CHAPTER 4

Iron-oxide Nanoparticle-based Contrast Agents

DIMITRI STANICKI,^a LUCE VANDER ELST,^{a,b} ROBERT N. MULLER,^{a,b} SOPHIE LAURENT,^{*a,b} DELPHINE FELDER-FLESCH,^c DAMIEN MERTZ,^c AUDREY PARAT,^c SYLVIE BEGIN-COLIN,^{*c} GEOFFREY COTIN,^c JEAN-MARC GRENECHE,^d OVIDIU ERSEN,^c BENOIT PICHON,^c VLAD SOCOLIUC,^e VICTOR KUNCSER,^{*f} RODICA TURCU,^g LADISLAU VÉKÁS,^{*e} PAULA FOSTER^{h,i} AND ROBERT BARTHA*^{h,i,j}

^a Laboratory of NMR and Molecular Imaging, Department of General, Organic and Biomedical Chemistry, University of Mons, 19 7000, Mons, Belgium; ^bCMMI – Center for Microscopy and Molecular Imaging, 8 6041, Gosselies, Belgium; ^cIPCMS UMR Centre National de la Recherche Scientifique, 67034, Strasbourg, France; ^d IMMM UMR CNRS 6283 Université du Maine, 72085, Le Mans, France; ^e Romanian Academy-Timisoara Branch, Center for Fundamental and Advanced Technical Research, Lab. Magnetic Fluids, RO-300223, Timisoara, Romania; ^fNational Institute of Materials Physics, 077125, Bucharest-Magurele, Romania; ^g National Institute R&D for Isotopic and Molecular Technologies, RO-400293, Cluj-Napoca, Romania; ^h Robarts Research Institute, University of Western Ontario, London, ON N6A 3K7, Canada; ⁱ Department of Medical Biophysics, University of Western Ontario, London, ON N6A 3K7, Canada; ^j Departments of Medical Imaging and Psychiatry, University of Western Ontario, London, ON N6A 3K7, Canada *Email: Sophie.Laurent@umons.ac.be; Sylvie.Begin@ipcms.unistra.fr; vekas@acad-tim.tm.edu.ro; rbartha@robarts.ca

New Developments in NMR No. 13

Contrast Agents for MRI: Experimental Methods

Edited by Valérie C. Pierre and Matthew J. Allen

[©] The Royal Society of Chemistry 2018

Published by the Royal Society of Chemistry, www.rsc.org

4.1 General Theory of the Relaxivity of Particulate Contrast Agents

DIMITRI STANICKI, LUCE VANDER ELST, ROBERT N. MULLER AND SOPHIE LAURENT*

4.1.1 General Introduction to Iron Oxide Nanoparticles

By reducing the size from bulk to the nanometer scale (<20 nm), ferrimagnetic iron oxide material acquires a magnetic property called superparamagnetism. When submitting superparamagnetic objects to an external magnetic field, their global magnetic moments align in the direction of the field. When the field is decreased to zero, however, the magnetic moment of the samples instantaneously returns to zero. Their magnetic properties and benign character led magnetic iron oxide nanoparticles to be extensively used in many biomedical applications, including magnetic resonance imaging (MRI). Some iron-oxide-based systems have been used clinically for the detection of hepatic or spleen anomalies. The emergence of areas such as cell tracking, magnetic hyperthermia, drug delivery, molecular imaging, and magnetic-particle imaging have encouraged researchers to pursue the development of efficient procedures to provide nanoparticles with specific desired properties. For example, to be used efficiently in biorelated applications, magnetic particles often must have specific magnetic properties, small size, narrow size distribution, specific surface functionality, and well-defined morphologies in addition to being nontoxic and biocompatible. Each of these characteristics can be achieved by the optimization of preparation processes.

The development of nanomaterials with interesting intrinsic properties has opened new exciting possibilities to solve unresolved challenges in several domains, such as medicine,^{1–3} data storage,⁴ and catalysis.⁵ In particular, magnetic nanomaterials like iron oxide are widely exploited in biomedical applications as contrast agents for MRI, for drug delivery, and in hyperthermia.^{6–12} The following characteristics are the main advantages of magnetic iron oxide nanostructures for such applications: a high concentration of superparamagnetic ions in a single unit; the ability to modulate size and surface as a function of desired applications; the possibility and biocompatibility; low toxicity for patients suffering from kidney disorders; and low price of production.

