Magnetic nanoparticles for MRI applications in medicine

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Abstract

Magnetic resonance imaging (MRI) has developed at an exponential rate since the last decades, and is now widely used as an anatomical and functional medical imaging modality. The development of magnetic nanoparticles as contrast agents for vascular, molecular, and cellular MRI applications has followed this trend. Nanoparticles that generate either “positive” or “negative” contrast in MRI figure among one of the most important direct applications of nanotechnology. The present chapter is an introduction to the principles underlying the performance of nanoparticulate-based MRI contrast agents. It addresses the main considerations guiding the design, the synthesis, and the physico-chemical characterization of magnetic nanoparticles based on the elements iron, manganese and gadolinium (Fe, Mn, Gd). The fundamental aspects of nanoparticle magnetism and relaxometric characterization are introduced, as well as examples of applications in biological models.
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1. Introduction to nanoparticles for MRI applications

Nanoparticles (NPs) made of the elements iron (Fe), gadolinium (Gd) or manganese (Mn) are currently used in many diagnostic applications performed under magnetic resonance imaging (MRI). In fact, MRI scanners do not directly detect magnetic nanoparticles (MNPs): they reconstruct images from the electromagnetic signals generated by the stimulation, and relaxation, of the large pool of hydrogen (\(^1\)H) protons found in biological tissues. By interactions taking place at the atomic and molecular level, MNPs influence the relaxation time of hydrogen protons contained in small, mobile molecules such as water. Thereby, MNPs induce contrast enhancement effects on the reconstructed MR images, either as a signal increase ("positive" contrast agents, CAs) or a signal decrease ("negative" CAs).

In fact, MRI is one of the most reliable, high resolution (75 – 300 µs), and multi-function imaging modalities of modern medicine. Compared to optical imaging and echography, it allows the acquisition of in-depth, whole-body images, from the mouse model to the humans. Contrarily to computer tomography (CT), another anatomical imaging modality, it does not rely on ionizing radiation. MRI also enables the tracking of cells, molecules, and drug delivery vehicles in the human body. These applications require the development of appropriate biomedical imaging probes (CAs or tracers). Such probes must be sensitively detected at the nanomolar concentration level, to allow the efficient detection of a reasonable amount of molecules, or cells, in the body.[1-3] Compared to MRI, positron emission tomography (PET) and luminescence/fluorescence imaging do not provide anatomical images, and neither are they considered as high resolution imaging modalities in general. However, both are truly efficient "molecular" imaging modalities (nanomolar detection). Unfortunately, MRI has a relatively poor sensitivity compared with such techniques. To fully exploit the wide range of advantages that MRI has to offer in molecular and cellular imaging applications, it has been necessary to design CAs which have the capacity to very efficiently interact with hydrogen protons.

Contrast agents (CAs) have been developed since the inception of MRI, to selectively change the longitudinal and transverse relaxation times (\(T_1\) and \(T_2\)) of \(^1\)H in biological tissues. Such energy transfers mainly occur though interactions taking place between the magnetic elements and the spins of hydrogen protons in their vicinity. Most clinically approved CAs are based on small molecules that sequestrate the paramagnetic ion Gd\(^{3+}\).[4, 5] They are mainly used as non-specific agents, to enhance the general contrast of organs, enabling thereby a better identification of anatomical changes occurring in the body. They are also applied to blood-pool and blood perfusion procedures. Gadolinium has 7 unpaired electrons in its 4f orbitals giving it a very large
magnetic moment; a relatively slow electronic relaxation rate compared with other paramagnetic elements, also enhances its proton relaxation properties.[6] Manganese can also be exploited in MRI applications, although its magnetic moment is weaker than that of Gd$^{3+}$ ($\text{Mn}^{2+}$ has 5 unpaired electrons on its 3d orbital). After the first marketing authorization of Gd-DTPA (Magnevist™) in the US, Europe and Japan in 1988, other Gd-based chelates were introduced to the market: Dotarem™, based on the DOTA chelator, as well as Omniscan™, Prohance™, Optimark™, and Gadovist™.[7] Today, contrast media are applied in approximately 30% to 40% of all MRI procedures.

In parallel with the development of paramagnetic CAs, progress in the field of MRI CAs also occurred through the unique properties of iron oxide NPs. By opposition to paramagnetic molecules, which produce “positive” contrast in MRI, iron oxide NPs are well known for their potential to increase the signal. Iron oxide NPs are, in general, divided in two classes: small particles of iron oxide (SPIO), and ultra-small particles of iron oxide (USPIO). SPIOs are made of iron oxide cores of mean diameters in the range 3 – 20 nm; however they form agglomerates of hydrodynamic diameter typically superior to 50 nm. The concept of hydrodynamic diameter (Figure 1) refers to the total effective diameter of a particle suspended in a fluid and forming a colloid. The hydrodynamic diameter is generally measured by means of laser analysis (dynamic light scattering, DLS), by evaluating the Brownian motion of the suspended NPs. USPIOs on the other hand, refer to the class of iron oxide NPs that are made of iron oxide cores (3 – 20 nm diam.) surrounded by a coating of molecules that preserve their individuality (mean diameter < 50 nm).

**Figure 1:** Schematic representations of a) the structure of functional magnetic nanoparticles (MNP s) for MRI applications and b) a colloidal nanoparticle suspended in biological media.
SPIOs and USPIOs have very different mean hydrodynamic diameters, as well as different “relaxometric” properties. Such properties can be advantageously exploited either in cell labeling (preferentially with SPIOs), liver cancer diagnostic (preferentially with SPIOs), molecular targeted imaging (preferentially with USPIOs), or blood pool/angiography procedures (preferentially with USPIOs). In fact, a very large fraction of molecular and cellular MRI applications are based on the design and fabrication of MNPs consisting of a) an inorganic nanocrystal core (e.g. \( \text{Fe}_2\text{O}_3/\text{Fe}_3\text{O}_4 \), \( \text{Gd}_2\text{O}_3 \), \( \text{MnO} \), \( \text{NaGdF}_4 \)), b) a coating made of small ligands, or biocompatible organic molecules, and c) a functional anchor that is used to graft specific molecules, complementary imaging functionalities (e.g. radioactive atoms or fluorescent molecules), or medicinal compounds for drug delivery. Injected intravenously, SPIOs are captured by macrophages. They end up in the Kupffer cells of the liver (part of the reticuloendothelial system, RES), where they are expected to degrade. As a result, an important shortening of the transverse relaxation time \( (T_2/T_2') \) is observed in the liver tissue, which is reflected by a strong signal loss.[8-10] An example of this is provided in Figure 2.

![Figure 2: Intravenous injections of polymer-coated iron oxide NPs in the mouse model (unpublished data, Fortin et al.). The presence of USPIOs in the blood enhance the vascular signal during at least 20 minutes (a: arrowhead, b: blood signal-enhancement ratio): USPIOs can be used as blood-pool agents. After injection, NPs are gradually sequestrated by the macrophages, and follow the reticuloendothelial (RES) route, mainly in the liver (a: t = 24 h., full arrow; and c). After several hours, a significant signal enhancement appears in the gall bladder, an indication of NPs excretion through the hepatobiliary way.](image)

By this mechanism, iron oxide NPs “passively”, but rather homogenously accumulate in the liver, where they are found to lower the MR signal. However, tumors and metastases are void of Kupffer cells, and they do not internalize iron oxide NPs. This
strategy has been used in the diagnostic of liver cancer and liver metastasis, as well as for imaging the spleen and of the lymph nodes.[11, 12] Because of their elimination by the liver, SPIOs are not efficient in applications requiring longer blood half-lives (MR angiography, tissue perfusion imaging, functional imaging of the brain). For vascular applications, USPIOs are better candidates since they stay in the blood for longer times. Their relaxation properties also allow them for use as positive CAs in $T_1$-weighted imaging (see next section) and angiography.[13-17] In the recent years, the research on targeted MRI contrast agents has focused on the development of targeted USPIOs that could enable the efficient, sensitive and selective detection of atherosclerosis, apoptosis, and amyloid deposition in Alzheimer’s disease.[18-25] This concept is frequently referred to as molecular targeted imaging.

In parallel with iron oxide NPs, a new generation of paramagnetic NPs has been developed, based on Gd and Mn-containing inorganic nanocrystals.[26, 27] These are based on the synthesis of Gd$_2$O$_3$[28-32], NaGdF$_4$[33-36] and MnO [37-39] nanocrystals. When adequately covered with biocompatible ligands and suspended in aqueous solutions, these are “positive” CAs in MRI. Because the core of inorganic nanoparticles is very small (< 20 nm), and because they contain hundreds or thousands of paramagnetic atoms, they can be used to label molecules or cells that are then much more sensitively detected than if they were labeled by traditional MRI contrast agents containing only one paramagnetic atom per unit of contrast agent (e.g. commercially available Gd-DTPA, or Gd-DOTA).[4, 5] Although they are not as sensitively detected as their “negative” CA counterparts based on iron oxide NPs, they provide contrast enhancement effects that are largely devoid of magnetic susceptibility image artifacts. In addition to this, the detection of signal-enhancement effects generated by “positive” CAs could enable more quantitative molecular and cellular imaging studies. Until now, CAs based on Gd and Mn nanocrystals, have been largely constrained to pre-clinical studies because of the inherent risks associated with the leaching of potentially toxic Mn$^{2+}$ and Gd$^{3+}$ ions. They nonetheless represent very useful contrast agents for research purposes in the field of cell labeling and tracking, as well as for molecular imaging with MRI.

