

Zn²⁺ and Mn²⁺ doping effect on spin dynamics of Fe₃O₄-based magnetic nanoparticles: A possible path to efficient MRI contrast agents

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Iron oxide-based magnetic nanoparticles (MNPs) play a major role as contrast agents for magnetic resonance imaging (MRI). The introduction of Zn²⁺ and Mn²⁺ ions within their crystal lattice alters the magnetic anisotropy and the spin dynamical properties of the systems, which in turn influence the nuclear relaxation mechanisms that drive contrast enhancement in MRI. This work explored two sets of doped-magnetite MNPs (chemical formula Zn_xMn_yFe_{3-x-y}O₄) with core diameters of $d \sim 4$ or 15 nm produced by the fast and reproducible microwave-assisted synthesis method, and using different solvents like water or polyols. The $d \sim 4$ nm MNPs were doped with Zn²⁺, from $x = 0$ to 0.9; for Zn²⁺ = 0.5, Mn²⁺ was introduced at $y = 0.2, 0.4$. The $d \sim 15$ nm MNPs were doped with Zn²⁺ from $x = 0$ to 0.4, with Mn²⁺ added at $y = 0.1$ for Zn²⁺ = 0.3 and at 0.3 for Zn²⁺ = 0.4. Both sets were dispersed in water with carboxymethyl-dextran or polyacrylic acid coatings to ensure their colloidal stability, even under high magnetic fields. We highlighted the influence of the size and of the Zn²⁺/Mn²⁺ doping on the static and dynamical magnetic properties, also through the ¹H-NMR-dispersion profile analysis over a wide frequency range and the application of the superparamagnetic relaxation heuristic model. Larger particles exhibited higher nuclear relaxometric efficiency and anisotropy compared to the smaller ones, consistently outperforming the commercial contrast agents. On the other hand, the magnetic response and the nuclear relaxivities are enhanced by a Zn²⁺ content of $x = 0.1$ ions for approximately 4 nm MNPs and of $x = 0.4$ for approximately 15 nm MNPs, while a slight enhancing effect was obtained by Mn²⁺ introduction. It is thus shown how a proper size and doping combination provide an attractive way to modify the static and dynamical magnetic properties of MNPs by acting on different spin-spin correlation times dominating in

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specific frequency intervals, thus enabling the fine-tuning of image contrast properties suitable for magnetic resonance imaging.

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I. INTRODUCTION

Iron oxide-based magnetic nanoparticles (MNPs) are widely utilized for diagnostic purposes in nanomedicine [1–4]. The MRI technique allows us to observe internal sections of the human body, and the related images depend on the proton density, the longitudinal (T_1), and the transverse (T_2) relaxation times of the different tissues and molecules [5]. The introduction of contrast agents (CAs) shortens the relaxation times of the surrounding hydrogen nuclei, resulting in black and/or white spots according to their biodistribution [6–8] and thus enhancing the image contrast. Consequently, it is of crucial importance to understand how both the magnetic anisotropy and the electron spin dynamics (i.e., the spectral density of the electronic spin fluctuations $J(\omega)$), led by the CAs magnetic center, influence nuclear relaxation. At a general level, the magnetic nature of MNPs induces inhomogeneities in the local magnetic field, and the related fluctuations of the hyperfine field at the nuclear site enhance the nuclear spin-spin interactions, making them effective T_2 contrast agents [9]. The involved correlation times arise from both inner and outer-sphere mechanisms (this last one being the dominant one in MNPs), which take into account the electronic relaxation, the coordinated-bulk water exchange, the rotational motion of the particles, and the relative diffusion of the hydrogen nuclei [10,11]. Consequently, there is a strong dependence of the relaxometric properties on the morpho-structural and magnetic characteristics of the MNPs. The role of the shape [12–14], size [15–17], coating [18–21], and magnetic core composition [13,22–25] is of major interest, being strongly influential on the spin dynamics of the particles [26,27]. In this context, the morpho-structural features of nanoparticles have also been explored for advanced functionalities beyond contrast enhancement, such as temperature sensors for MRI [28–30], heat releasers for tumor treatments through magnetofluid hyperthermia [31–34], and for quantitative imaging through the magnetic particle imaging (MPI) technique [35].

In previous works, our group investigated the role of the size of maghemite nanoparticles in MRI contrast efficiency (i.e., the nuclear relaxivity) [19]: a set of 4.4-nm particles was compared with an 8.9-nm set. The highest nuclear longitudinal relaxivity r_1 of the smallest set was 30% lower than that of the biggest. The nuclear transverse relaxivity r_2 values of the 8.9-nm particles reached the highest magnitude at 10 MHz, whereas those of the 4.4-nm set reached their highest magnitude above 100 MHz.

As concerns the ion species, Bordonali *et al.* [36] studied the effect of cobalt introduction in magnetite MNPs structure, underlying how strongly it enhances the anisotropy of the systems: by increasing the Co^{2+} content from 0 to 0.4, the anisotropy increases, and consequently r_1 goes from 10 to approximately $300 \text{ s}^{-1} \text{ mM}^{-1}$. Because of the proportionality between anisotropy and MNPs' volume, the authors utilized a wide range of sizes and doping amount combinations to map a broad interval of energy barriers and thus to study spin dynamics. The use of cobalt is questionable because, despite its significant enhancement of the MNPs' properties, its potential toxicity limits applications in the medical scenario.

Vuong *et al.* [37] developed a model to predict the maximum transverse relaxivity depending on the particle size and clustering level, which is based on the knowledge of the size of the particle and the saturation magnetization at the corresponding volume. As the magnetic properties are affected by the variations in the elemental composition, the authors analyzed the case of doped particles and showed that Zn-Mn doped particles can reach r_2 values of about $1200\text{--}1800 \text{ s}^{-1} \text{ mM}^{-1}$ above 15 nm.

Baricic *et al.* [38] investigated how the substitution of cobalt ions with Zn or Ni in cobalt ferrites improves the anisotropy of the systems and their magnetic response, reaching a 40% increase in saturation magnetization when 48% of Co is replaced by Zn. The authors underline how the variations in the doping elements influence the superexchange interactions, the coupling between the sublattices, and the spin-orbit interactions. These variations, in addition to the arrangement of the ions within the crystal structure, affect the magnetic response of the systems.

The influence on the magnetic properties of MNPs due to Mn^{2+} and Zn^{2+} doping elements has been investigated by Jang *et al.* [39] on 15-nm $\text{Zn}_x\text{Fe}_{1-x}\text{Fe}_2\text{O}_4$ MNPs, with x : 0, 0.1, 0.2, 0.3, 0.4, 0.8. It was shown that the saturation magnetization M_S is maximized when Zn^{2+} is 0.4, reaching 161 emu/g(ion) , i.e., 117 emu/g(NP) , meaning that the bulk value is overcome by 23%. Consequently, an enhancement of the transverse relaxivity r_2 is observed up to $687 \text{ s}^{-1} \text{ mM}^{-1}$ when $\text{Zn}^{2+} = 0.4$ at 4.7 T, i.e., 2.5 times more effective than the undoped 15-nm-sized sample. When the Mn is introduced ($\text{Zn}_{0.4}\text{Mn}_{0.6}\text{Fe}_3\text{O}_4$), the M_S is increased up to 175 emu/g(ion) , i.e., 124 emu/g(NP) (+38% of the bulk value), while the transverse relaxivity at 4.7 T is 2.5 times higher than that for similarly sized and undoped particles. It is worth noting that this relaxivity study is limited to one field (i.e., frequency) value, and it

does not allow to investigate the physical mechanisms inducing the nuclear relaxation.

Thus, one can observe that most previous works on Zn/Mn-doped systems report studies about static magnetic properties and relaxivity values extracted from MRI measurements at a single fixed field, without accounting for the underlying dynamic relaxation processes responsible for MRI contrast. In general, systematic studies of relaxation mechanisms are mainly restricted to one field and to cobalt-doped systems, whereas the role of Zn and Mn doping in governing spin relaxation dynamics remains largely unexplored.

In this work, we study the ¹H-NMR nuclear longitudinal and transverse relaxivities and the static averaged magnetic properties (M vs H curves) in Zn/Mn doped systems. We evince how the spin dynamics of noninteracting or weakly interacting particles plays a crucial role in the MRI image contrast.

This conclusion has been reached by investigating how the size and composition of Fe₃O₄-based MNPs doped with Zn²⁺ and/or Mn²⁺ ions affect their spin dynamics and thus their efficiency as MRI contrast agents. So, we focused on two different-sized sets of Zn_xMn_yFe_{3-x-y}O₄ MNPs. The first set is composed of eight batches of MNPs coated with carboxymethyl-dextran (CM-D) having a mean diameter of approximately 4 nm, with $x = 0, 0.1, 0.4, 0.5, 0.8, 0.9$, and when $x = 0.5$, $y = 0.2, 0.4$. The second set is composed of five batches of MNPs coated with polyacrylic acid (PAA) having a mean diameter of approximately 15 nm, with $x = 0, 0.3, 0.4$, $(x, y) = (0.3, 0.1)$, and $(x, y) = (0.4, 0.3)$. The contribution of zinc and manganese to the magnetic and relaxometric behavior of the MNPs was examined; the spin dynamics is led by M_S and the spectral density of the electronic spin fluctuations.

II. METHODS

A. Zn_xMn_yFe_{3-x-y}O₄ MNPs synthesis

The small 4-nm-sized set of MNPs (D_a) was synthesized by microwave-assisted synthesis method in a water medium, using hydrazine and iron(III)/zinc(II)/manganese(II) chlorides in a total molar amount of 0.28 mmol [40] at different ratios, and 100 mg of carboxymethyl-dextran sodium salt CM-D (MW: 10 000–20 000 g/mol). The synthesis was conducted in a Monowave300 reactor (Anton Paar GmbH). The sample was heated up to 120 °C as fast as possible (4 °C/s), the temperature was held for 10 min, and the sample was cooled down to room temperature. The final product was purified through a gel filtration column (PD-10) and finally filtered through a 0.22-μm pore size filter. This method produces particles coated with a hydrophilic CM-D layer.

The big 15-nm-sized set of MNPs (D_b) was synthesized by the microwave-assisted synthesis method in a polyol medium (diethylene glycol DEG), used as both solvent and

surfactant, and acetate precursors. For all samples, the total molar amount of acetate precursors was kept at 1.73 mmol [41,42], varying their ratios. The synthesis was conducted in a Monowave300 reactor (Anton Paar GmbH) by heating the samples to 230 °C in 1 h, maintaining this temperature for 30 min, and cooling to 55 °C [41]. The resulting MNPs are surrounded by a hydrophilic DEG layer.

To increase the colloidal stability of the particles, they were coated with poly(acrylic acid) PAA (MW: 2100 g/mol), a biocompatible polymer.

More details about the materials, synthesis procedure, and coating process are available in Supplemental Material [43].