Despite these advantages, a challenging aspect of using iron oxide nanoparticles is that the morphology of the nanomaterials strongly influences their physicochemical properties and, in particular, their magnetic properties. Consequently, a difficulty of this field is the synthesis of magnetic nanoparticles with controlled diameters and size distributions. The colloidal instability and agglomeration of nanostructures in aqueous suspension must also be carefully addressed. Because nanoparticle production is important, controlling the surface of nanoparticles is a challenge in the biomedical field.^{13,14}

Magnetite, $FeO \cdot Fe_2O_3$, and maghemite, γ -Fe₂O₃, exhibit inverse spinel structures with the general formula of AB₂X₄, where A and B are cations and X are anions.¹⁵ Both of these materials adopt a face-centered cubic crystalline structure. The inverse spinel structure of magnetite contains both ferric and ferrous ions. Thirty-two oxygen anions delimit a face-centered cubic unit cell in which the iron ions are located on eight tetrahedral sites surrounded by four oxygen ions and 16 octahedral sites surrounded by eight oxygen ions. The tetrahedral sites are exclusively occupied by ferric ions, whereas the octahedral sites are alternately occupied by ferrous and ferric ions (Figure 4.1). The composition of a unit cell of magnetite is thus $[Fe^{3+}]_{tetrahedral}[Fe^{3+}Fe^{2+}]_{octahedral}O_4$ or $FeO \cdot Fe_2O_3$. Because of superexchange-oxygen-mediated coupling, all the magnetic moments of the octahedral iron ions are aligned in a defined direction, and all magnetic moments of the tetrahedral iron ions are aligned in the opposite direction. Because ferric ions are equally distributed between octahedral and tetrahedral sites, they compensate for each other. Consequently, the resulting moment of a magnetite crystal arises solely from the uncompensated octahedral ferrous ions.

On the other hand, maghemite, which is the oxidized form of magnetite, is composed solely of trivalent iron ions. The oxidation of Fe^{II} to Fe^{II} creates vacancies in the octahedral sites. The formula of maghemite is $[Fe^{3^+}]_{tetrahedral}[Fe^{3^+}_{2/3\Upsilon 1/3}]_{octahedral}O_4$.

Magnetic materials can be classified in two categories according to their response to an applied magnetic field: (1) "weak" magnetic materials, which are diamagnetic and paramagnetic, and (2) "strong" magnetic materials,



Figure 4.1 Inverse spinel structure of (a) magnetite and (b) maghemite (the black balls are Fe^{II}, the green balls are Fe^{III}, and the red balls are O^{2⁻}). Adapted with permission from W. Wu, Z. Wu, T. Yu, C. Jiang and W.-S. Kim, *Sci. Technol. Adv. Mater.*, 2015, 16, 023501 (https://creativecommons.org/ licenses/by/3.0/).

which include ferromagnetic, ferrimagnetic, and antiferromagnetic structures.

Magnetic materials are generally defined by three important parameters: their dipolar magnetic moment, their magnetization, and their magnetic susceptibility. The dipolar magnetic moment is defined as an expression of the vector magnitude resulting from the electron motion in atoms, or from the electron spin, leading to the formation of a magnetic field. The magnetic moment is represented by the vector μ . If an external magnetic field is applied on the material, magnetic moments tend to align in the same direction. This phenomenon creates a magnetization (*M*) defined as a magnetic moment per volume. The magnetic susceptibility, χ , is a constant value that indicates the magnetization level of a material when it is subjected to an external magnetic field.

Diamagnetic compounds are molecules that have zero orbital angular momentum, like Eu^{III} in the ground state, or contain no unpaired electrons, such as noble gases, diatomic gases, and most organic molecules. When an external magnetic field is applied, these substances induce a low opposite magnetization close to zero. Consequently, in this case, the magnetic susceptibility is negative.

Paramagnetic materials do not exhibit a spontaneous magnetization. If a magnetic field is applied, however, their spins will align in the direction parallel to the magnetic field. As a consequence, magnetization increases as a function of the intensity of the applied magnetic field. Paramagnetic materials have unpaired electrons. Examples include some transition metal salts, such as potassium chromium(III) sulfate dodecahydrate (KCr(SO₄)₂ · 12H₂O), and some lanthanide ions, such as Gd^{III}.

In the absence of an external magnetic field, diamagnetic and paramagnetic compounds do not exhibit a permanent magnetization. Some other magnetic compounds, however, have a high magnetization state even without an external magnetic field. These compounds are either ferromagnetic, antiferromagnetic, or ferrimagnetic (Figure 4.2) and exhibit some particular magnetic properties.



Figure 4.2 Schematic representations of (a) ferromagnetic, (b) antiferromagnetic, and (c) ferrimagnetic materials.