This chapter first describes the general considerations that must be taken into account in the design and preparation of MNPs for MRI in vivo applications. Synthesis and coating procedures to prepare suspensions of MNPs of narrow particle size, appropriate magnetic and relaxometric properties, optimal colloidal stability, and good biocompatibility, are presented. The contrast-enhancement characteristics of superparamagnetic and paramagnetic NPs are discussed, in particular their respective use either as “negative” or “positive” CAs in $T_2/T_2^*$-weighted or $T_1$-weighted MRI sequences.
2. The basics of MRI in medicine

A brief introduction to the basic principles of MRI is provided at this step, to clarify important concepts guiding the design and use of MNP for MRI applications in molecular and cellular imaging. The reader is invited to expand his knowledge by referring to a selection of readings on the general topic of MRI. [40, 41] In brief, the strength of the magnetic field in common MRI scanners, ranges from 1 to 3 Tesla, which is 20 000 to 60 000 times stronger than the Earth’s magnetic field. When a patient is introduced in the gantry, the hydrogen protons (\(^1\)H) align their spins along the direction of the magnetic field (\(B_0\), Figure 3.a). The sum of each one of the magnetic moments of these spins represents the “macroscopic magnetization vector” (\(\vec{M}\)) of the biological tissue. This vector is globally oriented along the main magnetic field of the scanner. In their initial state, the spins “precess” at a certain frequency (\(\omega_0\): the Larmor frequency). They are not coherent in phase. Then, using a transmitter coil, a radiofrequency (RF) wave tuned at Larmor conditions, is applied to the biological tissue. This causes the excitation of \(^1\)H spins. At this step, spins lose their preferential orientation along the main magnetic field (Figure 3.b). After application of the RF excitation, \(\vec{M}\) oscillates around the main magnetic field (the “z” axis of the MRI scanner), and the spins are phase-coherent in the x-y plane (Figure 3.b).

From this moment, the oscillation motion along the x-y plane is detected by a receiver coil, and recorded. This represents the “MRI signal”. Then, the “x-y” phase coherence (\(M_{xy}\)) is gradually lost (within milliseconds) as the magnetic moments of neighboring \(^1\)H protons exert a mutual influence on each other (Figure 3.c). The time constant used to quantify this loss of phase coherence is called the transversal relaxation
time ($T_2$, Figure 3.d). Independently from this mechanism, the excited spins progressively release their energy and recover their initial orientation along the main magnetic field of the scanner ($M_z$ recovery, Figure 3.c). This return to the initial macroscopic magnetization state occurs within a time constant that is referred to as the longitudinal relaxation time ($T_1$, Figure 3.d). Both $T_1$ and $T_2$ are intrinsic characteristics of any biological tissue and, together with the density of $^1$H spins in the tissue ($\rho$), are the most important parameters influencing the signal. For instance, the signal recorded for a given tissue ($S$), using a basic spin-echo sequence, is given by the following equation:

$$S = \rho (1 - e^{(-TR/T_1)}) (e^{-TE/T_2})$$  \hspace{1cm} \text{Equation 1}$$

where TR and TE are the repetition and the echo times (parameters in the spin-echo sequence, [41]). Interactions between the paramagnetic elements Fe$^{3+}$, Gd$^{3+}$, Mn$^{2+}$ take place at the molecular level, causing $T_1$ and $T_2$ to decrease and, in turn, to modify the MR signal according to Equation 1. Such interactions accelerate the release of energy communicated to $^1$H protons during the RF excitation.

3. Relaxivity: the performance of MRI contrast agents

MNPs made of Fe, Gd, or Mn, influence the macroscopic relaxation times ($T_1$ and $T_2$) of $^1$H contained in neighboring mobile molecules. In turn, this effect modulates the MRI signal (Equation 1), which translates into contrast effects in anatomical MR images. The efficiency of MRI CAs to decrease both $T_1$ and $T_2$ of hydrogen protons contained in the solution, is referred to as the “relaxivity”. Relaxivity depends on the concentration, as well as on the physico-chemical characteristics of the CAs: hydrodynamic diameters, number of water binding sites at the magnetic ions, etc. The relaxivity of water protons in CAs also depends on temperature, pH, and on the magnetic field strength that is used to perform the MRI scans. Here is a list of suggested readings on the topic of MRI CA relaxivity.[4, 5, 42-44]

In brief, the effect of CAs on the relaxation time of protons ($^1$H protons), are usually measured by relaxometric analysis, which is the technical term that refers to the measurement of $T_1$ and $T_2$ of mobile $^1$H species in aqueous suspensions or in biological tissues. In MRI, the impact of CAs on the relaxation rate of protons, measured in fixed conditions of magnetic strength and temperature, is described by the following equation:

$$R_i = \frac{1}{T_i} = \left( \frac{1}{T_{i0}} \right) + r_1 C$$  \hspace{1cm} \text{Equation 2}$$
where $R_{i=1,2}$ is the relaxation rate of the aqueous solution, $T_{i0}$ is the relaxation time of the aqueous media in the absence of the CA, $r_{i=1,2}$ is the relaxivity (usually at $T = 20$ or $37^\circ C$, pH = 7, and $B_0 = 1.5$ or 3.0 Tesla), and C is the CA concentration (in mM of Gd, Fe, or Mn). The concentration of magnetic elements is measured by spectroscopic and spectrometric elemental analysis techniques (e.g. atomic absorption spectroscopy – AAS; inductively coupled plasma optical emission spectroscopy (ICP-OES); mass spectrometry, ICP-MS). Therefore, the relaxometric performance of MRI CAs is assessed first by measuring their relaxation rates ($1/T_1$ and $1/T_2$), followed by normalizing the data to the paramagnetic elemental concentration. Relaxivity values ($r_1$ and $r_2$) are extracted from the slope of the graph given by Equation 2. These are often referred to as relaxivity curves, and they figure among the most fundamental aspects of MRI CA quantification. A more detailed explanation of CA relaxivity mechanisms is found in section 6. Finally, CAs can be divided into two categories, based on the $r_2/r_1$ ratio. First, CAs having a similar impact on both $T_1$ and $T_2$, result in low $r_2/r_1$ ratios (close to 1), and a capacity to enhance the MR signal. This is in agreement with Equations 1 and 2. Such CAs are referred to as “positive”. On the other hand, CAs that more preferentially decrease $T_2$, with $r_2/r_1$ ratios superior to 5 and often as high as 100, are called “negative”. Examples of the performance of CAs are found in section 6.

4. Synthesis and characterization of magnetic nanoparticles

Comprehensive reviews have been written, describing the different ways to synthesize MNPs. These mainly report on iron oxide NPs, and most of these colloidal synthesis routes can also be adapted to other metal ions such as Mn$^{2+}$ and Gd$^{3+}$. The most important criteria guiding the selection and optimization of a particular colloidal NP synthesis route, with MRI applications as an objective, is a good control over nanocrystal size, shape, and NP size distribution (as narrow as possible). A reasonable synthesis yield is also critical: CA products must be concentrated enough to produce efficient contrast enhancement effects in MRI procedures; the colloidal synthesis technique must also minimize the loss of paramagnetic materials and surfactants used during the production, in order to enable industrial upscale of the process.

a. Synthesis of magnetic nanocrystals

Until now, NP cores have been made from different materials and with varying sizes, shapes, uniformities, and magnetic properties[45-49]. Apart from MRI applications, MNPs have been formed from iron and cobalt [50]. Procedures enabling the synthesis of CoPt$_3$[51] and FePt [52], as well as oxides [44] such as magnetite (Fe$_3$O$_4$) and maghemite ($\gamma$-Fe$_2$O$_3$), have been successfully developed and mastered [44, 53, 54]. Iron oxide NPs
have also been doped to enhance their magnetic properties to form MFe$_2$O$_4$ structures where M is a +2 cation such as Mn, Fe, Co or Ni[55, 56]. These MNPs make excellent MR contrast agents; because their magnetic susceptibility is relatively high, they can also be manipulated by external magnetic fields. However, NPs containing Co and Ni are toxic, making them poor candidates for clinical use. To a lesser extent, this also the case for Gd and Mn-based nanocrystals. However, these have their own advantages and, as preclinical CAs, can advantageously applied to molecular and cellular imaging in animal models. For clinical use, the biocompatibility profile of iron oxide NPs is well established, and adequately documented with a large range of clinical studies. Iron oxide NPs injected in vivo eventually degrade to their non-toxic iron and oxygen components, making them particularly attractive as clinical MRI CAs[57].

The present section reports the major colloidal nanoparticle synthesis procedures that must be selected to enable efficient, rapid, and high-yield production of small particles of well-controlled and narrow size distributions. For both paramagnetic and superparamagnetic nanocrystals, core size dictates both the magnetic and the relaxometric performance of CAs. A good control over this parameter is therefore critical in all aspects, and bottom-up approaches using chemical colloidal synthesis techniques are almost invariably privileged. For a matter of concision, and because Fe, Gd and Mn-based materials account for the huge majority of MRI NPs in pre-clinical and clinical applications, only synthesis routes enabling the production of nanocrystals containing these three elements, are described here. A selection of references is provided to the reader who wishes to read on the different variants to these colloidal synthesis techniques.