B. Characterization techniques

1. Inductively coupled plasma-optical emission spectroscopy ICP-OES

Their elemental composition was assessed through the inductively coupled plasma-optical emission spectrometry (ICP-OES) analysis by means of a PerkinElmer apparatus, OPTIME 2100DV, after acid digestion. The presence of the doping elements and the ratio in which they are present are thus evaluated.

2. Transmission electron microscopy TEM

The transmission electron microscopy (TEM) images were acquired on samples prepared by placing a few drops of an MNP dispersion, highly diluted on a 200-mesh carbon-coated copper grid, which was then air-dried. Micrographs were acquired for the samples coated with CM-D in the smaller set (D_a) and covered with a thin DEG layer in the case of the bigger set (D_b). A 100-keV JEOL-JEM 1010 microscope with a digital camera Gatan model Orius 200 SC was used for the acquisitions. To ensure statistical representativeness, TEM images were collected from tens of different regions of the grid, and particle diameters were manually measured for approximately 150 nanoparticles for each sample. During the analysis, only isolated particles with clearly defined boundaries were considered, whereas overlapping particles and agglomerated regions were excluded to avoid an overestimation of the particle size. The obtained size distributions were therefore considered representative of the analyzed nanoparticle populations. The analysis was performed with ImageJ software [44]. The diameters evaluated from the images were then fitted with a log-normal distribution to estimate the most probable diameter $\mu = R_0 e^{\frac{\sigma^2}{2}}$, where R and σ are, respectively, the mean radius and its standard deviation; and its error is $\sigma_\mu = R_0 \sqrt{e^{\sigma^2} (e^{\sigma^2} - 1)}$ [45].

3. X-ray diffraction XRD

The x-ray diffraction (XRD) samples of the bigger particles consist of fine powders pressed on a silicon crystal sample holder, while the XRD samples of the small

TABLE I. Morphostructural characteristics of the small (D_a) and big (D_b) sets of MNPs: elemental composition, core diameter d_{TEM} resulting from the TEM analysis, and crystal size d_{XRD} calculated from the x-ray diffraction.

Sample	Elemental composition	d_{TEM} (nm)	d_{XRD} (nm)	Sample	Elemental composition	d_{TEM} (nm)	d_{XRD} (nm)
D_a4	Fe_3O_4	4.0 (0.6)	6.5 (1)	D_b15	Fe_3O_4	15 (2)	20.1 (3)
D_a4_Z01	$\text{Zn}_{0.1}\text{Fe}_{2.9}\text{O}_4$	4.6 (0.7)	5.6 (1)	D_b15_Z03	$\text{Zn}_{0.3}\text{Fe}_{2.7}\text{O}_4$	14 (3)	29.6 (4)
D_a4_Z04	$\text{Zn}_{0.4}\text{Fe}_{2.6}\text{O}_4$	4.5 (0.6)	5.4 (1)	D_b15_Z04	$\text{Zn}_{0.4}\text{Fe}_{2.6}\text{O}_4$	14 (3)	18.1 (3)
D_a4_Z05	$\text{Zn}_{0.5}\text{Fe}_{2.5}\text{O}_4$	4.6 (0.5)	5.6 (1)	D_b15_Z03M01	$\text{Mn}_{0.1}\text{Zn}_{0.3}\text{Fe}_{2.6}\text{O}_4$	15 (3)	16.0 (3)
D_a4_Z08	$\text{Zn}_{0.8}\text{Fe}_{2.2}\text{O}_4$	4.0 (0.4)	4.8 (1)	D_b15_Z04M03	$\text{Mn}_{0.3}\text{Zn}_{0.4}\text{Fe}_{2.3}\text{O}_4$	12 (3)	12.9 (2)
D_a4_Z09	$\text{Zn}_{0.9}\text{Fe}_{2.1}\text{O}_4$	4.0 (0.5)	4.5 (1)				
D_a4_Z05M02	$\text{Mn}_{0.2}\text{Zn}_{0.5}\text{Fe}_{2.2}\text{O}_4$	4.1 (0.5)	4.5 (1)				
D_a4_Z05M04	$\text{Mn}_{0.4}\text{Zn}_{0.5}\text{Fe}_{2.1}\text{O}_4$	3.8 (0.5)	4.5 (1)				

particles are made of some dispersion drops, completely air-dried on the same silicon crystal sample holder. XRD pattern acquisitions were performed using a Bruker D8 Advance powder diffractometer with Cu $K\alpha$ radiation with an energy discriminator in a 2θ range $[10^\circ; 70^\circ]$ with an angular step of 0.04° . We accurately evaluated the position and width of the peaks to evaluate the crystal size of the MNPs by the Scherrer equation, considering the instrumental broadening.

4. Vibrating sample magnetometry VSM

The sample consists of a capsule filled with a water dispersion of the coated MNPs. Magnetization as a function of the external magnetic field was acquired in a vibrating sample magnetometer ADE system EV7 KLA-Tenco, from -1.8 to 1.8 T at room temperature.

5. Nuclear magnetic resonance NMR and relaxivity

The relaxometric efficiency of MNPs was evaluated by acquiring at room temperature the nuclear longitudinal T_1 and transverse T_2 nuclear relaxation times of the hydrogen nuclei in the presence of MNPs (i.e., CA). These measurements were performed at different Larmor frequencies ν , i.e., external static magnetic fields $\mu_0 H$, with $\nu = \frac{\gamma}{2\pi} \mu_0 H$, where $\frac{\gamma}{2\pi} = 42.58 \text{ MHz T}^{-1}$. It was considered a Larmor frequency range of 0.01 – 63.86 MHz , i.e., 0.00023 – 1.5 T . Two different instrumentations were used depending on the frequency range. For $0.01 \leq \nu \leq 7.2 \text{ MHz}$, Fast-Field-Cycling NMR SMARTracer Relaxometer (Stelar s.r.l., Mede, Italy) was used. The Saturation Recovery sequence pulse was utilized for T_1 measurements, and the Spin-Echo for T_2 ones. Acquisitions below 3.7 MHz resorted to prepolarization to enhance the low signal-to-noise ratio. For $7.2 \leq \nu \leq 63.86 \text{ MHz}$, a classic electromagnet MAGNET B-E 25 by Bruker (Billerica, MA, USA) plus a Tecmag (Houston, TX, USA) Apollo Fourier transform NMR spectrometer were utilized. The Saturation Recovery sequence pulse was used for T_1 measurement, and the Carr-Purcell-Meiboom-Gill for T_2 ones.

The NMR samples were made of MNPs dispersed in water at a millimolar (mM) concentration that ranged from 0.7 to 15 mM for the smallest D_a set, and from 0.04 to 0.2 mM for the largest D_b one. Relaxometric efficiency is evaluated through the relaxivity r_i from the longitudinal ($i = 1$) and transverse ($i = 2$) nuclear relaxation time measurements:

$$r_i = \frac{1}{c} \cdot \left(\frac{1}{T_{i,m}} - \frac{1}{T_{i,\text{dia}}} \right) \quad i = 1, 2. \quad (1)$$

In this expression, c is the concentration of the paramagnetic ions (usually expressed in mM units), $\frac{1}{T_{i,m}}$ are the measured relaxation rates of the ^1H in the presence of MNPs, and $\frac{1}{T_{i,\text{dia}}}$ are the relaxation rates of the sole dispersant (diamagnetic, dia). The NMR-d profiles represent the relaxivity values vs the Larmor frequencies.

III. RESULTS AND DISCUSSION

The elemental composition of the investigated samples is listed in Table I, and their schematic representation is shown in Fig. 1.

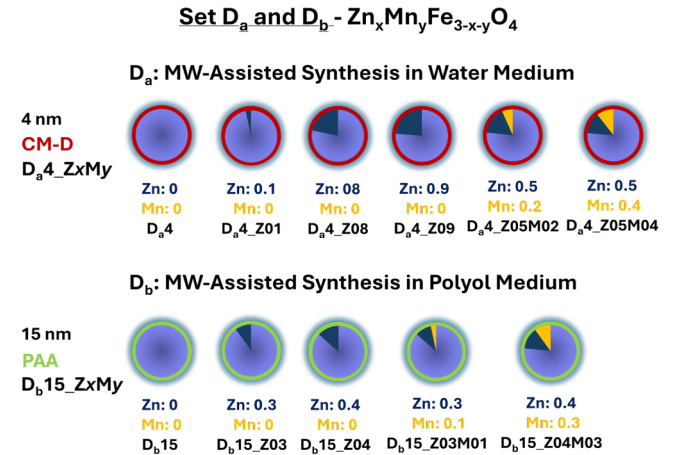


FIG. 1. Schematic representation of the two sets D_a and D_b of the samples studied.

A. Morphostructural properties

The mean diameter of the MNPs was evaluated from TEM images and ranged between 3.8 and 4.6 nm in the first set (D_a), and between 12 and 15 nm in the second one (D_b). TEM images of each set are shown in Fig. 2, and their mean diameters are listed in Table I. The hydrodynamic size was evaluated through dynamic light scattering (DLS) measurements (see Supplemental Material [43]).

The XRD patterns are shown in Fig. 3 and confirm a cubic spinel structure. Acquisitions of the smallest

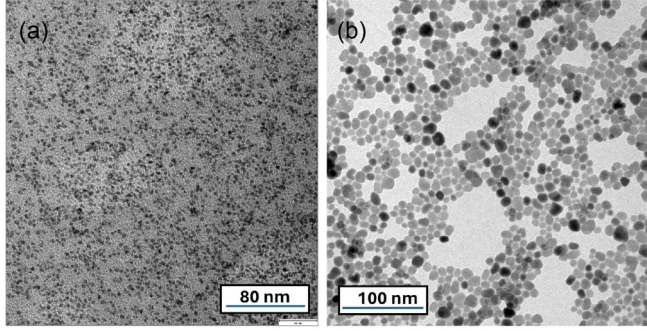


FIG. 2. Example of TEM images for the two sets of samples: (a) approximately 4 nm set D_a , (b) approximately 15 nm set D_b .