Ferromagnetism is the magnetism resulting from the alignment of permanent magnetic moments. In such materials, the magnetic moments are parallel to each other due to a strong interaction called ferromagnetic coupling.¹⁶ These materials display strong magnetization with an applied magnetic field and can retain magnetization when the external magnetic field is removed. This retention of magnetization is called magnetic remanence. Ferromagnetic materials can be considered to be permanent magnets.¹⁷

The electron spins of antiferromagnetic materials form a regular pattern with neighboring spins aligned in opposite directions. The resulting magnetization is lower than that of ferromagnetic compounds due to the interactions between spins.

As in antiferromagnetic materials, the electron spins of ferrimagnetic compounds are antiparallel. In ferrimagnetic compounds, however, the adjacent spins have different magnitudes and their magnetic moments are distributed in different subnetworks. The spontaneous magnetization of ferrimagnetic compounds stems from the unequal magnetization of spins.¹⁸

Most iron oxide material used as contrast agents for MRI are either magnetite or maghemite. Small crystals (4–15 nm diameter) of such materials are fully magnetized and have large magnetic moments. These nanocrystals are smaller than a magnetic domain and do not present magnetic remanence. They are thus called superparamagnetic nanoparticles.

Iron oxide nanoparticles can be classified into different categories according to their hydrodynamic size (see Section 4.4 for size measurement techniques): (1) ultrasmall superparamagnetic iron oxides (USPIO), which are characterized by a hydrodynamic diameter less than 50 nm: subsets of USPIO include very small particles of iron oxide (VSOP) that are between 7 and 9 nm wide and monocrystalline iron oxide nanoparticles (MION) that are between 10 and 30 nm wide; (2) superparamagnetic iron oxides (SPIO), which have a hydrodynamic diameter larger than 50 nm; and (3) micronsized iron oxide particles (MPIO).

UPSIO are single crystals usually embedded in a coating. SPIO, on the other hand, consist of several superparamagnetic cores distributed in a same nanosystem. Table 4.1 gives some examples of iron oxide nanoparticles together with their relaxivity (see Chapter 2.1 for a discussion of relaxivity) and their applications as contrast agents in MRI.

4.1.2 Mechanisms of Relaxation of Iron Oxide Nanoparticles

Relaxation induced by superparamagnetic iron oxide contrast agents cannot be described by a uniquely theoretical approach. The size, the distribution in size, and the morphological properties of the particles are all important parameters that need to be taken into account. In this section, the basic theory valid for USPIOs with diameters larger than 7.5 nm will first be described. The basic assumption of this model is that samples are composed of homogeneous dispersions of identically sized spherical iron oxide

Table 4.1 Examples of iro Oxide Nanopar L. Helm and É.	n-oxide-based contrast agents. Data from S. Lauren cicles for MRI, in <i>The Chemistry of Contrast Agents</i> Tóth, Wiley, 2nd edn, 2013, p. 427. © 2013 John V	ıt, L. Vander Elst and R. N. I s <i>in Medical Magnetic Resol</i> Wiley & Sons, Ltd.	Muller, Superparamagnetic Iron nance Imaging, ed. A. Merbach,
Short name/generic name/trade name	Iron oxide core diameter (TEM)/hydrodynamic diameter (PCS) coating	Relaxivity $(s^{-1} \text{ mM}^{-1})$ (37 °C, 1.5 T)	Ref. (with some examples of MRI applications)
AMI-227 Ferumoxtran	4–6 nm 20–40 nm	$r_1 = 19.5$ $r_2 = 87.6$	19–23
Sinerem [®] , Combidex [®] SHU 555C Ferucarbotran Supravist [®]	Dextran 3-4 nm 30 nm (17-46 nm) Carboxydextran	$r_1 = 10.7$ $r_2 = 38$	20,23–26
NC100150 Ferruglose PEG-feron Clariscan®	5–7 nm 20 nm Carbohydrate polyethyleneglycol	$r_1 = 20$ $r_2 = 35$ (0.47 T)	20,21
Ferumoxytol	 6.7±0.4 nm 30±2 nm Semi-synthetic carbohydrate (polyglucose sorbitol carboxymethyl ether) 	$r_1 = 15$ $r_2 = 89$	22,23,27,28
MION-46	4.5±1.2 nm 8-20 nm Dextran	$r_1 = 3.95$ $r_2 = 19.6$	21,29–34
VSOP-C184	4–5 nm 7–9 nm Citrate	$r_1 = 14$ $r_2 = 33.4$	20,35–39
AMI-25 Ferumoxide Endorem [®] , Feridex [®]	4.8–5.6 nm 80–150 nm Dextran	$r_1 = 9.95$ $r_2 = 158$	19-22,25,29,30
SHU 555A Ferucarbotran Resovist [®]	4.2 nm (3–5 nm) 62 nm Carboxydextran	$r_1 = 9.7$ $r_2 = 189$	19,21,24,25

Iron-oxide Nanoparticle-based Contrast Agents

323

nanocrystals. The relaxation induced by USPIOs with diameter smaller than 7.5 nm will then be discussed. The basic theory of USPIO can be adapted to structures of agglomerated SPIO particles.