**Iron oxide nanoparticles:** Among the most important colloidal synthesis methods that are used to fabricate iron oxide NPs, figure co-precipitation, co-precipitation in constrained environments, thermal decomposition and/or reduction, hydrothermal synthesis, and polyol synthesis. [44, 49, 58-61]. Each one has its own specific advantages. The most common, the simplest and possibly the most efficient is that of co-precipitation. It is based on the use of an ageing stoichiometric mixture of ferrous and ferric ions (Fe$^{2+}$/Fe$^{3+}$) in aqueous solutions. The chemical reaction for the formation of magnetite (Fe$_3$O$_4$) is:

$$\text{Fe}^{2+} + 2\text{Fe}^{3+} + 8\text{OH}^- \Rightarrow \text{Fe}_3\text{O}_4 + 4\text{H}_2\text{O}$$

According to the thermodynamics of this reaction, complete precipitation of Fe$_3$O$_4$ occurs at basic pH, with a stoichiometric ratio of 2:1 (Fe$^{3+}$:Fe$^{2+}$).[62] Therefore, the reaction takes place with the addition of base under an inert atmosphere.
In the presence of oxygen, magnetite oxidizes into maghemite, as follows:

\[
\text{Fe}_3\text{O}_4 + 2\text{H}^+ \Rightarrow \gamma\text{Fe}_2\text{O}_3 + \text{Fe}^{2+} + \text{H}_2\text{O}
\]

In the co-precipitation technique, NPs are formed by a nucleation and growth mechanism. NPs of relatively narrow size distributions can be synthesized, provided a short nucleation event takes place, followed by a slower growth phase. The type of salts employed (e.g. chlorides, sulfates, nitrates), the ratio between of ferrous and ferric ions, the temperature, pH and ionic strength figure are all parameters that must be finely tuned to yield NPs of desired size and narrow distribution.[49] Although they are totally appropriate to synthesize large amounts of MNPs, standard co-precipitation methods do not consistently deliver the same products (size, shape, and polydispersity). The presence of impurities and surface defects also affect the magnetic properties of such NPs.[46]

Adaptations to the co-precipitation approach have been investigated to improve the uniformity and stability of USPIOs and SPIOs. This was made through addition of polymers or polyelectrolytes to the ferric/ferrous ion solutions, with various improvements on size, shape, and crystallinity.[46, 63-66] The addition of chelating organic ions (carboxylate or α hydroxyl carboxylate ions, such as citric acid, gluconic, or oleic acid) or polymer surface complexing agents (dextran carboxydextran, starch, or polyvinyl alcool) during the formation of the magnetite crystals, help controlling NP size. The chelation of these charged organic molecules on the iron oxide surface can either prevent the nucleation and lead to larger particles, or inhibit the growth of crystal nuclei, leading to small nanoparticles. Adding polymers such as poly (acrylic acid) directly into the synthesis solution and in various concentrations, allows to tune the particle diameter between 7–14 nm[67]. Alternatively, polyethylene glycol (PEG) compounds such as PEG-g-poly(glycerol monoacrylate) are also used to modulate the size of USPIOs[68]. Importantly, these polymers may act as surface coatings when the nucleation and growth processes are complete. These are called in situ coating processes.

To achieve smaller sizes, narrower particle distributions and higher magnetic properties, new synthesis techniques have been developed, based on high-temperature decomposition methods using organic iron precursors[69]. For instance, a high-temperature reaction of iron (III) acetylacetonate, \(\text{Fe(acac)}_3\), in phenyl ether in the presence of alcohol, oleic acid, and oleylamine, yielded monodisperse, hydrophobic magnetite NPs with tunable sizes of 4–20 nm[70]. Because one of the major prerequisites of MNPs for MRI applications, is to achieve a good dispersion in aqueous solvents, an additional step must be introduced in the synthesis procedure, to replace the hydrophobic coating with an amphiphilic and biocompatible surfactant.
The polyol process is an alternative to thermal decomposition methods, for the synthesis of NPs with well-defined shapes and controlled sizes.[71, 72] Owing to their high dielectric constants, solvents as polyethylene glycol (PEG) are able to dissolve inorganic compounds on a large range of temperatures. Polyols also serve as stabilizers to control particle growth, and prevent particle aggregation. In polyol synthesis, the precursor compound is suspended in a liquid polyol, then heated to a given temperature. During this reaction, the solubilized metal precursor forms an intermediate, and is then reduced into metal nuclei that lead to NP growth. Good examples of iron oxide NP synthesis in different polyols (di-,tri-,tetra-) ethylene glycol, can be found in a selection of recent articles.[73-75] Overall, the nanoparticles synthesized by polyol routes have the smallest and narrowest size distributions, high water dispersion rates, and higher magnetization compared with particles produced by more conventional methods.

**Paramagnetic nanocrystals (Gd$_2$O$_3$ and NaGdF$_4$):** the main advantage of paramagnetic CAs compared to superparamagnetic NPs, is the possibility to generate “positive” signal at the accumulation of labeled molecules or labeled cells. Signal enhancement is in general easier to quantify than signal voids resulting from $T_2/T_2^*$ (e.g. magnetic susceptibility) effects (generated by iron oxide NPs). A good example of this is illustrated in Figure 4.[76] PEG-coated Gd$_2$O$_3$ NPs are used to label different types of cells[77-83], and, in this specific example, F98 brain cancer cells. A total of 3x10$^5$ labeled F98 cells were injected in mice brains (caudoputamen), to produce a brain tumor. This is a frequently used animal model in the field of brain cancer and oncology research. The same amount of SPIO-labeled cells was injected, to compare the capacity of both contrast media to allow cell tracking in MRI. Figure 4.a reveals the possibility to efficiently visualize the area of brain cancer cell implantation, at least 48 hours following the injection. Because this is a “positive” CA scanned with a $T_1$-weighted MR sequence, the anatomical information surrounding the area of cell implantation, is preserved. After one week, the tumor contours can be efficiently delineated without the use of a CAs. On the other hand, SPIO-labeled cells are much more sensitively detected than Gd$_2$O$_3$-labeled ones (Figure 4.b). However, the susceptibility artifact characteristic of iron oxide NPs largely exceeds the exact location of the implanted cells. Even after one week, the image artifact still obliterates important anatomical information directly in the area of the growing tumor. This is a good example of the potential of paramagnetic NPs, to replace SPIOs and UPSIOs in niche applications where the preservation of anatomical details is an issue (such as in cell implantation and tracking studies).
Figure 4: Mice brains implanted with 300,000 brain cancer cells (F98) labeled with a) PEG-Gd$_2$O$_3$ ($T_1$-weighted MR imaging) and b) SPIOs, ($T_2$-weighted MR imaging).[76]

The very high density of Gd atoms per unit of contrast agent, is an advantage over macromolecules containing Gd chelates: Gd$_2$O$_3$ and NaGdF$_4$ ultra-small nanoparticles (2–5 nm diameter) have narrow particle size distributions, and contain hundreds of Gd atoms per CA unit.[31, 76, 84] The production of Gd$_2$O$_3$ NPs, a pre-clinical CA, has required the development of advanced colloidal synthesis techniques in high boiling point alcohols (e.g. di-, tri-, poly-ethylene glycol) [31, 85-88]. The surface of Gd$_2$O$_3$ nanocrystals forms hydroxide in contact with water, and this form is susceptible to leach potentially toxic Gd$^{3+}$ ions, as in the case prolonged dialysis procedures.[30] Concerns related to the potential toxicity of Gd$_2$O$_3$ nanoparticles, even for pre-clinical applications in animal models, have led to the development of other forms of potentially more stable paramagnetic nanocrystals. NaGdF$_4$, in particular, is an attractive material due to its high concentration of Gd atoms in a crystalline form that is less susceptible to degrade in water. Sodium fluoride NPs are synthesized by thermal decomposition in oleic acid and octadecene, leading to hydrophobic surfaces that must be subsequently transferred to aqueous suspensions by using appropriate ligand exchange procedures.[89-91] Rare-earth fluorides doped with series of lanthanides, have very promising luminescent up-conversion properties. In particular, it has been demonstrated that ultra-small NaGdF$_4$ nanocrystals (3 nm diam.) doped with Tm and Tb, can be used for dual MRI and near-infra-red optical imaging, with a wide array of applications in biomedical research.[34]

**Antiferromagnetic MnO nanocrystals:** Although Mn$^{2+}$ ions are paramagnetic, MnO NPs express an antiferromagnetic behavior.[92] They overall behave as “positive” CAs. Thermal decomposition has been one of the most widely used and reliable routes to produce relatively large NP synthesis batches of small and narrow particle size.
distributions.[38, 93-95] One-pot synthesis techniques have also been developed in high boiling point solvents, enabling the production of 1–3 nm diameter MnO particles.[87]

b. Nanoparticle coatings for MRI applications

NPs must form stable colloids in physiological media (blood, plasma, lymph, urine) for being considered in MRI applications. They must also show good biocompatibility, and prolonged vascular retention. This is particularly critical in the case of targeted CAs, that must bring sufficient contrast at the molecular site where they are expected to bind to a molecular biomarkers (e.g. of atherosclerosis or Alzheimer’s disease). The ligands and polymers, which are used as particle coatings, must efficiently cover the particles and promote their individualization. They must also limit the adsorption of plasma proteins, which is the phenomenon that is precursor to their retrieval from the blood. Indeed, “opsonized” NPs are quickly recognized and ingested by macrophages. NPs must be coated with a surface ligand or a polymer that either induces an electrostatic repulsion between the particles, or exert a strong steric repulsion between them. Otherwise, particles are susceptible to agglomerate when they are submitted to strongly ionic conditions. Hence, any injection of magnetic nanoparticles in vivo, implies their preparation in a milieu that is close to the osmolality conditions of biological media. In general, the smaller the particle size, the more neutral and hydrophilic its surface, and the longer its plasma half-life.[96]