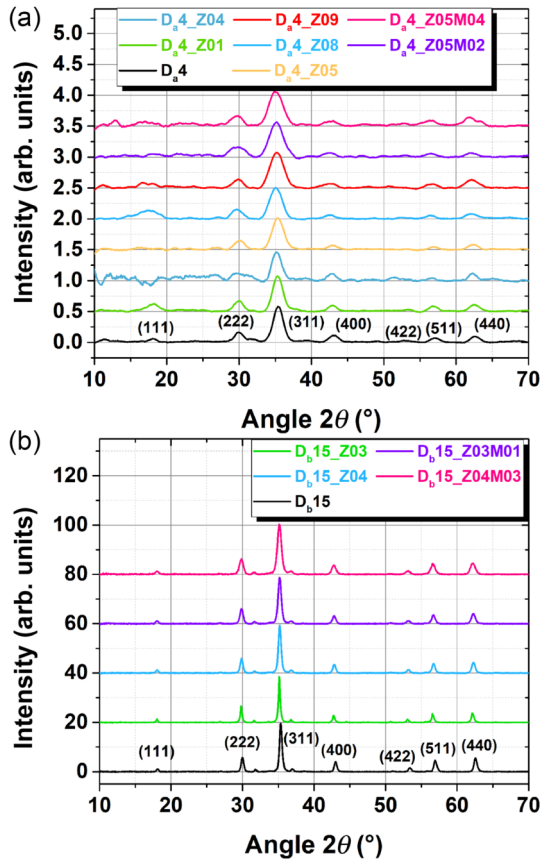


FIG. 3. XRD patterns of the smallest D_a set of CM-D coated particles (a) and of the biggest D_b set of particles coated with a thin DEG layer (b).

particles D_a resulted in broad peaks of diffraction pattern (high noise), which were smooth as shown in Fig. 3(a). The XRD patterns for the biggest D_b display sharper peaks [Fig. 3(b)]. The crystal sizes were evaluated from the analysis of the (3 1 1) plane and are listed in Table I.

$M \cdot \text{Fe}_2\text{O}_4$ ferrite, with M divalent cation, exhibits a cubic inverse spinel structure divided into two sublattices: the octahedral O_h and the tetrahedral T_h . The magnetic properties of the systems are determined by the metallic ion distribution within these sites, and they will be discussed in the next section.

B. Magnetic properties

The magnetization behavior as a function of the applied magnetic field is illustrated in Fig. 4; the saturation magnetization M_S values were extrapolated from the magnetic curves by fitting the high-field region of the magnetization curves with an approximate series expansion of the Langevin function ($M_S = (M) - \frac{A}{\mu_0 H} + \frac{B}{(\mu_0 H)^2}$, where M is the magnetization value, H is the applied magnetic field, and A and B are two constants) and are

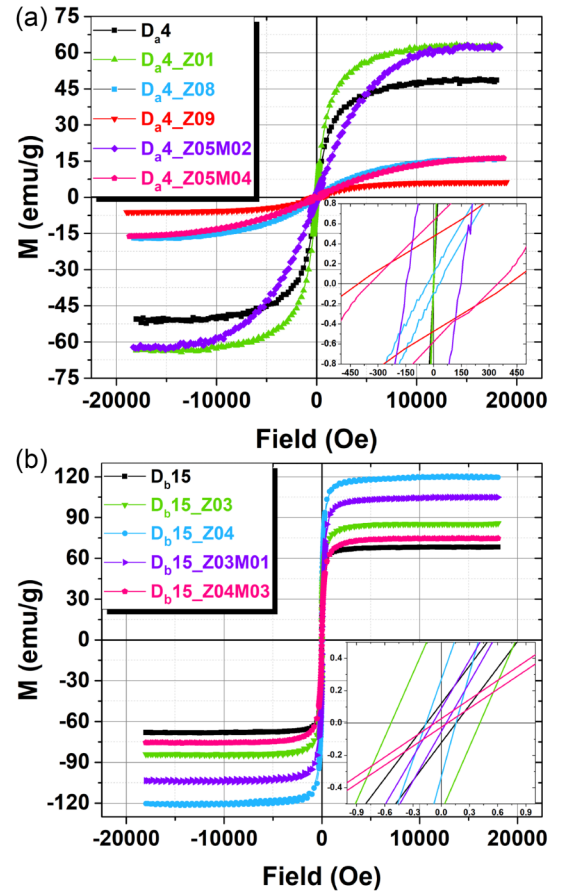


FIG. 4. Hysteresis curves of the D_a small set of MNPs coated with CM-D (a), and of the D_b big set of MNPs coated with PAA (b). The acquisitions were performed on water dispersions at room temperature. M values are in emu/g of magnetic core.

TABLE II. M_S and H_c of the samples of MNPs studied, evaluated on water dispersions acquired at room temperature. M_S values are reported in emu/g of magnetic core.

Sample	M_S (emu/g)	H_c (Oe)	Sample	M_S (emu/g)	H_c (Oe)
D _a 4	49 (12)	2.85	D _b 15	68 (7)	0.15
D _a 4_Z01	63 (16)	0.5	D _b 15_Z03	85 (9)	0.40
D _a 4_Z04	45 (11)		D _b 15_Z04	119 (12)	0.16
D _a 4_Z05	32 (8)		D _b 15_Z03M01	105 (10)	0.10
D _a 4_Z08	16 (4)	30	D _b 15_Z04M03	75 (8)	0.06
D _a 4_Z09	6 (2)	393			
D _a 4_Z05M02	62 (16)	132			
D _a 4_Z05M04	16 (4)	344			

listed in Table II with the coercive field H_c of each sample. All the following M_S values are reported in emu/g of magnetic core (emu/g[Zn_xMn_yFe_{3-x-y}O₄]).

For D_a *small set* (4 nm), the saturation magnetization M_S of the undoped sample (D_a4) resulted in 49 emu/g, and it is enhanced when introducing an amount of Zn = 0.1(D_a4_Z01), increasing up to 63 emu/g [Fig. 4(a)]. In contrast, the highest amounts of Zn, equal to 0.8 and 0.9 for D_a4_Z08 and D_a4_Z09, respectively, reduced M_S . Concerning the role of Mn, when Zn is fixed at 0.5, it turned out that an amount of Mn equal to 0.2 enhances M_S of the sample (D_a4_Z05M02), whereas an amount of Mn equal to 0.4(D_a4_Z05M04) reduces it.

For D_b *big set* (15 nm), the introduction of the selected amount of Zn enhanced the magnetic response of all the samples, reaching a maximum M_S up to 119 emu/g when Zn = 0.4 [Fig. 4(b)], in agreement with the literature [39]. Mn introduction in zinc-doped MNPs resulted in an enhancement of the M_S values in the case of D_b15_Z03M01, whereas they are strongly decreased for D_b15_Z04M03.

The undoped samples of different sizes have M_S values of 49 ($d = 4.0 \pm 0.6$) and 68 ($d = 15 \pm 2$) emu/g, and focusing on D_a4_Z01 with $M_S = 63$ emu/g, it is highlighted how the Zn = 0.1 in the small particles leads to a magnetic response comparable with that of D_b15, which has a volume 50 times larger than D_a4_Z01.

The introduction of dopant ions also affects the effective magnetic anisotropy K_{eff} by modifying the cation distribution, the magnetocrystalline anisotropy, and the surface spin configuration. These changes can be reflected in the coercive field H_c , which is approximately related to the effective anisotropy according to $H_c \sim \frac{2K_{\text{eff}}}{\mu_0 M_S}$.

For the D_a series (4 nm), both Zn and Zn-Mn incorporation lead to a progressive increase in the coercive field, from 0.5 Oe for Zn = 0.1 up to 344 Oe for Zn = 0.5/Mn = 0.4. Conversely, the D_b series (15 nm) exhibits coercivity values below 0.4 Oe, consistent with a superparamagnetic behavior at room temperature.

The effective anisotropy can be expressed as $K_{\text{eff}} = K_B + \frac{6K_S}{d}$ where K_B is the bulk magnetocrystalline

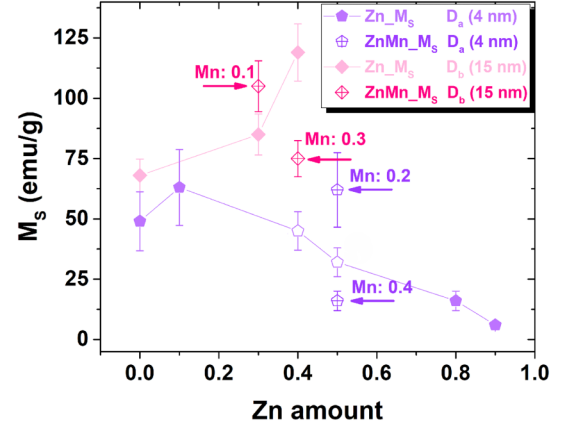


FIG. 5. M_S values vs Zn doping amount. Light full purple pentagons represent D_a set values, while light full pink diamonds represent those of D_b. When the Zn amount is fixed, the Mn introduction is depicted by dark empty purple pentagons (D_a set) and by dark empty pink diamonds D_b. M_S values of D_a4_Z04 and D_a4_Z05 samples are estimated from the RMG fit and are represented by light empty purple diamonds. M_S values are reported in emu/g of magnetic core.

anisotropy, K_S is the surface anisotropy, and d is the particle diameter. Therefore, while doping incorporation may modify both bulk and surface magnetic properties, its effect is expected to be more pronounced in the 4-nm nanoparticles because the surface contribution to K_{eff} is enhanced as the particle size decreases. Consequently, the increase in coercivity observed for the D_a series is more likely associated with doping-induced modifications of the effective anisotropy, particularly through surface-related effects.

In Fig. 5, M_S values as a function of doping introduction are displayed with the associated error bars, which were estimated by considering the electronic chain, the normalization to the magnetic content determined by ICP-OES, and the postprocessing corrections (diamagnetic background subtraction and drift correction). The M_S values for samples D_a4_Z04 and D_a4_Z05 were evaluated by fitting the NMR data with the Roch-Müller-Gillis (RMG) heuristic model, which describes the spin dynamics of MNPs (details in Sec. III C).

The octahedral (O_h) and the tetrahedral (T_h) sublattices, oriented oppositely, characterize the inverse spinel structure of $M \cdot \text{Fe}_2\text{O}_4$, where M is a divalent cation. In magnetite ($M = \text{Fe}^{2+}$), Fe^{2+} ions occupy the T_h site, while Fe^{3+} ions are divided between both sublattices. The balance between spins of oppositely oriented iron ions determines the net magnetic moment. The introduction of diamagnetic Zn^{2+} is expected to increase the overall magnetic moment, as Zn^{2+} preferentially occupies T_h site, leaving the O_h one unbalanced. Jang *et al.* reported that the optimal Zn^{2+} amount for enhancing the magnetic properties of the 15-nm-sized MNPs is 0.4 [39], in agreement with our result; higher Zn^{2+} content may populate the O_h

site after saturating the T_h one. When Mn^{2+} is considered, its magnetic moment of 5.9 μ_B , larger than the 4.9 μ_B of Fe^{2+} , suggests that it can replace divalent iron ions at the T_h site, thereby changing the magnetic response. The substitution with Zn^{2+} and Mn^{2+} changes the magnetic moment and anisotropy of the ferrite, as determined by the ion distribution within the sublattices [7,39]. Besides the selection of doping ions, their effect on structural interactions must be considered. Incorporation of diamagnetic Zn^{2+} disrupts the oxygen-mediated superexchange ($Fe^{3+}-O-Fe^{2+/3+}$), thereby reducing the magnetic coupling between T_h and O_h sites and limiting the increase of M_S beyond $Zn^{2+} \sim 0.5$ [38]. Whereas magnetic Mn^{2+} enhances the magnetic moment as it tends to occupy the T_h site [46,47] and reduces the monocrystalline anisotropy due to the weak spin-orbit coupling [48].

