particles and high MRI effect: Synthesis of 100 nm magnetoliposomes with high iron content, *Contrast Media Mol. Imaging* 7 (2012) 231–239, <https://doi.org/10.1002/cmmi.487>.
- [45] M. Holz, S.R. Heil, A. Sacco, Temperature-dependent self-diffusion coefficients of water and six selected molecular liquids for calibration in accurate 1H NMR PFG measurements, *Physical Chemistry Chemical Physics* 2 (2000) 4740–4742, <https://doi.org/10.1039/b005319h>.
- [46] A.F. Thünemann, D. Schütt, L. Kaufner, U. Pison, H. Möhwald, Maghemite Nanoparticles Protectively Coated with Poly(ethylene imine) and Poly(ethylene oxide)- b lock -poly(glutamic acid), *Langmuir* 22 (2006) 2351–2357, <https://doi.org/10.1021/la052990d>.
- [47] E. Amstad, M. Textor, E. Reimhult, Stabilization and functionalization of iron oxide nanoparticles for biomedical applications, *Nanoscale* 3 (2011) 2819, <https://doi.org/10.1039/c1nr10173k>.