The selected biocompatible coatings should not influence the cell viability. They must be grafted with a functionality (e.g. carboxylates, phosphates and sulfates) that strongly binds to the NP surface. It should not be too pH-sensitive. For instance, acidic functions (-COOH) can bind to metal oxide surfaces. However, if the bonding is monodendate only, their attachment at the particle surface is relatively weak and not strong enough for many applications. Citric acid is a small and very effective ligand that binds to the surface of iron oxide NPs through carboxylic binding.[97] In particular, this strategy was used to synthesize the commercial product VSOP C184 (4 nm core size).[98] However, citric acid cause a strong degradation of the surface, which affects the magnetic properties of iron oxide NPs.[99] It is also very rapidly and strongly deflected to the liver, due to its high surface charge. Other ligand molecules can be used for the stabilization of iron oxide NPs in aqueous medium (e.g. gluconic acid, dimercaptosuccinic acid, phosphorylcholine, as well as phosphate and phosphonates).[100, 101]
Among the polymer coatings that most efficiently enhance the blood retention of SPIOs and USPIOs, figure dextran and (carboxy, carboxymethyl)dextran.[102, 103] In particular, commercial USPIO products such as ferumoxtran-10 (AMI-227), ferumoxytol, and Supravist (SHU-555C), are all based on iron oxide of core diameters in the range 4 – 8 nm, and of hydrodynamic sizes not larger than 30 nm.[104] Dextran and starch-coated iron oxide nanoparticles have shown very good relaxometric properties; however, such coatings are relatively unstable when submitted to biological media. In order to improve colloidal stability, biocompatibility and blood retention, PEG is used at the surface of iron oxide nanoparticles.[105-107] Feruglose (Clariscan) is a commercial product that was developed using a PEGylated starch coating.[108] Such coatings are hardly recognized by the macrophage-monocytic system. Paramagnetic Gd$_2$O$_3$ and NaGdF$_4$, as well as MnO nanoparticles, have also been coated using similar strategies (e.g. citric acid, dimercaptosuccinic acid, glucoronic acid, PEG).[28, 30, 34, 109-112] PEG is widely applied as coatings for paramagnetic nanoparticles, through –OH[86, 113], –COOH[31, 114], -silane[30, 109, 115] and –phosphate grafting.[116] Silane coatings, for instance, could delay the degradation of Gd$_2$O$_3$ nanoparticles submitted to acidic environments. Unfortunately, silane coatings restrict the optimal water exchange with paramagnetic ions at the surface of NPs, which is the most important relaxation mechanism of “positive” CAs (see section 6). PEG-phosphate molecules can be used instead, with the significant advantage that they are not susceptible to homocondensation such as for silane-based products. [116] Recently, PEGylated phosphonate dendrons were successfully used to cover MnO and iron oxide nanoparticles, and this coating strategy provided enhanced NP excretion profiles through the urinary and gastrointestinal pathways.[117, 118]

**Figure 5:** a) Electron microscopy study of MnO nanoparticles produced by thermal decomposition, with b) particle-size distribution and c) hydrodynamic size measurements (from [39]).
c. Physico-chemical characterization

After synthesis and ligand exchange, NPs must be carefully cleaned from the residual magnetic ions (Fe$^{2+}$, Fe$^{3+}$, Gd$^{3+}$, Mn$^{2+}$ and other) that could contaminate the physico-chemical, magnetometric, and relaxometric measurements. This procedure can be achieved either by dialysis in saline water (e.g. 10 - 154 mM NaCl), by centrifugation-filtration cycles, or by size-exclusion chromatography. Dynamic light scattering (DLS) measurements are performed directly after the purification process, in order to assess the overall colloidal stability and to demonstrate the absence of large-size aggregates. No drastic divergence should be noted between “intensity”- and “number”-weighted results, as diverging results point to the presence of large-size agglomerates that must be eliminated prior to further use of the particles. Then, the NPs must ideally demonstrate the presence of only one major hydrodynamic diameter peak, for their magnetic and relaxometric properties to be adequately predicted and controlled. To demonstrate the colloidal stability of the particles, a weeklong DLS assay experiment is necessary, in parallel with zeta potential measurements (electrostatic charge of NPs). Then, the particles are submitted to a comprehensive set of physico-chemical characterization measurements using high-resolution electron microscopy (HRTEM: particle size, morphology, crystallographic parameters), X-ray photoelectron spectroscopy (XPS: surface elemental analysis), Fourier transform infrared spectroscopy (FTIR: molecular groups at the surface of particles; molecular grafting assessment), thermogravimetric analysis (TGA: mass ratio between inorganic cores and organic coating), $^1$H-NMR relaxometry (measurement of $T_1$ and $T_2$), and magnetometric measurements, only to name the most important techniques. Finally, the elemental concentration (Fe, Gd, Mn) of colloidal NPs is measured by spectrometric or spectroscopic measurements by AAS, GF-AAS, ICP-OES, ICP-MS (mentioned in section 3), after careful digestion in appropriate acidic conditions.

5. Physical properties of magnetic nanoparticles

NPs based on SPIOs, USPIOs, Gd$_2$O$_3$, NaGdF$_4$, and MnO, have core diameters typically in the range 2 – 20 nm. However, superparamagnetic and paramagnetic nanoparticles have very different magnetic behaviors, whose influence on their relaxometric performance is major. USPIOs and SPIOs are said to be superparamagnetic, because they have no remanence after being introduced into, and retracted from, the strong magnetic field. Upon introduction in the scanner, the global magnetic moment of superparamagnetic NPs aligns in the direction of this magnetic field. As soon as the magnetic field is set back to zero (e.g. when the patient is removed from the scanner), the magnetic moment of the NP also goes down to zero. This is not the same behavior as for “bulk” ferromagnetic magnetite/maghemite, which are materials that clearly show a
strong remanence. The absence of residual magnetization is a very critical and useful aspect of superparamagnetism applied to biomedicine. Macroscopically, the magnetic behavior of superparamagnetic particles is similar to paramagnetism (e.g. Gd$_2$O$_3$, NaGdF$_4$), except that they feature an exceptionally high magnetic moment per unit of CA (the “core” of iron oxide particles). This strong magnetization is largely responsible for the remarkable “negative” CA properties of iron oxide NPs. In fact, the absence of magnetic remanence for USPIOs and SPIOs, is due to the return to equilibrium of the magnetic moments through Néel relaxation. On the other hand, paramagnetic NPs, and to a lesser extent antiferromagnetic MnO nanocrystals, do not develop strong magnetization at clinical magnetic field strengths. Instead, they generate signal enhancement (“positive” contrast) mainly through direct interactions taking place between Gd and Mn ions, and mobile $^1$H protons in their surroundings, as described in section 6. Here are introduced the basic differences in magnetism between superparamagnetic and paramagnetic NPs.

a) **Superparamagnetic NPs**, are very efficient “negative” CAs in MRI.[119] Because of their strong impact on the transverse relaxation times ($T_2/T_2^*$) of aqueous solutions, they have for been considered very useful products for molecular and cellular MRI.[103] As mentioned above, each superparamagnetic iron oxide NP features a high magnetic moment, and Curie constants much higher than for paramagnetic NPs. As a result, they respond quickly to the application of an external magnetic field, and their magnetization quickly becomes saturated at relatively low magnetic field strengths (Figure 6).

![Figure 6](image_url)

**Figure 6**: Magnetometric measurements of USPIOs and Gd$_2$O$_3$ nanoparticles (adapted from [44, 120]).
Superparamagnetism only occurs when NPs are small enough to belong to single magnetic domains. It is worth mentioning that suspensions of iron NPs (and not iron oxide nanoparticles) would have a much higher magnetization than magnetite/maghemite (about 5 times higher); however, iron NPs are every quickly oxidized into iron oxide NPs in aqueous media, and this potential technology, until now, could not be applied in biomedicine. Magnetite (Fe$_3$O$_4$) and maghemite (γ-Fe$_2$O$_3$) are two relatively similar forms of iron oxide (crystal structure and magnetic properties).[121, 122] Both are present in superparamagnetic iron oxide NPs, and it is often difficult to distinguish between them using common X-ray diffraction analysis techniques. Magnetite is typically preferred due to its superior magnetic properties [44]. Maghemite (Fe$^{3+}$[Fe$^{2+}$Fe$^{3+}$]O$_4$) often results from the oxidation of magnetite (Fe$^{3+}$[Fe$^{3+}$]$_{5/3}$V$_{1/3}$O$_4$, where V represents a cation vacancy). Bulk magnetite is ferromagnetic. The occurrence of an oxygen-mediated coupling mechanism aligns all the magnetic moments of the iron ions located in the tetrahedral sites of the crystal (8 crystallographic sites per unit structure), whereas all the magnetic moments of the octahedral ions (16 crystallographic sites per unit structure) are aligned in the opposite direction. It is assumed that the magnetic properties of magnetite are provided by uncompensated Fe$^{2+}$ ions, whereas for maghemite, they are provided by that of Fe$^{3+}$ ions.[123]

The magnetic energy of iron oxide NPs, depends upon the direction of its magnetization vector, and this vector in turn depends on the crystallographic directions (the magneto-crystalline anisotropy field).[121] The directions that minimize the magnetic energy are called anisotropy directions, or easy axes (Figure 7.a, from [121]). The resulting magnetic moment of a magnetite/maghemite crystal is preferentially aligned along such specific directions. The magnetic energy increases with the tilt angle between the magnetic vector of the easy directions.[124] For the sake of simplicity, the anisotropy of magnetite particles is often assumed to be uniaxial, with a single anisotropy axis. In fact, there are several anisotropy axes dictated by the oxide’s crystallographic structure. The anisotropy energy (the amplitude of the curve), is given by the product of the crystal volume (V) times a constant ($K_a$: the anisotropy constant).

\[ E_a = K_aV \]  

Equation 3

The anisotropy energy, proportional to V, determines the Néel relaxation time.