Evaluating and understanding the efficiency of magnetic iron oxide nanosystems requires a theory describing the magnetic interactions between nanoparticles and water protons. Nuclear magnetic relaxation dispersion (NMRD) profiles, which describe the field dependence of the longitudinal proton relaxation rate, provide a powerful tool for evaluating the theory (see Chapter 2.3).^{40,41} Moreover, NMRD profiles are important for monitoring the quality of synthesized nanoparticles.⁴²

For superparamagnetic particles, inner-sphere contributions to relaxation are minor and outer-sphere contributions are dominant. The classical outersphere model provides longitudinal (R_1) and transverse (R_2) relaxation rates of water protons that diffuse near the unpaired electrons responsible for the magnetization of the particle.⁴³ The magnetic moments of superparamagnetic iron oxide nanoparticles are much larger than the electron moments but, at first approximation, are not qualitatively different. Curie relaxation⁴⁴ plays a central role in the reformulation of the basic theory and highlights the necessity to consider different time-scales. With respect to studying outersphere contributions, two parameters have to be examined separately: (1) the effect of water diffusion through the field inhomogeneities created by the time-averaged value of the particle magnetic moment—the dependence of this parameter on the external static field is governed by a Langevin function—and (2) the effect of the fluctuation of the magnetic moment itself.⁴⁵

This model fits well for the high-field part (>1 MHz) of NMRD profiles, but it fails to explain the low-field (<1 MHz) behavior characteristic of USPIO. The NMRD profiles of USPIO show a slight dispersion at low field below 1 MHz. This dispersion completely disappears for SPIO (Figure 4.3).⁴⁶ This behavior with respect to dispersions can be explained by considering the crystal anisotropy energy, which reflects the qualitative difference between superparamagnetic and paramagnetic compounds. Outer-sphere theory assumes an isotropic environment for unpaired electrons, a highly questionable assumption for superparamagnetic nanoparticles because an anisotropic field exists within such particles and forces the magnetic moment of the particle to align along the axes of easy magnetization.

The high magnetization of these compounds has a great influence on the surrounding water relaxation rate. The superparamagnetic relaxation phenomena are described by the Roch–Muller–Gillis model (also called RMG model or SPM model).^{46–48} This theory is based on the classical outer-sphere relaxation theory, but is extended to account for materials with a strong anisotropy. The relaxation induced by superparamagnetic nanoparticles is due to the coupling between the magnetic moments of water protons and the electron magnetic moments of particles. This modulation is caused by Néel relaxation (flipping of the magnetization vectors of particles from one easy axis to another), the diffusion of water protons, and the strength of the external magnetic field.





4.1.2.1 Theory for Large Crystals and Particles with High Anisotropy

Copyright (2008) Americal Chemical Society.

The anisotropy barrier is high for large crystals with a radius >7.5 nm. This high barrier induces locking of the magnetic moments of superparamagnetic materials on one anisotropy axis when high magnetic fields are applied. Depending on the strength of external magnetic field, three limiting cases can be described at low, high, and intermediate fields:

(1) Low magnetic fields. At low magnetic fields magnetic moments can be easily moved from one anisotropy direction to another one, thereby causing drastic magnetic fluctuations on water diffusion in the vicinity of magnetic particles. In this case, the dipolar interactions between water protons and magnetic cores are modulated by the translational correlation time of the water molecules (τ_D) and the Néel relaxation time (τ_N). Both modulations define the global correlation time (τ_{CI}) with $\tau_D = r^2/D$, where *r* is the crystal radius and *D* is the diffusion constant [eqn (4.1)].

$$\frac{1}{\tau_{\rm CI}} = \frac{1}{\tau_{\rm N}} + \frac{1}{\tau_{\rm D}} \tag{4.1}$$