Summarizing, magnetic curves revealed that saturation magnetization is dependent on the zinc introduction for both sets of particles D_a (small) and D_b (big).

Regarding MNPs with $d \sim 4$ nm (D_a), a Zn amount of 0.1 increases M_S , while higher Zn amounts decrease it. Taking into consideration the M_S values evaluated through the RMG model (see next paragraph) for sample $D_{a4}.Z04$, we can suppose that $0.1 \leq Zn < 0.4$ optimizes the magnetic response.

Bigger particles with $d \sim 15$ nm (D_b) displayed a growing trend of M_S up to $Zn = 0.4$, in agreement with the literature findings [39] that also show a M_S decrease above that amount. From these results, we could assume that the T_h site of the MNPs crystal structure is rather populated by Zn^{2+} ions, with a consequent unbalancing of Fe^{3+} allocated at the opposite O_h site and growth of the net magnetic moment.

By considering the Mn^{2+} introduction, in the smallest D_a set of MNPs, when Zn is fixed at 0.5, the inclusion of $Mn = 0.2$ approximately doubles M_S , while the highest amount of $Mn = 0.4$ decreases M_S below the value of the only zinc-doped sample. In the biggest set D_b , the additional amount of $Mn = 0.1$ when $Zn = 0.3$ increased M_S by approximately 23%, while $Mn = 0.3$ when $Zn = 0.4$ decreased it by about 37%.

Our results suggest that additional introduction of Mn could enhance the M_S of Zn-doped MNPs only at reduced amounts ($Mn \sim 0.1$).

C. Relaxometry

1. Relaxivity profiles—group D_a

The ¹H-NMR-dispersion longitudinal (r_1) and transverse (r_2) profiles (relaxivity vs frequency) of the smallest group D_a of samples are displayed in Fig. 6. The uncertainty associated with the relaxivity values (15%) was assessed by accounting for the contributions arising from the selected acquisition parameters, pulse sequences,

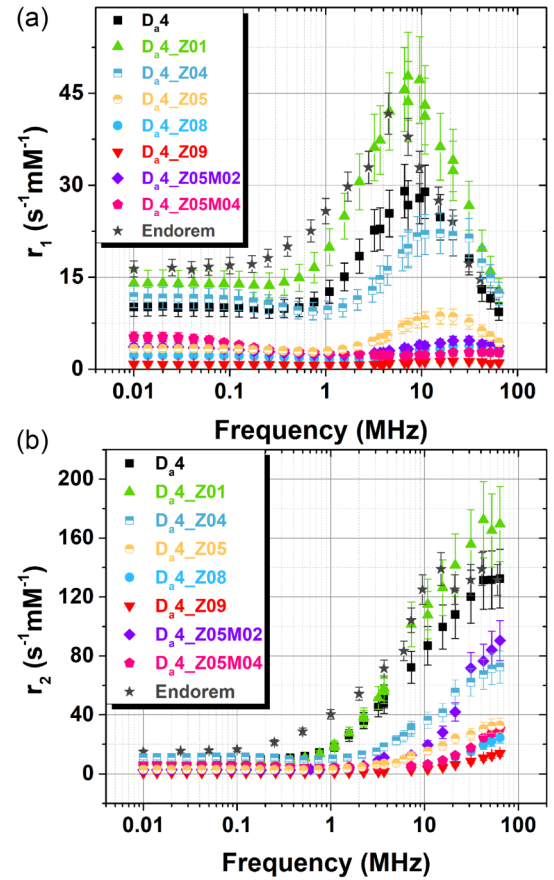


FIG. 6. Longitudinal (a) and transverse (b) nuclear relaxivity of the small D_a set of MNPs coated with CM-D, as a function of the frequency. The error bar is calculated *a priori* to be 15%. Gray stars refer to $r_{1,2}$ values of the commercial contrast agent Endorem.

instrumental factors (including the electronics), and errors in the dilution procedure.

The longitudinal r_1 profiles [Fig. 6(a)] display a frequency behavior dependent on the introduction of different divalent cations, i.e., different r_1 peak intensity and/or position, or “dispersion” degree (presence of a dip at around some MHz). It is worth reminding that the peak position and the “dispersion” degree are related to the magnetic and morphostructural characteristics, such as particle size or elemental composition. Furthermore, the intensity of the r_1 peak is proportional to the squared saturation magnetization as $r_{\max} \sim C \cdot M^2 \cdot \tau_D$ [10], with $r_{\max}$ the maximum relaxation rate, C a constant, and τ_D the diffusion time.

In our case, the undoped reference sample D_{a4} (black squares) displayed a frequency behavior similar to $D_{a4}.Z01$ (green triangles), which has the highest iron amount of 2.9 and the lowest iron substitution. In the other samples, iron is more replaced, with a maximum value of 2.1 for $D_{a4}.Z05M04$. Higher substitutions with Zn and Mn ($D_{a4}.Z05M02$ and $D_{a4}.Z05M04$ samples) led to a marked

dispersion level, which is almost absent in the other samples. The peak position is shifted from 8 MHz to 20–30 MHz. The highest r_1 peak intensity, i.e., highest relaxometric efficiency, was observed for the sample D_a4.Z01, with the lowest zinc amount of 0.1. Additional amounts of Zn led to a progressive decrease in the relaxometric efficiency of the particles, down to the case of Zn = 0.9 (D_a4.Z09). Magnetic measurements agree with this trend; in fact, they highlight an M_S peak for the D_a4.Z01 sample and a reduced magnetic response when Zn = 0.9.

Regarding the transverse relaxivity r_2 [Fig. 6(b)], all samples are still approaching their plateau at 63.86 MHz, which appears to have a different value depending on the doping introduction. Sample D_a4.Z01 is slightly more efficient than the undoped reference D_a4, while higher Zn/Mn doping amounts decrease transverse relaxometric efficiency and delay the growth of the profile from approximately 1 to 3–5 MHz. The introduction of manganese at the fixed value of Zn = 0.5 enhances the transverse r_2 relaxivity at the highest field when Mn is 0.2, while it decreases when Mn is 0.4.

Despite reduced size, sample D_a4.Z01 is moderately more efficient than the commercial contrast agent Endorem (product currently out of production but used as reference for contrast agent relaxometric efficiency studies, marked with gray stars in Fig. 6), in terms of highest intensity for the r_1 , and at higher frequencies for r_2 .

The magnetic and relaxometric properties are enhanced when the amount of Zn is 0.1 and reduced when it is ≥ 0.4 . It is suggested that the optimal amount of Zn that maximizes these properties could be ≥ 0.1 and < 0.4 for particles of this small size (approximately 4 nm). For 15-nm-sized MNPs, the preferential Zn amount is found to be 0.4, in agreement with the literature [39], but at reduced size, we can guess that it might no longer be verified.

2. Roch-Müller-Gillis heuristic model—group D_a

The Roch-Müller-Gillis (RMG) heuristic model [49] was applied to the experimental r_1 data to extrapolate microscopic information about MNPs subject to this study. In this model, the r_1 expression is

$$\begin{aligned} \frac{1}{T_1} = & \left(\frac{32\pi}{135000} \right) \mu^2 \gamma_I^2 \left(\frac{N_A C_{\text{Fe}}}{\text{RD}} \right) \\ & \times \left\{ 7P \frac{L(x)}{x} J_F[\Omega(\omega_S, \omega_0), \tau_D, \tau_N] \right. \\ & + \left[7Q \frac{L(x)}{x} + 3 \left(1 - L^2(x) - 2 \frac{L(x)}{x} \right) \right] J_F(\omega_I, \tau_D, \tau_N) \\ & \left. + 3L^2(x) J_A(\sqrt{\omega_I \tau_D}) \right\} \end{aligned} \quad (2)$$

where C_{Fe} is the iron ion concentration, μ the magnetic moment of a single particle, γ_I the proton gyromagnetic

ratio, D the diffusion coefficient of MNPs in the water medium, and R the distance of minimum approach. The other factors expressed in the r_1 function are as follows:

- (i) the Langevin function $L(x)$ with $x = \frac{\mu H_0}{k_B T}$.
- (ii) the Freed spectral density functions $J_{F_1}(\Omega(\omega_S, \omega_0), \tau_D, \tau_N)$ and $J_{F_2}(\omega_I, \tau_D, \tau_N)$.
- (iii) the Ayant spectral density function $J_A(\sqrt{\omega_I \tau_D})$.

The term $\Omega(\omega_S, \omega_0)$ is introduced to represent the dispersion at low fields, and its empirical formulation is

$$\begin{aligned} \Omega(\omega_S, \omega_0) = & (\omega_S^{1/4} - \omega_0^{1/4})^4 \quad \text{if } \omega_S > \omega_0 \\ = & 0 \quad \text{if } \omega_S < \omega_0, \end{aligned} \quad (3)$$

where ω_0 is a factor dependent on the anisotropy energy E_a and it is null for $E_a = 0$. The parameters P and Q quantify E_a , being $P + Q \leq 1$. $P = 1$ and $Q = 0$ denote $E_a = 0$, while $P = 0$ and $Q = 1$ represent high E_a . It is worth noting that in Eq. (2) τ_N is considered to follow the Arrhenius law $\tau_N = \tau_0 e^{\frac{KV}{k_B T}}$ (with τ_0 the preexponential factor, K the anisotropy constant, V the particle volume, k_B the Boltzmann constant, and T the temperature), i.e., interparticle interactions are neglected and are assumed to give a negligible contribution in nuclear relaxation mechanisms. Such approximations are considered good enough for particles having the dimensions reported here.

This heuristic model describes the relaxometric response of nanoparticle systems in the intermediate anisotropy regime, where the Freed and Ayant spectral densities that represent the limiting cases of low and high anisotropy, are combined through weighting parameters P and Q . Within this framework, the model interpolates between the two regimes where diffusion and Néel relaxation, respectively, dominate. The inflection points observed in NMRD profiles can be interpreted as crossover regions, where the characteristic correlation diffusion time τ_D of the local dipolar field fluctuations induced by nanoparticle dynamics becomes comparable to the inverse Larmor angular frequency, marking a transition between distinct spin-relaxation regimes.

The interpretation of the fitting parameters provides access to microscopic properties of the particles. The average core radius r_{fit} is determined from the relaxation behavior in the intermediate-field region. Since $\tau_D = R^2/D$, the characteristic minimum approach distance R can be directly inferred from the inflection point occurring at $\omega_I \tau_D \sim 1$. The specific magnetization M_S is estimated from the amplitude of the high-field relaxation peak, scaling as $M_S \propto (R_{\text{max}}/\tau_D)^{1/2}$, and therefore reflects the strength of the average saturation magnetic moment of the nanoparticle. The anisotropy energy E_a governs the low-field behavior: the presence or absence of low-field dispersion directly indicates the entity of the anisotropy barrier and the related Néel dynamics. Finally, the Néel relaxation time τ_N determines the relaxation rate in the low-field limit when

$\tau_N < \tau_D$, thus providing information on the internal magnetization reversal dynamics of the crystal [50].