Large samples of bulk ferrimagnetic magnetite/maghemite, are divided into Weiss domains (represented in Figure 7.b) Inside each one of these volumes, the magnetic moments are aligned into one preferential direction; however, between each one of these domains, the magnetic moment is not oriented along the same direction. As iron oxide
nanocores (such as in USPIOs) are smaller than one of these domains, each NP is composed of a single domain whose magnetic moment is oriented in a specific direction.

![Diagram of magnetic behavior](image)

**Figure 7**: Magnetic behavior of iron oxide NPs (radius = 5 nm): a) uniaxial anisotropy for magnetite/maghemite nanoparticles (i.e. the probability of alignment of the magnetic moment in one direction with respect to the angle between this direction and the anisotropy axis); b) representation of Weiss domains in a large magnetite/maghemite crystal, compared with the dimensions of a typical NP (the small circle), much smaller than a Weiss domain; c) magnetization versus magnetic field for magnetite/maghemite NPs. With permission from [100, 121, 125].

In these single domains, the direction of the magnetic moment can flip from one orientation to the other. When the thermal energy, given by kT (k: Boltzman constant; T: absolute temperature) is sufficient to overcome this anisotropy energy barrier, the magnetization fluctuates between the different anisotropy directions, according to a characteristic time: the Néel relaxation time ($\tau_N$).[126] Although $\tau_N$ relaxation influences the hydrogen relaxation times by inducing changes to the magnetic moment of MNPs, it is a phenomenon entirely distinct from the nuclear relaxation mechanisms of hydrogen protons ($^1$H) described in section 6. Néel relaxation refers to the relaxation of the global electronic moment of a superparamagnetic crystal constituted by a ferri, ferro, or
antiferromagnetic compound. For dry powders of monodomain iron oxide NPs, \( \tau_N \) indicates the time it takes to the magnetization to come back to a state of equilibrium, after it was submitted to a strong magnetic field. For highly anisotropic crystals, the crystal magnetization is “locked” in the easy axes. The Néel relaxation defines the rate of fluctuations that arise from the jumps of the magnetic moment between the different easy axes (Figure 7.c). In order to flip from one easy direction to the other, the magnetization of a NP must jump over an anisotropy energy hump. For a superparamagnetic NP of specific \( V \) and \( K_a \), the Néel relaxation time \( (\tau_N) \) is given by an Arrhenius law that is similar to that describing the activation energy for a chemical reaction [127]:

\[
\tau_N = \tau_0(E_a) e^{\frac{E_a}{kT}}
\]  

Equation 4

where \( \tau_0(E_a) \) is the pre-exponential factor of the Néel relaxation time expression, that depends on factors such as the volume \( (V) \), the specific magnetization of the nanocrystal, and the gyromagnetic ratio of the electron.[44, 128, 129] Whereas the pre-exponential factor decreases as the value of anisotropy energy increases, \( \tau_N \) increases as an exponential function of \( V \) because of the second factor of Equation 4. For small values of the anisotropy energy and at high temperatures, \( E_a << kT \) (the exponential term tends to 1), and \( \tau_N \) is mainly determined by the preexponential term. These conditions are fulfilled, for instance, with individual ultra-small NPs of iron oxide of \( r < 4 \) nm. On the other hand, for high anisotropy energies, when \( E_a >> kT \) the evolution of \( \tau_N \) is mainly dictated by the exponential factor (fast increase with \( E_a \)).

According to equation 4, the flipping of the magnetic moment of magnetite/maghemite crystals is observed only for NPs of size \( r < 12 \) nm. Indeed, for magnetite (\( \tau_0 \approx 10^{-9} \) s, \( K \approx 13500 \) J m\(^{-3} \)), \( \tau_N \) goes from \(~500\) years for particles of \( r =15 \) nm, down to the ms for particles of about \( r = 10 \) nm. Practically, this means that for particles having a Néel relaxation time \( (\tau_N) \) longer than the measurement time, the magnetization curve of the NP system is irreversible and shows an hysteresis loop. These are referred to as “frozen single domain” NPs. NPs that demonstrate Néel relaxation times of several years, or centuries, can be used in computer hard disks. Indeed, such long-term data storage applications require as less as possible magnetic material, with a maximal anisotropy constant \( (E_a) \).

Such NPs cannot be used in MRI applications. First, the Néel relaxation of larger nanoparticles showing high \( E_a \), has no effect on the nuclear relaxation of neighboring water protons. Indeed, the diffusive rotation time of the particles in water, and the diffusion time of water around the particles are much shorter than 1 ms. This is incompatible with the long Néel relaxation times of large particles.
For magnetic iron oxide NPs to provide efficient properties as MRI CAs, the condition \( \tau_N < 1 \, \text{s} \) should be respected. Then, for NPs dispersed in a liquid media (a colloid), the return of the magnetization to equilibrium after application of a strong magnetic field is determined by both \( \tau_N \) and the Brownian relaxation \( \tau_B \) of the particles. The latter characterizes the rotation of the particle in the fluid, and takes into account the viscosity of the solvent (Figure 7.c). The global magnetic relaxation rate is a sum of two processes:

\[
\frac{1}{\tau} = \frac{1}{\tau_N} + \frac{1}{\tau_B}
\]

Equation 5

where \( \tau \) is the global magnetization time and \( \tau_B \) is the Brownian relaxation time, given by:

\[
\tau_B = \frac{3V\eta}{kT}
\]

Equation 6

where \( \eta \) is the viscosity of the solvent. For large particles, \( \tau_B < \tau_N \) because the Brownian component of the magnetic relaxation is proportional to the crystal volume (Equation 6) and the Néel relaxation is an exponential function of the volume (Equation 4). Therefore, in suspensions of large NPs, the viscous rotation of the particles in the liquid becomes the dominant process determining the global magnetic relaxation properties (Figure 7.d). As a result, the magnetic relaxation of suspensions of magnetic nanoparticles is much faster than for dry powders of iron oxide NPs.

In resume, superparamagnetism refers to a specific magnetic condition for which ultra-small particles of a size well inferior to typical Weiss domains of magnetite/maghemite materials, can be submitted to high magnetic field strengths, without evidences of magnetic remanence once they are retrieved (Figure 6). Macroscopically, the magnetization of iron oxide NPs suspensions is described by a Langevin function, whose shape depends on the saturation magnetization (\( M_{sat} \)) and the size of the magnetite crystals:

\[
M(B_0) = M_{sat}L(x)
\]

Equation 7

where \( M_{sat} \) is the magnetization at saturation, and \( L(x) \) is the Langevin function as:

\[
L(x) = \left[ \coth(x) - \frac{1}{x} \right]
\]

Equation 8

with:
Equation 9

\[
\chi = \frac{M_x(T)\nu B_0}{kT}
\]

Magnetization curves of ultra-small iron oxide NPs are perfectly reversible because the fast magnetic relaxation allows the system to be always at thermodynamic equilibrium.[130] Finally, because iron oxide superparamagnetic NPs obey this law, it is possible to estimate the NP core size from the fit of the magnetization curves (Figure 6).

\(b)\) Paramagnetic nanoparticles also respond to the application of an external magnetic field, by developing a magnetization vector oriented along the direction of this field, that slightly increased the local magnetic field strength.[131] Paramagnetic nanocrystals follows Curie’s law:

\[
M = \chi H = \frac{C}{T} H
\]

where \(\chi\) is the magnetic susceptibility, \(H\) the applied magnetic field (e.g. that of the MRI scanner), \(T\) the absolute temperature, and \(C\) a material-specific Curie constant. Unlike ferromagnets, paramagnetic materials do not retain magnetization in the absence of an external magnetic field, and the thermal energy is sufficient to randomize the induced magnetization. At similar concentrations of metal elements (Fe, Gd, or Mn), the “positive contrast effect” of paramagnetic NPs is less efficiently detected that the “negative contrast” effect generated by superparamagnetic agents. The more limited magnetization response of the rare-earth ions, compared with USPIOs and SPIOs, is due to their magnetic moment that is not saturated at magnetic fields strengths typical used in MRI.[6, 132] The difference of magnetization response between iron oxide (USPIOs) and paramagnetic Gd\(_2\)O\(_3\) nanoparticles is shown in Figure 6.