The proton longitudinal (R_1) and the transversal (R_2) relaxation rates can in turn be expressed by the Freed spectral density [eqn (4.2)–(4.5)], where γ is the proton gyromagnetic ratio, μ is the electron magnetic moment, N_A is Avogadro's number, *C* corresponds to the molar concentration of superparamagnetic particle, r is the crystal radius, and $\omega_{\rm I}$ is the proton angular frequency.⁴⁹

$$R_{1} = \frac{1}{T_{1}} = \frac{32\pi}{405000} \gamma^{2} \mu^{2} \left(\frac{N_{\rm A}C}{r^{3}}\right) [10J_{\rm F}(\omega_{\rm I}, \tau_{\rm D}, \tau_{\rm N})]$$
(4.2)

$$R_2 = \frac{1}{T_2} = \frac{32\pi}{405000} \gamma^2 \mu^2 \left(\frac{N_{\rm A}C}{r^3}\right) [8J_{\rm F}(\omega_{\rm I},\tau_{\rm D},\tau_{\rm N}) + 2J_{\rm F}(0,\tau_{\rm D},\tau_{\rm N})] \quad (4.3)$$

with

$$J_{\rm F}(\omega_{\rm I},\tau_{\rm D},\tau_{\rm N}) = R_{\rm e} \left[\frac{1 + \frac{1}{4}\Omega^{\frac{1}{2}}}{1 + \Omega^{\frac{1}{2}} + \frac{4}{9}\Omega + \frac{1}{9}\Omega^{\frac{3}{2}}} \right]$$
(4.4)

and

$$\Omega = i\omega_{\rm I}\tau_{\rm D} + \frac{\tau_{\rm D}}{\tau_{\rm N}} \tag{4.5}$$

(2) High magnetic fields. At high magnetic fields, the magnetization vector aligns along one of the easy axes. In this case, the Néel relaxation time is relatively long due to the high anisotropy of the particles. Therefore, the modulation of their relaxation results solely from the diffusion of water protons. This modulation is described by the Ayant spectral density.⁵⁰ The relaxation rates can then be described using eqn (4.6)-(4.7).

$$R_{1} = \frac{1}{T_{1}} = \frac{32\pi}{405000} \gamma^{2} \mu^{2} \left(\frac{N_{\rm A}C}{r^{3}} \right) \left[9L^{2}(\alpha) J_{\rm A}(\sqrt{2\omega_{\rm I}\tau_{\rm D}}) \right]$$
(4.6)

$$R_{2} = \frac{1}{T_{2}} = \frac{32\pi}{405000} \gamma^{2} \mu^{2} \left(\frac{N_{\rm A}C}{r^{3}}\right) \left[\frac{9}{2} J_{\rm A}\left(\sqrt{2\omega_{\rm I}\tau_{\rm D}}\right) + 6J_{\rm A}(0)\right]$$
(4.7)

with

$$J_{\rm A}(\mu) = \frac{1 + \frac{5\mu}{8} + \frac{\mu^2}{8}}{1 + \mu + \frac{\mu^2}{2} + \frac{\mu^3}{6} + \frac{4\mu^4}{81} + \frac{\mu^5}{81} + \frac{\mu^6}{648}}$$
(4.8)

(3) Intermediate magnetic fields. The water relaxation induced by superparamagnetic particles at intermediate fields is modulated both by the low and the high field contributions. A linear combination of the equations with a Langevin function $[L(\alpha)]$ must be used to take into account the fact that the magnetization is divided into two parts: the first part is locked with the external magnetic field, and the second part is influenced by Néel relaxation [eqn (4.9) and (4.10)].

Iron-oxide Nanoparticle-based Contrast Agents

$$R_{1} = \frac{1}{T_{1}} = \frac{32\pi}{405000} \gamma^{2} \mu^{2} \left(\frac{N_{A}C}{r^{3}}\right) \left\{ \left(\frac{L(\alpha)}{\alpha}\right) 21 J_{F}(\omega_{I}, \tau_{D}, \tau_{N}) + 9 \left[1 - L^{2}(\alpha) - 2\left(\frac{L(\alpha)}{\alpha}\right)\right] J_{F}(\omega_{I}, \tau_{D}, \tau_{N}) + 9L^{2}(\alpha) J_{A}(\sqrt{2\omega_{I}\tau_{D}}) \right\}$$

$$(4.9)$$

$$R_{2} = \frac{1}{T_{2}} = \frac{32\pi}{405000} \gamma^{2} \mu^{2} \left(\frac{N_{A}C}{r^{3}}\right) \left\{ \left(\frac{L(\alpha)}{\alpha}\right) 19.5 J_{F}(\omega_{I}, \tau_{D}, \tau_{N}) + \left[1 - L^{2}(\alpha) - 2\left(\frac{L(\alpha)}{\alpha}\right)\right] \frac{9}{2} [J_{F}(\omega_{I}, \tau_{D}, \tau_{N}) + 6J_{F}(0, \tau_{D}, \tau_{N}] + L^{2}(\alpha) \left[\frac{9}{2} J_{A}(\sqrt{2\omega_{I}\tau_{D}}) + 6J_{A}(0)\right] \right\}$$

$$(4.10)$$

In conclusion, for large crystals, relaxation is modulated differently depending on the strength of the external magnetic field. This theory predicts the evolution of water proton relaxation rate with magnetic field, as shown in Figure 4.4.