The model adaptation to the experimental data is ruled by the saturation magnetization M_S , the core radius r_{fit} , the Néel relaxation time τ_N , the distance of minimum approach R , the diffusion coefficient of the solvent D , the P parameter, the temperature T , and a multiplicative constant. This multitude of parameters is managed by fixing some of them at the experimental/theoretical values (as reported in the discussion below) and by introducing realistic constraints. Specific description of the used parameters is subsequently described. Overall, from the fit procedure, we determined the saturation magnetization M_S , the core radius r_{fit} , the Néel relaxation time τ_N , and the distance of minimum approach R .

About the initialization of the physical quantities: (i) the initial value of the saturation magnetization M_S was set to the results of the magnetic measurements carried out on liquid dispersions at room temperature, where the significant diamagnetic contribution led us to consider an experimental error of 25% on the magnetic moment. However, for D_a4_Z04 and D_a4_Z05 M_S , it was a free parameter, and the first fit results gave 45 emu/g for D_a4_Z04 and 32 emu/g for D_a4_Z05, a behavior in agreement with the magnetic response as a function of Zn doping values. (ii) After a first fit, the M_S was fixed at the obtained value to decrease the number of free parameters in the subsequent final fitting procedure; (iii) the core radius r_{fit} was a free parameter; (iv) the Néel relaxation time τ_N initial value was set to 1 ns; (v) the distance of minimum approach R was left free to range between $r_{\text{fit}} + 1$ nm and $r_{\text{fit}} + 3.5$ nm, an estimation based on chemical characteristics.

The resulting fitting curves are shown in Fig. 7, and the parameters obtained from the fit are summarized in Table III. The model gives an accurate representation of the r_1 frequency behavior at both low and high frequencies, where the Néel relaxation and the Curie one dominate, respectively.

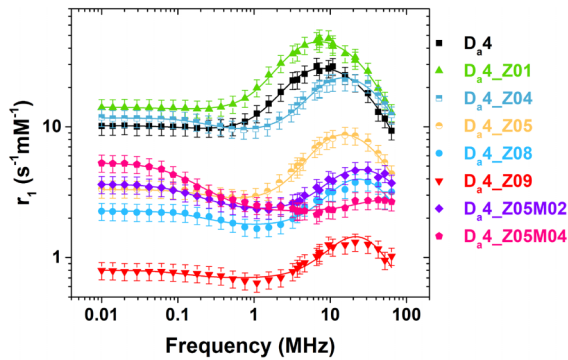


FIG. 7. Longitudinal relaxivity r_1 ¹H-NMR-d profiles of the small D_a set of MNPs coated with CM-D (symbols), fitted with the RMG model (continuous lines).

TABLE III. Parameters estimated from the fit of the r_1 ¹H-NMR-d profiles of the small D_a set of MNPs: the saturation magnetization M_S , the core radius r_{fit} resulting from the fit and r_{TEM} from the TEM analysis, the Néel relaxation time τ_N , and the minimum approach distance R .

Sample	M_S (emu/g)	r_{fit} (nm)	r_{TEM} (nm)	τ_N (ns)	R (nm)
D _a 4	57 (14)	4.8 (0.1)	2.0 (0.3)	4.4 (4)	5.7 (0.1)
D _a 4_Z01	78 (19)	4.5 (0.1)	2.3 (0.4)	8 (10)	6.2 (0.2)
D _a 4_Z04	45 (11)	3.9 (0.3)	2.3 (0.3)	1.6 (0.4)	4.9 (0.1)
D _a 4_Z05	32 (8)	4.4 (0.3)	2.3 (0.3)	1.6 (0.6)	5.3 (0.2)
D _a 4_Z08	20 (5)	4.0 (0.3)	2.0 (0.2)	2 (1)	5 (1)
D _a 4_Z09	7.5 (2)	5.2 (0.7)	2.0 (0.3)	3.5 (3)	6.2 (2)
D _a 4_Z05M02	61 (15)	2.9 (0.1)	2.0 (0.3)	4.1 (1)	3.9 (0.7)
D _a 4_Z05M04	20 (5)	2.7 (0.4)	2.4 (0.3)	5 (3)	3.7 (0.7)

The values of the core radius r_{fit} of the undoped D_a4 and Zn-doped samples obtained from the fit exceeded approximately 2 times the radius resulting from the TEM evaluation, envisaging the possibility of small aggregates of MNPs which are not detectable by TEM but might be evidenced by dynamic light scattering (DLS) measurements and supported by thermogravimetric analysis (TGA) data (Supplemental Material [43]): the hydrodynamic size results approximately 5 times larger than r_{TEM} and the TGA indicates the presence of 80%–90% of organic material, suggesting the possibility of soft agglomeration within the solvation shell. The Néel relaxation time τ_N was of the order of 10^{-9} s, typical for particles with this reduced dimension, indicates a small energy barrier that allows the magnetic moment to fluctuate. The distance of minimum approach R exceeded r_{fit} by approximately 1–1.5 nm in all cases, suggesting that CM-D is a flexible, hydrophilic polysaccharide, forming a highly hydrated and dynamic polymeric shell around the nanoparticles. While this interfacial layer may locally hinder direct approach of water molecules up to approximately 1–1.5 nm from the magnetic core, the high organic content observed by TGA suggests that a more extended and water-permeable polymer region is also present, which is likely accessible to solvent penetration over a larger spatial extent.

3. Relaxivity profiles—group D_b

The longitudinal r_1 and transverse r_2 nuclear relaxivity of the group D_b of samples are displayed in Fig. 8.

The longitudinal r_1 ¹H-NMR-d profiles [Fig. 8(a)] do not have the peaked shape typical of ultrasmall particles (e.g., samples of the groups D_a) and show growing r_1 values at the lowest frequencies, except for sample D_b15_Z05M03. The absence of “dispersion” and the low-frequency r_1 increase suggest a high anisotropy. In particular, at 0.01 MHz, the r_1 value is enhanced (~40%) when the Zn amount is increased up to 0.4, meaning an even higher anisotropy. On the other hand, when Mn

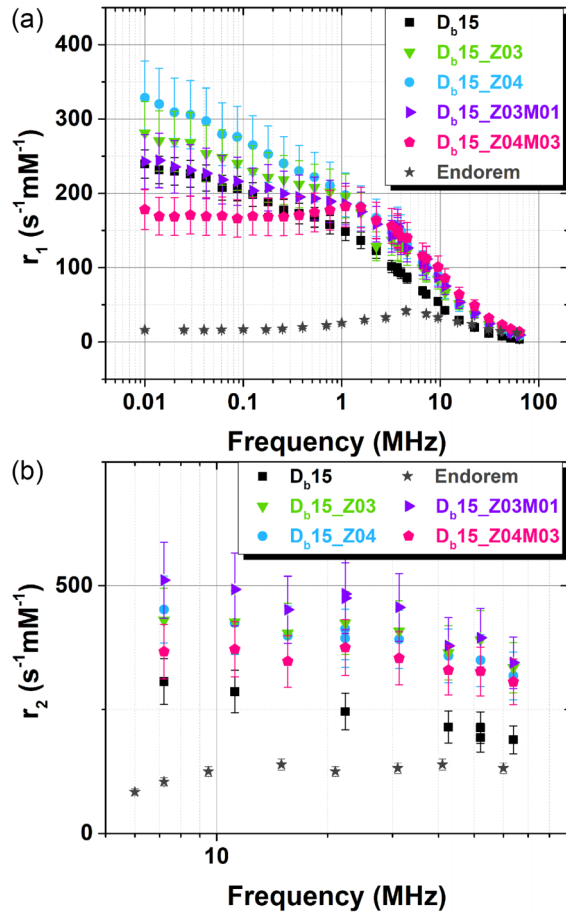


FIG. 8. Longitudinal (a) and transverse (b) nuclear relaxivity of the big D_b set of MNPs coated with PAA, as a function of the frequency. The error bar is calculated *a priori* to be 15%. Gray stars refer to $r_{1,2}$ values of the commercial contrast agent Endorem.

is introduced, a decrease in anisotropy is observed. Moreover, in the case of $Zn = 0.4$ and $Mn = 0.3$ (D_b15_Z04M03), the r_1 profile is peaked, but one has to consider that this sample has slightly lower dimensions (approximately 12 nm) compared to the others, an occurrence that can promote the peaked shape and the reduced anisotropy.

The r_2 values are shown in Fig. 8(b). Values below 5 MHz are not shown because the low-frequency T_2 acquisitions are very sensitive to aggregation processes, and particles with a mean diameter of approximately 15 nm are prone to form small clusters. Sample D_b15_Z03M01 showed an effective electronic spin dynamics over the hydrogen nuclear relaxation rate, hinting at the prevalence of dynamics contributions.

Within the high-frequency range, all the doped samples turned out to be more efficient than the undoped reference one D_b15 , for both longitudinal r_1 and transverse r_2 cases. We compared our results with the commercial contrast agent Endorem (gray stars in Fig. 8), whose r_2

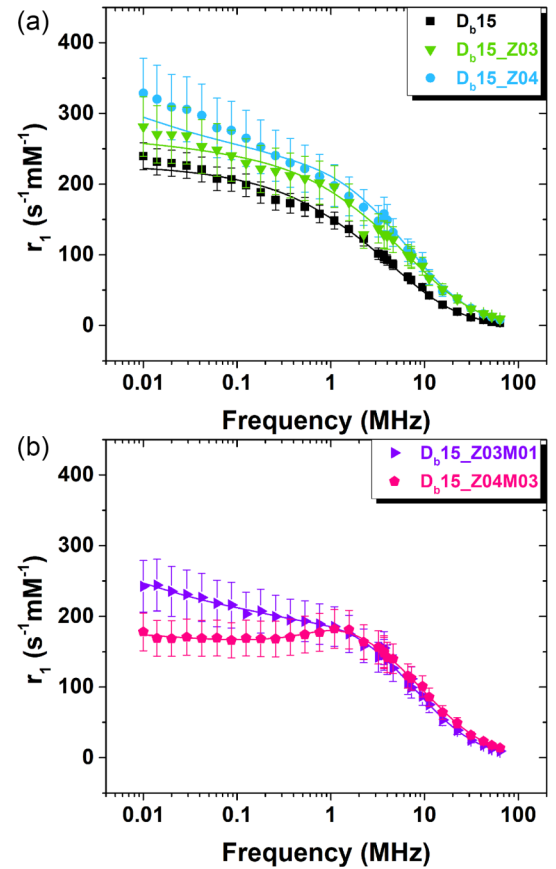


FIG. 9. Longitudinal relaxivity r_1 ^1H -NMR-d profiles (symbols) of the big D_b set of MNPs coated with PAA, fitted with the RMG model (continuous lines).

relaxometric efficiency was overcome in all cases. In particular, D_b15_Z03M01 exceeds the Endorem efficiency approximately 2.5 times at 63.86 MHz and approximately 5 times at 7.2 MHz. These findings highlight the possibility of reducing the amount of MNPs injected into patients during MRI diagnostic examinations.