6. MR relaxation properties of magnetic nanoparticles

For a given concentration of magnetic atoms, the magnetization of paramagnetic CAs is much lower than that of superparamagnetic nanocrystals. However, Gd chelates are still, by far, the most widely used CAs in routine MRI. This is mainly due to their exceptional relaxometric properties. Both paramagnetic and superparamagnetic CAs interact with the small, hydrogen-containing mobile molecules contained in biological media. In fact, one of the most important factors influencing signal enhancement in MRI, is the binding of water molecules to the paramagnetic ions. Then, the motion of contrast agents in the fluid also has an impact, as well as the magnetic field strength-dependent electronic relaxation of the paramagnetic elements Finally, Gd\(^{3+}\) chelates are, in general, very efficiently excreted by the kidneys, and this also accounts for their widespread use in clinical MRI. This section does not aim at bringing a comprehensive explanation of the
complex relaxometric mechanisms taking place between paramagnetic – or superparamagnetic – CAs, and the water molecules. Several books and reviews have described this subject.[5, 6, 44, 133-135] Here is a resume of the most important aspects that must be taken into account when designing optimal nanoparticulate contrast agents for MRI applications.

a. Relaxivity of paramagnetic CAs

Theories to explain the relaxivity of paramagnetic CAs, have been developed since the inception of MRI. In fact, the efficiency of CAs is linked to molecular motions of the CA unit, as well to the motions of small molecules containing the “spins” (\(^1\)H protons). They are also linked to intrinsic properties of the nuclei (magnetic moment, gyromagnetic ratio, spin). The relaxation of paramagnetic solutions is mainly explained by two mechanisms: the “inner sphere” (IS) and “outer sphere” (OS) contributions.[6] From Equation 2, it is possible to dissociate two contributions for \(T_1\), one from the water protons in contact with the paramagnetic elements (p), and one for the rest of the water protons in the matrix (diamagnetic contribution, d):

\[
\left( \frac{1}{T_{1,obs}} \right) = \left( \frac{1}{T_{1,d}} \right) + \left( \frac{1}{T_{1,p}} \right)
\]

Equation 11

Where, \(i = 1, 2\). From the paramagnetic contribution, it is possible to dissociate the IS and the OS contributions, as follows:

\[
\left( \frac{1}{T_{1,obs}} \right) = \left( \frac{1}{T_{1,d}} \right)^{IS} + \left( \frac{1}{T_{1,p}} \right)^{OS}
\]

Equation 12

The IS relaxation relies on the exchange of energy between the spins and the electrons of the paramagnetic elements, which is facilitated when water molecules bind to the paramagnetic ions (Figure 8). The water molecules that bind to the paramagnetic centers (Gd\(^{3+}\), Mn\(^{2+}\)) ions, and denoted “p” water molecules (red circles in Figure 8), rapidly leave the first coordination sphere, and are immediately replaced by “fresh” molecules from the matrix (“d” water molecules: orange circles in Figure 8). In contact with the paramagnetic ions, hydrogen relax faster. The water residence time in the inner sphere (\(\tau_M\)) is in the order of \(\sim\) 1 ns and this means the relaxation effect propagates very fast to the rest of the solution (to “d” protons). Each one of the water protons that relax energy, participate in the decrease of the overall longitudinal relaxation time of the water solvent. The IS model has been described by the Solomon-Bloembergen-Morgan theory (SBM).[136, 137]
Figure 8: Schematic representation of the inner sphere (IS), outer sphere (OS), chemical exchange, and rotational correlation times guiding the paramagnetic relaxation.

From the inner sphere contribution, $T_1$ in the first coordination sphere is:

$$\left(\frac{1}{T_1}\right)^{IS} = f q \left(\frac{1}{T_{1M} + T_M}\right)$$  \hspace{1cm} \text{Equation 13}$$

where $f$ is the relative concentration of paramagnetic complex over water molecules, and $q$ is the number of water molecules in the first coordination sphere. The calculation of $T_{1M}$ is based on a model that includes the amplitude of the magnetic interaction, its temporal modulation and the effect of the strength of the external magnetic field, as follows:

$$\frac{1}{T_{1M}} = \frac{2}{15} \left(\frac{\mu_0}{4\pi}\right)^2 \gamma^2 \gamma_s^2 \hbar^2 S(S+1) \frac{1}{r^6} \left[ \frac{7\tau_{c2}}{1+(\omega_s \tau_{c2})^2} + \frac{3\tau_{c1}}{1+(\omega_H \tau_{c1})^2} \right]$$  \hspace{1cm} \text{Equation 14}$$

where:

$$\frac{1}{\tau_{cl}} = \frac{1}{\tau_R} + \frac{1}{\tau_M} + \frac{1}{\tau_{si}}$$  \hspace{1cm} \text{Equation 15}$$

$$\frac{1}{\tau_{s1}} = \frac{1}{5\tau_{SO}} \left[ \frac{1}{1+\omega_s^2 \gamma_s^2} + \frac{4}{1+4\omega_s^2 \gamma_s^2} \right]$$  \hspace{1cm} \text{Equation 16}$$

$$\frac{1}{\tau_{s2}} = \frac{1}{10\tau_{SO}} \left[ 3 + \frac{5}{1+\omega_s^2 \gamma_s^2} + \frac{2}{1+4\omega_s^2 \gamma_s^2} \right]$$  \hspace{1cm} \text{Equation 17}$$
and where $\gamma_S$ and $\gamma_H$ are the gyromagnetic ratio of the electron (S) and of the proton (H), respectively, $\omega_{S,H}$ is the angular frequencies of the electron and of the proton, $r$ is the distance between coordinated water protons and unpaired electrons spins, $\tau_{c1,2}$ is the correlation time modulating the interaction (defined by Equation 15), $\tau_R$ is the rotational correlation time of the hydrated complex, $\tau_{s1,2}$ is the longitudinal and transverse relaxation of the electron, $\tau_{S0}$ is the value of $\tau_{s1,2}$ at zero field, and $\tau_v$ is the correlation time characteristic of the electronic relaxation times.

The second term of Equation 12, the outer sphere relaxation (OS), is explained by the dipolar interaction at long-distance between the magnetic moment of the paramagnetic substance, and the nuclear spin of hydrogen protons. In fact, paramagnetic centers influence the local magnetic field around the $^1$H protons flowing in their vicinity. The complete equations explaining the outer sphere contribution of the longitudinal relaxation rate $\left(\frac{1}{T_1}\right)_{OS}$, are too complex to be detailed here, and the reader is invited to refer to the description of the OS model by Freed.[44, 138] The most important aspect to retain from the OS contribution in the case of paramagnetic substances, is the fact that the dipolar intermolecular mechanism is modulated by the translational correlation time ($\tau_D$) that takes into account the relative diffusion (D) of the paramagnetic center and the solvent molecule, as well as their distance of closest approach (d), as follows:[138]

$$\tau_D = \frac{d^2}{D} \quad \text{Equation 13}$$

This expression indicates that viscous solvents and large particles lead to high translational correlation times. In resume, the equations describing IS and OS contributions to the relaxivity of paramagnetic CAs are rather complex, and a large number of parameters influence the overall relaxometric performance of CAs: $\tau_M$, q, $\tau_R$, D, r, d, $\tau_v$, $\tau_{S0}$). Because of this high number of parameters, it is often difficult to perform an accurate theoretical estimation of the performance of MRI CAs at different magnetic field strengths. Relaxometric measurements must be performed experimentally, by using a technique called proton nuclear magnetic relaxation dispersion (NMRD). NMRD curves characterize the efficiency of CA at different magnetic fields. For more information about the interpretation of NMRD curves, the reader is invited to read a selection of references on the subject.[6, 44, 134]

Here are a few points that are important to know prior to the interpretation of NMRD profiles for paramagnetic solutions:

- **The rotational correlation time ($\tau_R$):** it characterizes the reorientation of the vector between the paramagnetic ions and the protons of the water molecule. For
a low molecular weight complex, $\tau_R$ limits the relaxivity of paramagnetic CAs at magnetic field strengths used in clinical MRI (0.5 – 3 Tesla). The value of $\tau_R$ cannot be measured by proton NMRD, but instead by $^{17}$O NMR measurements (longitudinal relaxation of the nucleus $^{17}$O), and other methods.[6]

- **Electronic relaxation times ($\tau_{S1}$ and $\tau_{S2}$):** Longitudinal and transverse electronic relaxation times describe the process of return to equilibrium of the magnetization associated to electrons that transit between electronic levels of the paramagnetic center. These transitions produce fluctuations that allow the relaxation of protons; $\tau_{S1}$ and $\tau_{S2}$ are magnetic field-dependent.

- **Number of coordinated water molecules ($q$)** strongly influences the IS contribution. For small complexes such as Gd-DTPA, the number of coordinated water molecules is equal to 1. It means that, in general, only one water molecule can bind to the paramagnetic Gd$^{3+}$ ion sequestrated in the DTPA molecule. The value of $q$ can be estimated either in solid phase (X-rays or neutron diffraction) or in solution [fluorescence of Eu or Yb complexes, LIS (lanthanide-induced shift) method in $^{17}$O-NMR].

- **Proton-metal distance ($r$):** The efficiency of the IS dipolar mechanism is proportional to $1/r^6$, where $r$ is the metal-proton distance. Small changes to this distance have a considerable impact on the relaxivity.

- **Coordinated water residence time ($\tau_M$):** The mechanism of IS relaxation is based on an exchange between water molecules surrounding the complex and the water molecules coordinated to the lanthanide ion. Consequently, the exchange rate ($k_{ex} = 1/\tau_M$) is an essential parameter for transmitting the relaxation effect to protons in the water matrix.[6]

### b. Relaxivity of superparamagnetic CAs

For superparamagnetic particles, the IS contribution to the relaxation is minor and often completely negligible as compared to the dominant OS contribution. As mentioned in the previous section, this contribution is largely dependent on the movement of water molecules near the local magnetic field gradients generated by the superparamagnetic nanoparticles. The relaxation of superparamagnetic NP suspensions, is generally governed by Freed’s equations when $\tau_{S1}$ is the Néel relaxation time.[139] When $\tau_D$ is much shorter than the Néel relaxation time, Freed’s equation are simplified. In fact, the ability of a fluctuation to relax the $^1$H proton spins depends upon whether its correlation time is longer or shorter then the precession period of the spins within the external magnetic field $B_0$. If the global correlation time $\tau_C (\tau_C^{-1} = \tau_D^{-1} + \tau_N^{-1})$ is longer than this period, the fluctuation is averaged by the precession and it is inefficient. It is efficient in the opposite situation. Other parameters such as electron polarization and crystal anisotropy have also a considerable influence on the relaxation times of water protons.
submitted to different magnetic fields. As in the case of paramagnetic CAs, the theoretical models explaining the relaxometry of aqueous suspensions of iron oxide NPs, must be validated by NMRD profiles (Figure 9). First, it is possible to precisely measure, with NMRD, the relaxometric potential of NP as contrast agents for MRI.[140] Then, NMRD profile analysis also represents a powerful tool to control the reproducibility of synthetic MNP suspensions, as well as for optimizing the parameters of nanomagnet synthesis.[141] Finally, the fitting of NMRD profiles by adequate theories provides information about the average radius (r) of the nanocrystals, their specific magnetization (Mₘ), their anisotropy energy (Eₐ), as well as their Néel relaxation time (τₙ).[119]