The RMG model enables a nearly perfect fitting of experimental data for large crystals (Figure 4.3A). However, this model does not fit well the dispersion profiles of particles with low anisotropy energies (r < 7.5 nm).



Figure 4.4 Contributions to proton relaxation in the simplified model for crystals of large anisotropy.¹³
 Adapted with permission from S. Laurent, D. Forge, M. Port, A. Roch, C. Robic, L. Vander Elst and R. N. Muller, *Chem. Rev.*, 2008, 108, 2064. Copyright (2008) American Chemical Society.

In these cases, experimental NMRD profiles have dispersions at low frequencies that do not agree with theoretical predictions. An example is shown in Figure 4.3B. For particles with relatively low anisotropies, an adaption of the RMG model thus had to be developed.

4.1.2.2 Theory for Small Crystals and Particles with Relatively Low Anisotropy

Small nanoparticles (particle radius <7.5 nm) have small anisotropic energy, and their magnetic moments can fluctuate between easy magnetization axes. A different model that incorporates the anisotropy energy as a quantitative parameter is thus needed to explain the superparamagnetic relaxation of small magnetic cores. Roch and co-workers developed an alternative method to include this parameter.^{13,46} This model reproduces the gradual vanishing of the low field dispersion through a linear combination of the rate for infinite and zero anisotropy energy, as described by eqn (4.11) and (4.12), where *P* is a weighting factor of the linear combination. The modified model enables a good interpretation of the relaxation for small particles (Figure 4.3B).

$$R_{1} = \frac{1}{T_{1}} = \frac{32\pi}{405000} \gamma^{2} \mu^{2} \left(\frac{N_{A}C}{r^{3}}\right) \left\{ \left(\frac{L(\alpha)}{\alpha}\right) 21PJ_{F}(\omega_{I},\tau_{D},\tau_{N}) + 21(1-P)J_{F}(\omega_{I},\tau_{D},\tau_{N}) + 9\left[1-L^{2}(\alpha)-2\left(\frac{L(\alpha)}{\alpha}\right)\right] J_{F}(\omega_{I},\tau_{D},\tau_{N}) + 9L^{2}(\alpha)J_{A}(\sqrt{2\omega_{I}\tau_{D}}) \right\}$$

$$(4.11)$$

$$R_{1} = \frac{1}{T_{2}} = \frac{32\pi}{405000} \gamma^{2} \mu^{2} \left(\frac{N_{A}C}{r^{3}}\right) \left\{ \left(\frac{L(\alpha)}{\alpha}\right) 19.5 J_{F}(\omega_{I}, \tau_{D}, \tau_{N}) + \left[1 - L^{2}(\alpha) - 2\left(\frac{L(\alpha)}{\alpha}\right)\right] \frac{9}{2} \left[J_{F}(\omega_{I}, \tau_{D}, \tau_{N}) + 6J_{F}(0, \tau_{D}, \tau_{N}) + L^{2}(\alpha)J_{A}\left[\frac{9}{2} J_{A}\left(\sqrt{2\omega_{I}\tau_{D}}\right) + 6J_{A}(0)\right] \right\}$$

$$(4.12)$$

4.1.2.3 Nuclear Magnetic Relaxation Dispersion Profiles

As mentioned previously, NMRD profiles are an essential tool to evaluate the relaxometric properties of MRI contrast agents as a function of magnetic field (Chapter 2.3). The method enables rapid analysis of the properties of new contrast agents and can be exploited to monitor the reproducibility of nanoparticle synthetic protocols. The fitting of NMRD profiles with suitable theoretical models provides information about the magnetic crystals (Figure 4.5), such as their average radius (r), their saturation magnetization (M_s), their anisotropic energy (E_a) and their Néel relaxation times (τ_N).



Figure 4.5 NMRD profile of magnetite particles in colloidal solution.¹³
 Adapted with permission from S. Laurent, D. Forge, M. Port, A. Roch, C. Robic, L. Vander Elst and R. N. Muller, *Chem. Rev.*, 2008, 108, 2064. Copyright (2008) American Chemical Society.

The NMRD profile is a function of several parameters.