4. Roch-Müller-Gillis heuristic model—group D_b

Also, for the bigger samples, the r_1 experimental data were fitted with the heuristic model of Roch-Müller-Gillis [49]. The resulting fitting curves are displayed in Fig. 9, and the fitting parameters obtained are listed in Table IV.

About the initialization of the physical quantities involved in the fitting procedure: (i) the saturation magnetization M_S has been set to the value obtained from the magnetic measurement carried on in liquid dispersions at room temperature; an error of 10% was considered; (ii) then, M_S was fixed at the value resulting from a first fit to reduce the number of free parameters; (iii) the Néel relaxation time τ_N initial value was set at 1 ns and it was constrained to not become larger than 10^{-4} s; (iv) the distance of minimum approach R was left free to range between 1 and 3.5 nm over the outcome core radius.

TABLE IV. Parameters estimated from the fit of the r_1 $^1\text{H-NMR-d}$ profiles of the D_b set of MNPs: the saturation magnetization M_S , the core radius r_{fit} resulting from the fit and r_{TEM} from the TEM analysis, and the minimum approach distance R .

Sample	M_S (emu/g)	r_{fit} (nm)	r_{TEM} (nm)	R (nm)
D_b15	61 (6)	8.5 (6)	7.5 (1)	12 (1)
D_b15_Z03	86 (9)	6 (7)	7 (2)	9.6 (0.3)
D_b15_Z04	107 (11)	7 (8)	7 (1)	10.5 (3)
D_b15_Z03M01	95 (9)	6.7 (1)	7.5 (2)	10.2 (0.2)
D_b15_Z04M03	79 (8)	6.6 (0.6)	6 (2)	8.7 (0.2)

Regarding the parameter values obtained from the fit, we can see that the saturation magnetization M_S as a function of doping follows the same trend as the experimental magnetic data. The estimation of the core radius r_{fit} was comparable, within the experimental error, with the core radius evaluated from the TEM analysis r_{TEM} . The Néel relaxation time τ_N and the distance of minimum approach R saturated to the highest limit, denoting a thick nonpermeable coating layer of about 2–5 nm, except for sample D_b15_Z04M03 , which displayed $\tau_N = 69 \pm 8$ ns and $R = 8.7 \pm 0.2$ nm. This result is compatible with the following observations: the PAA is a highly hydrophilic polyelectrolyte polymer forming a hydrated interfacial layer around the nanoparticles; TGA results mark approximately 40%–50% of organic material, which is coherent with the thickness estimation.

According to the Arrhenius (or Vogel-Fulcher) law $\tau_N = \tau_0 e^{\frac{KV}{RT}}$, a bigger volume and/or anisotropy constant leads to longer Néel relaxation times, due to the increase of the anisotropy energy barrier. Times of about 10^{-4} s suggest that the nanoparticles are no longer in the fast superparamagnetic fluctuation regime, likely due to the combined effects of large magnetic volume, enhanced effective anisotropy, and possible interparticle magnetic interactions. The evaluation of the spin dynamical regime showed that these nanosystems fall into the motional averaging regime (MAR). $R_2 = \frac{16}{45} f \tau_D (\Delta\omega)^2$, with the angular frequency shift $\Delta\omega = \frac{\gamma\mu_0 M_V}{3}$ and f the volume fraction [37].

5. Relaxivity at 7.2 and 63.86 MHz

We studied the effect of Zn²⁺ and Mn²⁺ doping at two typical MRI frequencies commonly used in clinical practice: 7.2 and 63.86 MHz (i.e., 0.17 and 1.5 T).

Concerning the small D_a subset (Fig. 10), the longitudinal relaxivity is more effective at 7.2 MHz, while the transverse one has a higher efficiency at the highest frequency (63.86 MHz). Theoretical simulated curves [49] of particles with this size are consistent with our findings. It is highlighted that zinc introduction improves

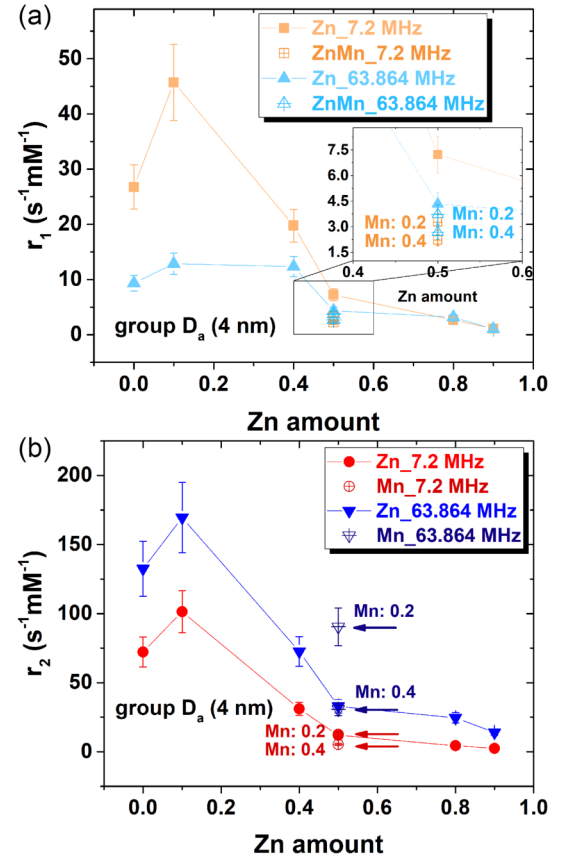


FIG. 10. Longitudinal r_1 (a) and transverse r_2 (b) relaxivity values of the small D_a set of particles as a function of Zn doping amount. The undoped and zinc-doped particles are represented with full symbols, while particles doped with zinc and manganese are denoted with empty symbols.

both r_1 and r_2 at lower amounts (0.1), improving the r_1 at 7.2 MHz by about 70%. On the other hand, the addition of manganese led to a reduction in $r_{1,2}$ compared to the undoped reference sample D_a4 . However, at 63.86 MHz, the r_2 is enhanced when Mn is 0.2 for an amount of Zn fixed at 0.5.

Concerning the big D_b subset (Fig. 11), it is shown that the Zn introduction increases r_1 and r_2 values until an amount of 0.4, in agreement with the literature [39], with an increment of approximately 70% in the case of r_2 at 63.86 MHz.

By comparing the D_a and D_b sets, we observe that the ideal zinc amount for MRI contrast enhancement is related to the particle size.

However, as several parameters contribute to relaxation processes, the doping contribution is tricky to isolate. In this scenario, first we assume that the synthesis processes used to produce the smaller and bigger sets of particles do not significantly affect the resulting products. Second, the concentration is expected not to influence the relaxivity, in the limit of their mutual linear dependence. Then, as concerns M_S role, we highlighted in both sets of particles

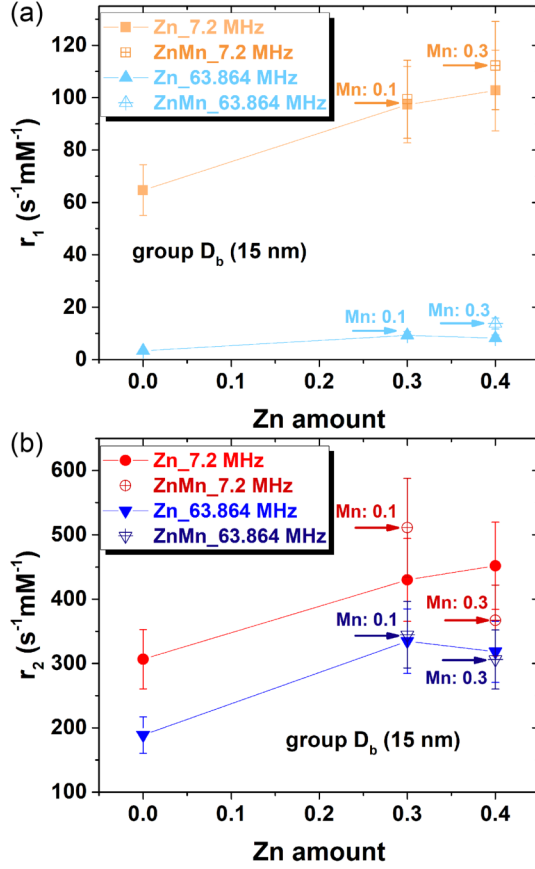


FIG. 11. Longitudinal r_1 (a) and transverse r_2 (b) relaxivity values of the big D_b set of particles as a function of Zn doping amount. The undoped and zinc-doped particles are represented with full symbols, while particles doped with zinc and manganese are denoted with empty symbols.

its relevant contribution to the r_2 behavior, as the magnetic moment induces inhomogeneities in the local magnetic field, promoting ¹H spin dephasing and thus accelerating the transverse relaxation (out of equilibrium) time T_2 . Such T_2 decrease is also due to out of equilibrium dynamical processes, led by mainly the diffusion correlation time τ_D and the Néel relaxation time τ_N .

Another factor influencing T_2 is the size distribution (due, e.g., also to the aggregation), which is correlated to a distribution of magnetic moments and might influence the correlation times and spin interaction dynamics. On this respect, we observe, however, that the RMG model reproduces correctly the experimental data despite it does not include distribution of size and spin-spin interactions. On the other hand, the inclusion of such quantities requires a formidable theoretical work. Additionally, it is worth noting that the coating might play a role in the relaxometric response of MNPs: in our previous works [19,21], we observed that, for diameters of about 14 nm, the coating does not seem to influence the r_2 , while at 4 nm, where the surface/volume ratio increases, its influence is singled out. However, as for each set size, the coating is the same

(CM-D for 4 nm, and PAA for 15 nm), we assumed that for each set of particles, the coating effect cannot be present.

As a consequence of the above considerations, we might be confident that our discussion on the role of Zn/Mn doping is quite well founded.

From applicative point of view, although the proposed nanoparticles show improved relaxometric performance, their biomedical relevance is ultimately dictated also by additional biological and clinical translational factors. Particle's cytotoxicity is influenced by composition, surface coating, and colloidal stability [51]. Zinc and manganese are biocompatible elements and are incorporated into the spinel ferrite structure as divalent cations (Zn^{2+} and Mn^{2+}), where they are stabilized within the crystalline. Moreover, as Zn^{2+} and Mn^{2+} ions are involved in several physiological processes, their potential biological impact is associated with their free divalent ionic form, which may contribute to oxidative stress or modulation of cellular pathways [52–54].