**Figure 9**: NMRD profile for magnetite particles in colloidal solution (from [134]).

The main information extracted from NMRD profiles, for superparamagnetic NP suspensions, is:

- **The average radius (r)**: at high magnetic fields, the relaxation rate depends only on τᵟ and the inflection point corresponds to the condition ωᵢτᵟD ∼ 1 (Figure 14). According to Equation 13, the determination of τᵟ gives the crystal size r (good complement to HRTEM measurements).
- **The specific magnetization (Mₘ)**: at high magnetic fields, Mₘ can be obtained from the equation Mₘ ≈ [(Rₘax/ (C · τᵟD)]¹/², where C is a constant and Rₘax the maximal relaxation rate.
• **The crystal anisotropy energy** ($E_a$): the absence or the presence of an inflection point at low magnetic field strengths ($10^{-2} – 1$) is an indication of the anisotropy energy. For crystals characterized by a high $E_a$ compared to the thermal agitation, the low field dispersion disappears. This was confirmed in a previous work with cobalt ferrites, a high anisotropy energy material.[142]

• **The Néel relaxation time** ($\tau_N$): the relaxation rate at very low fields ($R_0$) is governed by a “zero magnetic field” correlation time $\tau_{c0}$, which is equal to $\tau_N$ if $\tau_N <\tau_D$. However, this situation is often not met, and in this case $\tau_N$ is only reported as qualitative information.

c. **Relaxometric performance of MRI CAs at clinical magnetic field strengths**

Among polymer coatings that have been successfully used to enhance the blood retention of USPIOs, figure dextran and (carboxy, carboxymethyl)dextran. In particular, commercial products such as Ferumoxtran-10 (AMI-227), Ferumoxytol, and Supravist, are all based on iron oxide of core diameters in the range 4 – 8 nm, and of hydrodynamic sizes not larger than 30 nm (Table 1). At 1.5 Tesla, the longitudinal relaxivities ($r_1$) of those particles range between 9 and 15 mM$^{-1}$s$^{-1}$.143] As mentioned in the previous sections, the relaxometric ratio at clinical magnetic strengths (typically at 1.5 T) is used to classify the behavior of CAs between “positive” (i.e. $r_2/r_1 < 5$), and “negative” ones (i.e. $r_2/r_1 >> 10$). The relaxometric ratio of dextran and carboxy/carboxymethyl/dextran-coated USPIOs, is found between 2 and 5 (1.5 T). Finally, the blood half-lives of these products, from 6 to 36 hours, figure among the longest of all iron oxide NP systems. For such reasons, and in spite of the potential instability of dextran grafting at their surface, ferumoxtran, ferumoxyltol and supravist particles have been widely applied to MR molecular imaging. The following table summarizes the performance of a selection of commercial and pre-clinical products, based on both superparamagnetic and paramagnetic NPs.

**Table 1**: Relaxometric properties of MNPs measured at clinical MRI field strengths

<table>
<thead>
<tr>
<th>Product, commercial name, coating</th>
<th>NP core diam., TEM (nm)</th>
<th>Hydrodyn. size, DLS (nm)</th>
<th>$r_1$ (1.5T, 37°C)</th>
<th>$r_2/r_1$</th>
<th>Blood half-life, $T_{1/2}$ (species)</th>
<th>Use</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPIO (Ferumoxides, Endorem, Feridex), (AMI-25), dextran T10</td>
<td>~5</td>
<td>120 - 180</td>
<td>10.1</td>
<td>12</td>
<td>2 h (humans)</td>
<td>LI, CL</td>
<td>[144, 145]</td>
</tr>
<tr>
<td>SPIO, Resovist, Ferucarbotran (SHU-555A), Resovist</td>
<td>~10</td>
<td>60</td>
<td>9.7</td>
<td>19.5</td>
<td>2.4 – 3.6 h (humans)</td>
<td>LI, CL</td>
<td>[146]</td>
</tr>
</tbody>
</table>
USPIO, Ferumoxtran-10 (AMI-227), dextran T10 | 4.5 | 15 - 30 | 9.9 | 6.57 | 24-36 h (humans) | MLNI, MI, BPA, CL | [102]  
USPIO, Ferumoxytol-7228, carboxymethyldextran | 6.7 | 30 - 35 | 15 | 5.93 | 10-14 h (humans) | MI, BPA, CL | [144, 147]  
USPIO, Supravist (SHU-555C), carboxydextran | 3 - 5 | 21 | 10.7 | 3.55 | 6 h (humans) | BPA, CL | [148]  
USPIO, Feruglose NC100150, Clariscan, PEGylated starch111 | 6.43 | 11.9 | ~18 (* at 0.5 T) | n.a. | 6 h (humans) | BPA | [17, 149]  
VSOP (iron oxide), C184 citrate | 7 | 14 | 14 | 2.4 | 0.6 – 1.3 h (humans) | BPA, CL | [150]  
ESION (iron oxide), PO-PEG | 3 | 4.77 (* at 3 T) | 6.12 | > 10 min. (rat) | BPA | [151]  
USPIO bis-phosphonate-PEG | 5.5 ± 0.6 | 24 ± 3 | 9.5 (*at 3T, RT) | 2.97 | < 40 min. (mouse) | BPA | [152]  
Gd$_2$O$_3$ PEG-diacid | 2 | 9 – 11 | 14.2 | 1.2 | _ | CL | [76]  
NaGdF$_4$ citrate | < 5 | 6 - 9 | 3.37 | 1.18 | > 90 min. (mouse) | BPA, MRI-OI | [34]  
MnO bis-phosphonate-PEG dendrons | 6 - 8 | 13.4 – 16.2 | 4.4 | 8.6 | < 20 min. (mouse) | BPA | [118]  

*Use: LI: liver imaging; CL: cellular labeling; MLNI: metastatic lymph node imaging; MI: macrophage imaging; BPA: blood pool agent; MRI-OI: MRI-optical imaging

### 7. Biological performance of magnetic nanoparticles for MRI

MNPs for MRI applications are complex pharmaceutical constructs that must navigate the body either to provide a general contrast enhancement effect, or in search of a target. They are made of at least two, if not four or five different components (Figure 1): a central magnetically active core, a stabilizing shell, or coating, made of one or many types of biocompatible molecules, to which targeting ligands and additional imaging modalities are anchored. Therapeutic agents can also be embedded in the structure. MNPs must be biocompatible, and should not harm the patient. The behaviour of the nanoconstruct in vivo, as well as that of each one of the different components (blood retention, clearance kinetics, possible degradation resulting in metal ion leaching, polymer or drug elution, etc.), must be comprehensively investigated prior to approval by the health authorities. Finally, MNPs coated and functionalized, should preserve their relaxometric properties.

**a. In vivo barriers**

MNPs injected in vivo, must overcome several biological barrier either to reach their target, or to remain in the blood for prolonged times. Most MRI CAs are administrated through intravascular (i.v.) injections. MNPs immediately encounter blood, a high ionic...
strength, heterogeneous solution that contains high concentrations of organic molecules. Chemical binding and electrostatic interactions occur between these molecules and the MNPs, that can lead to dramatic changes in their hydrodynamic diameter, relaxometric properties, and colloidal stability. Agglomeration and surface charge effects can also accelerate the opsonization of MNPs by the immune system, which results in stronger and more rapid uptake by the macrophages. Depending on their molecular coating, MNP can interact more or less strongly with the extracellular matrix of cells and, in the case of binding, this can cause the NPs to be taken up by cells prematurely before they reach their target tissue.[153]

The NPs must also overcome different anatomical size restrictions which limit their access to target tissues (e.g. extravasation of lymph node-targeting NPs from the blood).[154] These size limitations are very stringent in the case of certain organs such as the brain and the kidneys.[155] For instance, only NPs of sufficient small sizes and appropriate physicochemical properties may pass the blood brain barrier, a structural and metabolic barrier consisting of endothelial cells and reinforcing astrocyte cells that protect the brain.[156] In addition to biological barriers present in the extracellular space (blood vessels, lymphatic conduits), intracellular barriers may also restrict the function of several biomedical NP systems. NPs that bind at the surface of targeted cells, are typically taken-up by such cells through a receptor-mediated endocytosis mechanisms. Upon ingestion in the cells, NPs are entrapped and “trafficked” through endosome compartments where they progressively degrade by acidification. The endosomes are progressively translocated into lysosomes, compartments in which hydrolytic and enzymatic reactions metabolise or evacuate macromolecules and NP debris.[157] Different strategies, more or less complex or potentially cytotoxic, have been developed to facilitate the escape of NPs from the endosomes.[158] Overall, each one of these biological obstacles illustrates the different levels of complexity that must be addressed when designing optimal MNPs as CAs for blood pool, cell labeling and tracking, targeted imaging, and drug delivery procedures.

b. Impact of nanoparticle size and surface on colloidal stability and blood retention

The ability of MNPs to remain in the blood for prolonged times and to pass biological barriers is largely related to their main physico-chemical characteristics: hydrodynamic size, surface charge, and type of coating.[155, 159, 160] The retention of NP in the blood vessels, the mechanisms of NP clearance, and the permeability of NPs from the vasculature, all strongly depend on these three parameters.[161-163] In particular, NP charge and hydrophobicity can affect NP biodistribution by limiting or enhancing interactions of NPs with the adaptive immune system, with plasma proteins,
with extracellular matrices and with non-targeted cells.[153] Specifically, hydrophobic and strongly charged NPs have short circulation times due to the adsorption of plasma proteins such as the opsonins, which are responsible for the recognition of MNPs by the reticuloendothelial system (RES). As a next step, the macrophages recognize opsonins and remove MNPs from the blood circulation.[160] Most of particles end up in the liver and in the spleen, where the particles eventually degrade. The sequestration of MNPs by the RES, can been advantageously used in the diagnosis of hepatic lesions (e.g. liver cancer). However, particles that are too rapidly sequestered and removed from the blood stream, cannot optimally fulfill targeted applications such as targeted imaging (e.g. for cancer, atherosclerotic plaque, and Alzheimer’s disease), and drug delivery to specific organs.