- (1) Average radius (r): At high magnetic fields, the Ayant theory determines that the relaxation rates depend only on the diffusion correlation time (τ_D). The inflection point corresponds to the condition of $\omega_I \tau_D \gg 1$, where ω_I is the proton Larmor frequency. The diffusion correlation time is determined by the ratio of the square of crystal radius divided by diffusion constant ($\tau_D = r^2/D$), enabling estimations of average radii. An increase of crystal size modifies the position of the inflection point, moving it toward lower frequencies;
- (2) Saturation magnetization (M_s) : At high magnetic fields, saturation magnetization is reached and can be estimated from the maximum relaxation rate using the relationship of $M_s \approx (R_{\text{max}}/c\tau_D)^{1/2}$, where *c* and R_{max} correspond to a constant and the maximum relaxation rate, respectively;
- (3) Crystal anisotropy energy (E_a) : The dispersion observed at low magnetic fields provides information regarding the presence of crystals with low anisotropic energy. For materials with high anisotropic energy, no pitch dispersion is observed. The dispersion at low

frequencies gives qualitative information about the magnitude of anisotropic energy of magnetic compounds in solution; and

(4) Néel relaxation time (τ_N) : The Néel relaxation time obtained from the theoretical fitting is an approximate value. It provides qualitative information in addition to the crystal size and the specific magnetization.

Magnetic and relaxometric measurements provide a thorough description of the physicochemical properties of iron oxide nanoparticles. It is important to note that the RMG model is based on the assumption that nanoparticle size is uniform and, consequently, the theoretical parameters extracted from the theoretical fitting are average values. However, in practice, size heterogeneity of magnetic crystals is often observed. If the size distribution is large or if the nanoparticle suspension contains agglomerated nanoparticles, the theoretical parameters calculated might not accurately represent the effective characteristics of the nanoparticle.

4.1.3 Acknowledgements

This work was performed with the financial support of the Fonds National pour la Recherche Scientifique (F.R.S.-FNRS), the FEDER, the Walloon Region, the COST Action TD1402, the Centre for Microscopy and Molecular Imaging (CMMI) supported by the European Regional Development Fund of the Walloon region, the ARC and UIAP programs.

4.2 Synthesis of Iron Oxide Nanoparticles

DIMITRI STANICKI, LUCE VANDER ELST, ROBERT N. MULLER AND SOPHIE LAURENT*

4.2.1 Mechanism of Formation

Because the physicochemical properties of superparamagnetic nanosystems depend on the homogeneity of samples, characterization of uniformity in size and composition is important (Section 4.4). Although absolutely monodisperse systems are rare outside of biology, systems are often considered to be monodisperse when the standard deviation in particle size is less than 5%.

The first model of nanoparticle formation was presented by LaMer in the 1950s (*i.e.* the LaMer and Dinegar model).⁵¹ This model describes the different mechanisms behind the synthetic process. This theory is based on nucleation–growth mechanisms and considers nucleation as the limiting step in the process. Typically, the diagrams presenting the evolution of the monomer concentration *versus* time (*i.e.* the LaMer diagram) can be divided into three stages as shown in Figure 4.6.⁵²

• In stage 1, the concentration of monomer increases until the nucleation concentration $(C_{\rm MIN})$ is reached. At this minimal concentration, the system becomes heterogeneous, and solute molecules combine to produce small nuclei due to the collision of solute molecules in solution. Such nuclei are continuously being formed and dissolved.



Figure 4.6 Nucleation and growth model according to LaMer's theory (left).⁵⁴
Comparison of nucleation and growth rates *versus* monomer concentration (right). Left: reprinted with permission from C. Hui, C. Shen, T. Yang, L. Bao, J. Tian, H. Ding, C. Li and H.J. Gao, *J. Phys. Chem. C*, 2008, 112, 11336. Copyright (1950) American Chemical Society. Right: comparison of nucleation and growth rates *vs* monomer concentration (right), adapted from ref. 55.

The stability of the nuclei depends on the free-energy barrier (ΔG). If ΔG is high compared to the thermal energy (*kT*), the chance of forming such nuclei is negligible and no particles will form. However, if the free-energy barrier is low enough, the rate of nucleation tends to be infinite.

- During stage 2, nuclei formation partially reduces supersaturation, leading to a decrease of nucleation rate. When the critical limiting supersaturation (C_{MAX}) falls to C_{MIN} , particle growth replaces particle nucleation.
- If the system remains supersaturated, the growth of stable nuclei (stage 3) into discrete particles proceeds by diffusion of species from the solution to the nuclei.

This theory suggests that the synthesis of monodisperse nanoparticles requires a net separation of nucleation and growth.

4.2.2 Methods for the Preparation of Magnetic Nanoparticles

Due to their potential for use in a wide range of applications, numerous innovative protocols have been reported for the synthesis of iron oxide nanoparticles with control over size, shape, and composition. The many methods reported for the synthesis of nano-scaled magnetite include hydrothermal synthesis, sol-gel methods, micro-emulsion processes, and electrochemical or aerosol methods (Table 4.2). The most common ones are the coprecipitation method and the thermal decomposition of organometallic precursors.