On the other hand, the biological response to ferrite nanoparticles is strongly affected by experimental conditions, including cell model and surface functionalization, which govern nanoparticle uptake, colloidal stability, and interactions with biological media [55]. In this context, degradation of Zn/Mn-doped ferrites has been reported as a relatively slow process under physiological conditions, with negligible adverse effects associated with ion release at relevant exposure levels [56]. Remarkably, the choice of biocompatible coatings such as PAA and CM-D plays a crucial role in enhancing colloidal stability and reducing cytotoxicity, as previously demonstrated for similarly coated Zn-Mn ferrites showing high cell viability in both cancer-derived and normal cell models [57,58].

Finally, it could be observed that the investigated nanoparticles are intended as blood-pool MRI contrast agents, where their performance is primarily governed by colloidal stability and circulation time rather than extensive cellular internalization. In this context, maintaining stability in physiological media is essential to ensure signal enhancement within the imaging time window, prior to significant clearance or tissue uptake processes.

IV. CONCLUSIONS

We explored the role of $\text{Zn}^{2+}/\text{Mn}^{2+}$ doping in nanoparticles of different sizes on the magnetic static (M vs H) and spin dynamical (¹H-NMR) relaxation properties of $\text{Zn}_x\text{Mn}_y\text{Fe}_{3-x-y}\text{O}_4$ systems, with the aim of tailoring such characteristics for MRI applications. The smallest set D_a, mean diameter of approximately 4 nm, is constituted of CM-D-coated particles, with doping: (i) x : 0, 0.1, 0.4, 0.5, 0.8, 0.9; (ii) x : 0.5 and y : 0.2. The biggest D_b set, mean diameter of approximately 15 nm, is composed of PAA-coated particles with doping: (i) x : 0, 0.3, and 0.4; (ii) x : 0.3, y : 0.1, and x : 0.4, y : 0.3.

As the magnetic properties are concerned, the contribution of zinc and manganese to the M vs H behavior of the MNPs was investigated, including the corresponding variation in saturation magnetization. The results suggest that for particles D_a ($d \sim 4$ nm) $0.1 \leq \text{Zn} < 0.4$ optimizes the magnetization saturation M_S , while for particles D_b ($d \sim 15$ nm), a growing M_S was observed up to $\text{Zn} = 0.4$, consistent with the literature [39], where a decrease for higher Zn amounts was also observed. This is a consequence of the spin balance within the crystal sublattices of the ferrite, and we hypothesize that the best amount of Zn to enhance the magnetic response is size-dependent. By considering the Mn role in Zn-doped particles, it resulted in M_S enhancement only at lower amounts for both sizes, doubling it for small D_a particles when $\text{Mn} = 0.2$ and $\text{Zn} = 0.5$, and improving it by about 23% when $\text{Mn} = 0.1$ and $\text{Zn} = 0.4$.

On the spin dynamics side, the relaxometric efficiency follows roughly the M_S trend and displays a maximum in r_1 and r_2 when $\text{Zn} = 0.1$ and $\text{Zn} = 0.4$ for the biggest D_b set of particles.

To exploit more spin dynamics details, the longitudinal ^1H -NMRD profiles were fitted with the heuristic RMG model, from which: (i) the resulting saturation magnetization follows the same trend of the experimental magnetic data in both sets; (ii) a τ_N of about 10^{-9} s resulted for the 4-nm particles, while it results much longer for the 15-nm ones; (iii) the minimum approach distance denotes the presence of a 1- to 1.5-nm nonpermeable covering layer for the 4-nm particles, and a thicker layer of about 2–5 nm for the 15-nm ones.

Summarizing, our experimental results highlighted that (i) as already well known from previous literature, the size strongly affects both the longitudinal and transverse nuclear relaxometric efficiency and the anisotropy of the systems; (ii) the enhancement of magnetic response, anisotropy, and the relaxometric efficiency induced by Zn²⁺ doping is size-dependent, with the optimal Zn²⁺ content varying with particle size; (iii) the Mn²⁺ introduction enhances the magnetic response and the relaxometric efficiency at the lowest ion amount and decreases the anisotropy of the biggest set D_b ; (iv) all the samples of the biggest D_b set resulted better T_2 contrast agents than the commercial Endorem, reaching an efficiency r_2 about 2.5 times higher at 1.5 T, and about 5 times higher at 7.2 MHz.

Overall, this work highlights the importance of probing spin relaxation dynamics across a broad frequency range, to unravel the involved physical mechanisms and more specifically the spin-spin correlation times, here namely the Néel and diffusion relaxation ones. Thus, our results remark that, acting properly on the size and doping of coated Fe₃O₄-based MNPs, the MRI image contrast and sensitivity can be optimized.

ACKNOWLEDGMENTS

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DATA AVAILABILITY

The data that support the findings of this article are not publicly available. The data are available from the authors upon reasonable request.

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- [1] M. F. Casula, P. Floris, C. Innocenti, A. Lascialfari, M. Marinone, M. Corti, R. A. Sperling, W. J. Parak, and C. Sangregorio, Magnetic resonance imaging contrast agents based on iron oxide superparamagnetic ferrofluids, *Chem. Mater.* **22**, 1739 (2010).
 - [2] D. K. Kim, Y. Zhang, W. Voit, K. V. Rao, J. Kehr, B. Bjelke, and M. Muhammed, Superparamagnetic iron oxide nanoparticles for bio-medical applications, *Scr. Mater.* **44**, 1713 (2001).
 - [3] X. Zhao, H. Zhao, Z. Chen, and M. Lan, Ultrasmall superparamagnetic iron oxide nanoparticles for magnetic resonance imaging contrast agent, *J. Nanosci. Nanotechnol.* **14**, 210 (2014).
 - [4] A. R. Puente-Santiago and D. Rodríguez-Padrón, Magnetic nanoparticles as mri contrast agents, *Top. Curr. Chem.* **378**, 49 (2020).
 - [5] G. Katti, S. A. Ara, and A. Shireen, Magnetic resonance imaging (MRI)—a review, *Int. J. Dent. Clin.* **3**, 65 (2011).
 - [6] Y.-D. Xiao, R. Paudel, J. Liu, C. Ma, Z.-S. Zhang, and S.-K. Zhou, MRI contrast agents: Classification and application, *Int. J. Mol. Med.* **38**, 1319 (2016).
 - [7] H. Shokrollahi, Contrast agents for MRI, *Mater. Sci. Eng.: C* **33**, 4485 (2013).
 - [8] S. Caspani, R. Magalhães, J. P. Araújo, and C. T. Sousa, Magnetic nanomaterials as contrast agents for MRI, *Materials* **13**, 2586 (2020).
 - [9] N. Kostevšek, A review on the optimal design of magnetic nanoparticle-based t 2 MRI contrast agents, *Magnetochemistry* **6**, 11 (2020).
 - [10] S. Laurent, D. Forge, M. Port, A. Roch, C. Robic, L. Vander Elst, and R. N. Muller, Magnetic iron oxide nanoparticles: Synthesis, stabilization, vectorization, physicochemical