Hydrodynamic size strongly affects the clearance of NPs from the vascular circulation [37,47–51]. In general, small NPs (< 20 nm) can be more efficiently excreted by the kidneys,[164-166], whereas large NPs (> 50 nm) are mostly found in the liver and spleen. However, strongly charged particles such as citrate-capped iron oxide and paramagnetic nanocrystals, follow the RES route and are cleared in the liver.[98] As a consequence, and a minor fraction of them find their way through the urinary tracts.[150] Most endothelial barriers allow nanoparticles < 150 nm hydrodynamic diameter to pass; however, other barriers, such as the BBB, are far more restrictive. Because the liver can metabolize very large amounts of iron, USPIOs and SPIOs are in general well-tolerated products. As a matter of fact, Ferumoxides, Feromoxtran, Ferucarbotran, Supravist, Clariscan and a few other iron oxide-based NP systems have either been commercialized, or at least investigated in the clinics (see Table 1). In the case of paramagnetic nanoparticles that are not based on Gd or Mn chelates (MnO, Gd₂O₃, NaGdF₄), the toxicity risk that represents the massive injection of Mn and Gd-based crystals potentially leaching toxic Mn²⁺ and Gd³⁺ ions, greatly restricts their eventual transfer to clinical applications. However, emerging coating strategies, in particular those based on silane-PEG or phosphate/phosphonate-PEG (Figure 10), have proved to promote the rapid excretion of paramagnetic nanocrystals through the gastrointestinal and urinary routes.[118, 166] It is too soon for the moment to predict if these strategies could potentially allow the clinical use of paramagnetic nanocrystals in the future. However, they facilitate the development of more complex and specific “positive” CAs for molecular and celluar pre-clinical research.
Figure 10: Injections of MnO NPs PEGylated with phosphonate dendrons, and scanned in 1 T MRI; a) after 1h, strong evidences of CA elimination is found in the gall bladder (hepatobiliairy way); b) blood signal-enhancement persists at lest 20 minutes after injection, while evidences of CA clearance are found in the kidneys (b, 1h and c). Adapted from [118].

In order to be truly efficient for targeted molecular imaging applications, it is necessary for the MNP CAs to reach blood half-lives of many hours (such as for Feromuxtran, Ferumoxytol and Feruglose, Table 1). This way, the CAs have more chances to effectively bind to molecular epitopes and receptors that are expressed at the surface of vascular cells, and to which the targeted CAs are designed to reach. Surface modification with hydrophilic molecules dextran and polyethylene glycol (PEG), have been shown to reduce opsonization, leading to prolonged NP circulation times (Table 1, and [167]).

c. Directing nanoparticles in vivo

The specificity of NPs for select tissues is critical in MRI-based diagnostic imaging. [16,74,75]. NPs can be engineered to have an affinity for target tissues through passive, active, and magnetic targeting approaches. Passive targeting uses the predetermined physicochemical properties (size and surface charge) of a given NP to specifically migrate to a given tissue region. In particular, the enhanced permeation and retention effect (EPR) can be used to target solid tumour tissue.[76] Tumor cells, in an effort to grow rapidly, stimulate the production of new blood vessels (termed « neovasculature »). Such vessels are poorly organized and have leaky fenestrations. This enables the extravasation of NPs out of the vasculature, into the tumor tissue [77,78]. Then, because the lymphatic drainage is relatively inefficient in solid tumors, NPs tend to accumulate at this site[79,80]. However, the EPR effect is limited to specific metastatic solid tumors, and the successful implementation of CA systes relying on the EPR effect,
is dependent upon a number of factors including the degree of capillary disorder, blood flow, and the lymphatic drainage rate. As a result, it is not possible to base a therapeutic treatment, or a MR diagnostic, only by relying on the EPR effect.

However, passive targeting does work only for very specific biomedical application, such as for solid tumor of leaky vasculature. Neither does it guarantee their internalization by targeted cells. To achieve this, it is necessary to modify the NPs with molecular targeting ligands, to provide an “active” approach. NPs functionalized with molecules that specifically bind to molecular epitopes expressed at the surface of cancer cells, or other diseases tissues, allow a more efficient way to concentrate NPs selectively to the tissue to be diagnosed, or treated (81, 82). In particular, a number of iron oxide nanoparticle systems have been developed and tested in vivo, with varying success. Among targeting molecules that have been used so far to achieve active targeting with iron oxide NPs, figure small organic molecules [81,83,84], peptides [71,85–88], proteins [89], antibodies [90–92], and aptamers [93–95].

In addition to engineering NPs for tissue targeting, external magnetic fields can be used to assist the diffusion of MNPs to given organs. This strategy is referred to as “magnetic targeting” [18,104]. It consists in focusing high fields, high gradients, or strong rare earth magnets on the target organ, or biological site. This is a good strategy to accumulate high-susceptibility MNPs at specific sites, to conduct hyperthermia treatments.[100] This technique was successfully implemented in a clinical trial to deliver the chemotherapeutic doxorubicin to hepatocarcinoma cells [105]. The effectiveness of magnetic targeting is unfortunately limited to target tissues that are located close to the surface of the body (rapid loss of magnetic field strength away from the magnets).

d. Toxicity

In order to generate significant signal-enhancement effects, NPs used as MRI CAs must be injected at relatively high dosage compared with common tracers used in nuclear medicine (e.g. PET). Therefore, NPs must be demonstrated safe for cells and for different tissues, in particular when high quantities find their way to critical organs (liver, kidneys, spleen). The impact of MNPs on the proliferation and on the viability of different cell types is always demonstrated in vitro prior to injection in vivo. Depending on the concentration, type of particle, surface charge, and class of coating ligand, the presence of high concentration of nanoparticles in the vicinity of cells, can have a transitory effect on their cell division cycle, and sometimes influence their viability. Apoptose measurements can also be performed at high dosages of MNPs, to evaluate the risk induced by high concentrations of MNPs to cells. The cells selected for in vitro tests should ideally represent either the tissues that are expected to receive the highest
concentrations of MNPs, or the cells that are the most susceptible to be affected by MNPs. As an example, for assessing the biocompatibility of ultra-small MNPs for targeted vascular imaging, epithelial vascular, kidney, and hepatic cell lines could be used.

Finally, MNPs are not intact when they are excreted by body. They are confronted to different biological mechanisms that impact their integrity. Nanosystems for medical applications face as very important challenge: it is necessary to evaluate the toxicity of both the intact products, and of their different components. For instance, the potential leaching on metal ions from MNPs in different organs must be carefully and comprehensively quantified. Also, the impact of the different NP coatings, as well as their degradation products, on specific cells and organ in which they could potentially accumulate, is also an important aspect of the toxicity evaluation of MNPs. The nature of the degradation by-products, must also be addressed.[168] Nanotoxicology is an emerging and expanding research area, and a selection of works have been written to specifically address this topic.[155, 169, 170]

8. Conclusion

The recent advances in synthesis and characterization of MNPs as MRI CAs, has allowed the emergence of a variety of new biomedical applications: stem cell labeling and tracking in vivo, imaging-assisted drug and gene delivery, molecular targeting of chronic diseases such as atherosclerosis and cancer. Because of their very strong impact on the transverse relaxivity, superparamagnetic NPs in particular, have been used in a variety of clinical applications (liver, spleen, lymph nodes imaging). In this chapter were reviewed the basic principles of MNPs for MRI applications. The main parameters and conditions guiding their optimal design, use, and performance in biological applications, were presented. Because the relaxometric potential of MNPs is very dependent on their size, fine particles of narrow size distributions are developed, then coated with biocompatible molecules that provide enhanced colloidal stability in physiological environments through electrostatic and steric repulsion mechanisms. The biocompatibility of nanoparticles must be assessed in vitro, prior to measuring their magnetic and relaxometric properties. Finally, the biological kinetics (blood retention, organ uptake, clearance) and contrast-enhancement effects of each new nanoparticulate system, must be carefully studied in vivo. NPs are complex systems, and the medical health authorities enforce very strict requirements over the design, production reproducibility, potential toxicity, and pharmacokinetics performance of such injectable products. Finally, the expansion of hybrid imaging modalities (MRI/PET, MRI/luminescence, MRI/SPECT, MRI/echography), call for the development of multifunctional and increasingly complex imaging tracers. For instance, stem cell therapies could advantageously been conducted
using MRI/PET, which would enable a more sensitive detection and quantitation of areas of implanted stem cells. The delivery of targeted drugs through nanovectors could also be performed under MRI/PET guidance, to provide quantitative measurements of the residual concentration of drug delivery vehicles still present in the blood, then accumulating into specific organs. The use of MNP-labeled nanosized targeted drug carriers would enable to record real-time, personalized pharmacokinetic profiles of drugs that more efficiently finding their ways to target organs.

9. References