4.2.2.1 Coprecipitation Method

Coprecipitation is the simplest and most efficient procedure to prepare iron oxide nanoparticles at the gram scale. In this method, nanoparticles are produced by mixing 2/1 stoichiometric mixtures of ferric and ferrous salts in alkaline medium [eqn (4.13)].

$$M^{2^+} + 2Fe^{3^+} + 8OH^- \rightarrow MFe_2O_4 + 4H_2O$$
 (4.13)

A complete precipitation of Fe_3O_4 occurs for pH values between 9 and 14 under a nonoxidizing, oxygen-free environment. The size and shape of the particles can be adjusted by the experimental conditions, including stoichiometry, pH, ionic strength, and temperature.^{53,54} In aqueous syntheses, coprecipitation is widely employed to obtain different ferrites (MFe₂O₄, M = Fe, Mn, Co, Mg, Zn, and Ni),⁵⁵ as shown in eqn (4.13). Although this procedure is fast, relatively easy to implement, and economical, it provides poorly crystalline particles that are characterized by a broad size distribution and that, consequently, requires size-sorting procedures to reduce polydispersity.

Better control over the properties of particles can be obtained by incorporating surfactants into the syntheses of particles. Small ferrites,

Synthetic method	Reaction time	Solvent	Surface capping molecule	Size distribution	Shape control	Yield
Coprecipitation	Minutes	Water	No	Broad	Not good	Medium
Thermal	Minutes-hours	High boiling	Yes	Very narrow	Very good	Medium
Polyol method	Hours	apual solvents (Poly)glycol	Yes	Narrow- hroad	Good	Medium
Microemulsion	Hours	Organic	Yes	Narrow	Good	Low
Laser pyrolysis	Seconds	Gas	No	Narrow	Good	Hi <i>e</i> h

Table 4.2 Principal methods for the synthesis of iron oxide nanoparticles.

Iron-oxide Nanoparticle-based Contrast Agents

including Fe_3O_4 , $CoFe_2O_4$, and $MnFe_2O_4$, were obtained using aminoalcohols such as isopropanolamine or diisopropanolamine⁵⁵ as the alkaline source. Such alkaline molecules act as complexing agents that control particle size during synthesis. These molecules also change the spin rearrangement at the surface (thinner magnetic "dead" layers), conferring better magnetic properties compared to synthesis in the absence of surfactants. Other surfactants, such as CTAB, PVP, or sodium cholate, were also shown to greatly influence mean size and size distribution.⁵⁶ Some solvents can also play the role of surfactants. For example, diethyleneglycol (DEG) can efficiently control the size and improve crystallinity of nanoparticles because of its high boiling point.^{57,58}

Stable colloids can be obtained by treating samples with acidic or alkaline medium, a process called peptisation. However, biomedical applications require stable particles in physiological conditions. The stability of magnetite colloids can be improved by performing the reaction in the presence of polymers such as dextran or polyacrylic acid.⁵⁴ Such processes often lead to the formation of particle clusters embedded within a polymeric matrix, which can be undesirable for certain applications.

4.2.2.2 Thermal Decomposition of Organometallic Precursors

USPIO with high monodispersity in both size and morphology can be synthesized by thermal decomposition of complexes of iron. Because these syntheses are performed at high temperatures (<200 °C), nanoparticles prepared *via* this method exhibit high crystallinity and high saturation moments. Generally, the reaction is performed in the presence of surfactants, typically long-chain hydrophobic hydrocarbons, which guarantee near control over growth and good dispersibility of particles in nonpolar solvents, such as petroleum ether, dichloromethane, or tetrahydrofuran.

According to LaMer diagrams, the synthesis of monodisperse colloids through homogeneous nucleation requires a temporal separation of nucleation and growth steps. Initially, the concentration of monomers, which are the smallest subunits of the crystal, increases either by external addition or by *in situ* generation. In the case of thermal decomposition, the poly iron oxo clusters obtained from thermal degradation of organometallic complexes (intermediate species between the iron complexes and the final nanosized products) have been demonstrated to act as the smallest building blocks or monomers (Figure 4.7).⁵⁹

Experimentally, such separation between nucleation and growth steps can be achieved by the direct and fast injection of a solution of reagent into a hot solvent. In this process, referred to as a "hot injection", the concentration of the precursor is rapidly raised above $C_{\rm MIN}$, leading to instantaneous nucleation. Because the injected solution is at ambient temperature, the nucleation step is quickly quenched by the rapid cooling of the reaction mixture. The number of germs formed in solution will depend on the ease of formation of the monomers, which depends on the thermal stability of the