- characterizations, and biological applications, *Chem. Rev.* **108**, 2064 (2008).
- [11] M. Avolio, A. Guerrini, F. Brero, C. Innocenti, C. Sangregorio, M. Cobianchi, M. Mariani, F. Orsini, P. Arosio, and A. Lascialfari, In-gel study of the effect of magnetic nanoparticles immobilization on their heating efficiency for application in magnetic fluid hyperthermia, *J. Magn. Magn. Mater.* **471**, 504 (2019).
- [12] H. Gavilán, O. Posth, L. K. Bogart, U. Steinhoff, L. Gutiérrez, and M. P. Morales, How shape and internal structure affect the magnetic properties of anisometric magnetite nanoparticles, *Acta Mater.* **125**, 416 (2017).
- [13] A. Pardo, S. Yáñez, Y. Piñeiro, R. Iglesias-Rey, A. Al-Modlej, S. Barbosa, J. Rivas, and P. Taboada, Cubic anisotropic Co- and Zn-substituted ferrite nanoparticles as multimodal magnetic agents, *ACS Appl. Mater. Interfaces* **12**, 9017 (2020).
- [14] M. Basini, M. Mariani, Q. L. Vuong, Y. Gossuin, S. Slimani, G. Singh, D. Peddis, and A. Lascialfari, Unravelling the surface local spin dynamics in magnetic nanoparticles by means of NMR relaxometry, *Phys. Rev. Lett.* **134**, 216703 (2025).
- [15] M. Basini, T. Orlando, P. Arosio, M. F. Casula, D. Espa, S. Murgia, C. Sangregorio, C. Innocenti, and A. Lascialfari, Local spin dynamics of iron oxide magnetic nanoparticles dispersed in different solvents with variable size and shape: A 1 h NMR study, *J. Chem. Phys.* **146**, 034703 (2017).
- [16] F. Liu, S. Laurent, A. Roch, L. Vander Elst, R. N. Muller, and M. A. Correa-Duarte, Size-controlled synthesis of CoFe_2O_4 nanoparticles potential contrast agent for MRI and investigation on their size-dependent magnetic properties, *J. Nanomater.* **2013**, 462540 (2013).
- [17] R. Costo, M. P. Morales, and S. Veintemillas-Verdaguer, Improving magnetic properties of ultrasmall magnetic nanoparticles by biocompatible coatings, *J. Appl. Phys.* **117**, 064311 (2015).
- [18] F. Brero, M. Basini, M. Avolio, F. Orsini, P. Arosio, C. Sangregorio, C. Innocenti, A. Guerrini, J. Boucard, E. Ishow *et al.*, Coating effect on the 1 h—NMR relaxation properties of iron oxide magnetic nanoparticles, *Nanomaterials* **10**, 1660 (2020).
- [19] F. Brero, P. Arosio, M. Albino, D. Cicolari, M. Porru, M. Basini, M. Mariani, C. Innocenti, C. Sangregorio, F. Orsini, and A. Lascialfari, 1 h-NMR relaxation of ferrite core-shell nanoparticles: Evaluation of the coating effect, *Nanomaterials* **13**, 804 (2023).
- [20] J. Pellico, J. Ruiz-Cabello, I. Fernández-Barahona, L. Gutiérrez, A. V. Lechuga-Vieco, J. A. Enríquez, M. P. Morales, and F. Herranz, One-step fast synthesis of nanoparticles for MRI: Coating chemistry as the key variable determining positive or negative contrast, *Langmuir* **33**, 10239 (2017).
- [21] M. Porru, F. Brero, C. Díaz-Ufano, M. Mariani, F. Orsini, P. Arosio, M. del Puerto Morales, and A. Lascialfari, Iron oxide nanospheres: Dual functionality as MRI contrast agents and magnetic fluid hyperthermia therapeutics, *Dalton Trans.* **54**, 9057 (2025).
- [22] M. Albino, E. Fantechi, C. Innocenti, A. López-Ortega, V. Bonanni, G. Campo, F. Pineider, M. Gurioli, P. Arosio, T. Orlando *et al.*, Role of Zn^{2+} substitution on the magnetic, hyperthermic, and relaxometric properties of cobalt ferrite nanoparticles, *J. Phys. Chem. C* **123**, 6148 (2019).
- [23] Y. Miao, Q. Xie, H. Zhang, J. Cai, X. Liu, J. Jiao, S. Hu, A. Ghosal, Y. Yang, and H. Fan, Composition-tunable ultrasmall manganese ferrite nanoparticles: Insights into their in vivo t1 contrast efficacy, *Theranostics* **9**, 1764 (2019).
- [24] H. Mohseni, H. Shokrollahi, I. Sharifi, and K. Gheisari, Magnetic and structural studies of the Mn-doped Mg–Zn ferrite nanoparticles synthesized by the glycine nitrate process, *J. Magn. Magn. Mater.* **324**, 3741 (2012).
- [25] E. Lima Jr., A. L. Brandl, A. D. Arelaro, and G. F. Goya, Spin disorder and magnetic anisotropy in Fe_3O_4 nanoparticles, *J. Appl. Phys.* **99**, 083908 (2006).
- [26] Z. Zhou, L. Yang, J. Gao, and X. Chen, Structure–relaxivity relationships of magnetic nanoparticles for magnetic resonance imaging, *Adv. Mater.* **31**, 1804567 (2019).
- [27] P. Arosio, F. Orsini, F. Brero, M. Mariani, C. Innocenti, C. Sangregorio, and A. Lascialfari, The effect of size, shape, coating and functionalization on nuclear relaxation properties in iron oxide core–shell nanoparticles: A brief review of the situation, *Dalton Trans.* **52**, 3551 (2023).
- [28] N. Alghamdi, J. Hankiewicz, N. Anderson, K. Stupic, R. Camley, M. Przybylski, J. Żukrowski, and Z. Celinski, Development of ferrite-based temperature sensors for magnetic resonance imaging: A study of $\text{Cu}_{1-x}\text{Zn}_x\text{Fe}_2\text{O}_4$, *Phys. Rev. Appl.* **9**, 054030 (2018).
- [29] P.-Y. Lin, D. Chalise, and D. G. Cahill, Improving nuclear magnetic resonance and electron spin resonance thermometry with size reduction of superparamagnetic iron oxide nanoparticles, *Phys. Rev. Appl.* **22**, 044082 (2024).
- [30] D. Chalise and D. G. Cahill, Highly sensitive and high-throughput magnetic resonance thermometry of fluids using superparamagnetic nanoparticles, *Phys. Rev. Appl.* **19**, 014055 (2023).
- [31] R. Behbahani, M. L. Plumer, and I. Saika-Voivod, Micro-magnetic simulations of clusters of nanoparticles with internal structure: Application to magnetic hyperthermia, *arXiv:2110.11178*.
- [32] D. P. Valdés, E. Lima, R. D. Zysler, and E. De Biasi, Modeling the magnetic-hyperthermia response of linear chains of nanoparticles with low anisotropy: A key to improving specific power absorption, *Phys. Rev. Appl.* **14**, 014023 (2020).
- [33] M.-K. Kim, J. Sim, J.-H. Lee, M. Kim, and S.-K. Kim, Dynamical origin of highly efficient energy dissipation in soft magnetic nanoparticles for magnetic hyperthermia applications, *Phys. Rev. Appl.* **9**, 054037 (2018).
- [34] G. Barrera, P. Allia, and P. Tiberto, Magnetic nanoparticle hyperthermia enhanced by a rotating field, *Phys. Rev. Appl.* **21**, 064037 (2024).
- [35] G. Barrera, P. Allia, and P. Tiberto, Magnetic tracers for magnetic particle imaging: Insight on the roles of frequency-sustained hysteresis and interactions in quantitative imaging, *Phys. Rev. Appl.* **19**, 034029 (2023).
- [36] L. Bordonali, T. Kalaivani, K. Sabareesh, C. Innocenti, E. Fantechi, C. Sangregorio, M. F. Casula, L. Lartigue, J. Larionova, Y. Guari *et al.*, NMR-D study of the local spin dynamics and magnetic anisotropy in different nearly monodispersed ferrite nanoparticles, *J. Phys. Condens. Matter* **25**, 066008 (2013).

- [37] Q. L. Vuong, J.-F. Berret, J. Fresnais, Y. Gossuin, and O. Sandre, A universal scaling law to predict the efficiency of magnetic nanoparticles as MRI T2-contrast agents, *Adv. Healthcare Mater.* **1**, 502 (2012).
- [38] M. Baričič, P. Maltoni, G. Barucca, N. Yaacoub, A. Omelyanchik, F. Canepa, R. Mathieu, and D. Peddis, Chemical engineering of cationic distribution in spinel ferrite nanoparticles: The effect on the magnetic properties, *Phys. Chem. Chem. Phys.* **26**, 6325 (2024).
- [39] J.-T. Jang, H. Nah, J.-H. Lee, S. H. Moon, M. G. Kim, and J. Cheon, Critical enhancements of MRI contrast and hyperthermic effects by dopant-controlled magnetic nanoparticles, *Angew. Chem., Int. Ed.* **48**, 1234 (2009).
- [40] J. Pellico, J. Ruiz-Cabello, M. Saiz-Alía, G. Del Rosario, S. Caja, M. Montoya, L. Fernandez de Manuel, M. P. Morales, L. Gutiérrez, B. Galiana *et al.*, Fast synthesis and bioconjugation of ⁶⁸Ga core-doped extremely small iron oxide nanoparticles for PET/MR imaging, *Contrast Media Mol. Imaging* **11**, 203 (2016).
- [41] M. Porru, M. d. P. Morales, A. Gallo-Cordova, A. Espinosa, M. Moros, F. Brero, M. Mariani, A. Lascialfari, and J. G. Ovejero, Tailoring the magnetic and structural properties of manganese/zinc doped iron oxide nanoparticles through microwaves-assisted polyol synthesis, *Nanomaterials* **12**, 3304 (2022).
- [42] Á. Gallo-Cordova, A. Espinosa, A. Serrano, L. Gutiérrez, N. Menéndez, M. del Puerto Morales, and E. Mazarío, New insights into the structural analysis of maghemite and (MFe₂O₄, M = Co, Zn) ferrite nanoparticles synthesized by a microwave-assisted polyol process, *Mater. Chem. Front.* **4**, 3063 (2020).
- [43] See Supplemental Material at <http://link.aps.org/supplemental/10.1103/n4jy-9t8m> for details on the materials used for MNP production, the synthesis and coating procedures, and the hydrodynamic size and zeta potential measurements obtained by DLS.
- [44] C. A. Schneider, W. S. Rasband, and K. W. Eliceiri, NIH image to imagej: 25 years of image analysis, *Nat. Methods* **9**, 671 (2012).
- [45] R. E. Rosensweig, Heating magnetic fluid with alternating magnetic field, *J. Magn. Magn. Mater.* **252**, 370 (2002).
- [46] J. M. Hastings and L. M. Corliss, Neutron diffraction study of manganese ferrite, *Phys. Rev.* **104**, 328 (1956).
- [47] X. Liang, Y. Zhong, S. Zhu, H. He, P. Yuan, J. Zhu, and Z. Jiang, The valence and site occupancy of substituting metals in magnetite spinel structure Fe_{3-x}M_xO₄ (M = Cr, Mn, Co and Ni) and their influence on thermal stability: An XANES and TG-DSC investigation, *Solid State Sci.* **15**, 115 (2013).
- [48] D. García-Soriano, R. Amaro, N. Lafuente-Gómez, P. Milán-Rois, Á. Somoza, C. Navío, F. Herranz, L. Gutiérrez, and G. Salas, The influence of cation incorporation and leaching in the properties of Mn-doped nanoparticles for biomedical applications, *J. Colloid Interface Sci.* **578**, 510 (2020).
- [49] A. Roch, R. N. Muller, and P. Gillis, Theory of proton relaxation induced by superparamagnetic particles, *J. Chem. Phys.* **110**, 5403 (1999).
- [50] S. Laurent, J.-L. Bridot, L. Vander Elst, and R. N. Muller, Magnetic iron oxide nanoparticles for biomedical applications, *Future Med. Chem.* **2**, 427 (2010).
- [51] J. Schubert and M. Chanana, Coating matters: Review on colloidal stability of nanoparticles with biocompatible coatings in biological media, living cells and organisms, *Curr. Med. Chem.* **25**, 4553 (2018).
- [52] P. Huang, G. Li, C. Chen, H. Wang, Y. Han, S. Zhang, Y. Xiao, M. Zhang, N. Liu, J. Chu *et al.*, Differential toxicity of Mn²⁺ and Mn³⁺ to rat liver tissues: Oxidative damage, membrane fluidity and histopathological changes, *Exp. Toxicol. Pathol.* **64**, 197 (2012).
- [53] A. Sun, S. Ma, and W.-X. Wang, Two-sided cellular and physiological effects of zinc oxide nanoparticles (nZnO): A critical review, *Environ. Sci.: Nano* **12**, 1059 (2025).
- [54] O. Bondarenko, K. Juganson, A. Ivask, K. Kasemets, M. Mortimer, and A. Kahru, Toxicity of Ag, CuO and ZnO nanoparticles to selected environmentally relevant test organisms and mammalian cells in vitro: A critical review, *Arch. Toxicol.* **87**, 1181 (2013).
- [55] I. Rozman, A. Gallo-Cordova, M. del Puerto Morales, M. A. M. Ovalle, G. F. Goya, K. Kološa, D. Hočevár, B. Žegura, and A. Štern, Safety of ferrite nanoparticles for biomedical applications: Cyto- and genotoxic effects of M_xFe_{3-x}O₄ (M = Fe, Zn, Mn) in an advanced 3d human hepatic in vitro model, *Biomed. Pharmacother.* **195**, 118950 (2026).
- [56] L. M. Slavu, R. Rinaldi, and R. Di Corato, Application in nanomedicine of manganese-zinc ferrite nanoparticles, *Appl. Sci.* **11**, 11183 (2021).
- [57] T. Patel, K. Parekh, and N. Jain, Apoptosis-inducing effect of cytocompatible poly (acrylic acid)-coated temperature-sensitive Mn_{0.9}Zn_{0.1}Fe₂O₄ magnetic fluid on breast cancer cells MCF-7, *ACS Appl. Bio Mater.* **8**, 8101 (2025).
- [58] M. Latorre-Esteves, A. Cortés, M. Torres-Lugo, and C. Rinaldi, Synthesis and characterization of carboxymethyl dextran-coated Mn/Zn ferrite for biomedical applications, *J. Magn. Magn. Mater.* **321**, 3061 (2009).