Effect of Spin Clustering on Basic and Relaxometric Properties of Magnetic Nanoparticles

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An increasing awareness about novel medical applications of smaller, inorganic-based nanoparticles, possessing unique properties at the nanoscale, has led to a burst of research activities in the development of “nanoprobes” for diagnostic medicine and agents for novel, externally activated therapies. In this research field magnetic nanoparticles are prominent due to fundamental peculiar properties particularly appealing for their use in materials and biomedical applications. Aiming to study the relationship between the topology of the magnetic nanoparticles and their efficacy as MRI contrast agents (relaxometric properties), we prepared three different stable colloidal suspension (ferrofluid) of magnetic nanobeads (MNBs) constituted by a discrete number of maghemite nanoparticles, arranged in disordered clusters or ordered in a polymeric matrix. An accurate morpho-dimensional and magnetic characterization displays the close correlation between the magnetic fundamental properties and the topology of our systems. The NMR relaxometry profiles confirmed the nature of the physical mechanisms inducing the increase of nuclear relaxation rates at low (magnetic anisotropy) and high (Curie relaxation) magnetic fields. Moreover transverse relaxivity (r_2) values for all the MNBs are higher than those of common contrast agents and the differences between the three MNBs are suggested to be due to the spin topology effect.

Keywords:

1. INTRODUCTION

Superparamagnetic nanoparticles (SPNs) represent nowadays a research field of huge interest, due to their peculiar properties. A great impulse to study SPNs was given by their application in medicine, where they can ideally bring diagnostic and therapeutic improvements for different diseases. SPNs, covered by a proper coating and dispersed in stable suspensions, are currently used as contrast agents (CAs) in magnetic resonance imaging (MRI) and as magnetohyperthermic (MH) agents.^{1–4} Superparamagnetic iron oxide nanoparticles (SPIONs) are particularly intriguing as CAs for MRI because of their low level of toxicity, superparamagnetic properties and biocompatibility.^{5–8} The essential parameters characterizing SPIONs are their magnetic, surface and molecular targeting potential properties.

In this respect the coating design is fundamental in terms of stability and biocompatibility, but also for the Magnetic Resonance efficiency in contrasting images. In addition the coating offers the possibility to functionalize the nanoparticles with drugs and targeting agents, such as antibodies, short peptides, nucleotides and small organic molecules. Furthermore in the last decades great efforts were spent to improve the synthesis routes in order to obtain monodisperse SPIONs which allow an investigation of fundamental properties and the optimization of applicative performances. Some fundamental aspects of SPIONs such as the surface, size and spin topology are currently subject of intense theoretical and experimental research activity.^{1–3}

In literature a particular attention was devoted to the role of the coating, that must be non-toxic, biocompatible and should allow proper circulation times of the nanoconstruct.^{6,7} Among different coatings, sugars, polymers and small molecules (i.e.: acids) are commonly used.^{9–12}

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Figure 1. (a) cryoTEM images of samples A1 and B samples, (b) TEM images of A1, A2 and B samples, and (c) pictorial representation of core and coating structures in the samples.

In this research context, we investigated the effect of spin topology and of the composition of the coating on 3 classes of SPIONs.

The investigated samples are stable colloidal suspension (ferrofluid) of magnetic polymer nanobeads (MNBs) constituted by a discrete number of maghemite nanoparticles ($\gamma\text{-Fe}_2\text{O}_3\text{NPs}$), arranged in disordered clusters (A1 and A2 samples) or ordered in a polymeric matrix (B sample). The pictorial representation of samples A1, A2 and B is shown in Figure 1(b) and takes into account the results of the morphological and structural characterization. The effect of the presence of a hydrophobic part in the coating was also investigated (the comparison between sample A1 and A2 is in that light). The strategies for synthesized the polymers and the final MNBs were chosen to provide multifunctionalization.

A similar investigation was recently performed by Hak et al. who proposed a systematic study on clusters as a function of the number of particles in a single cluster and as a function of the cluster radius.¹³ Our work is original with respect to the one of Hak et al. as we proposed to investigate the effect of the coating (sample A1 and A2) and the effect of the nanoparticle's arrangement within the cluster (sample A2 and B) on the magnetic and relaxometric properties. Moreover, with respect to the r_2 presented by Hak et al. at two different fields, we present here a complete NMR dispersion curve of all the investigated samples.

In order to achieve the suitable properties for the MNBs' biomedical applications, a fully understanding of the fundamental mechanisms that drive the magnetic behavior for the different spin topologies is needed. Thus we performed a wide chemico-physical characterization. In particular Transmission Electron Microscopy and Cryogenic Transmission Electron Microscopy (TEM/cryo-TEM) measurements were performed to determine the size of the metal-oxide cores, while the crystallographic nature of the compounds was studied by Electron diffraction (ED). DC and AC magnetic measurements were performed in order to study the system's static magnetic answer and the macroscopic spin dynamics respectively. The local spin dynamics at different frequency was also investigated by means of ^1H -NMR wide-band characterization. Longitudinal and transverse relaxivity dispersion curves (r_1 and r_2) were calculated not only to test the MNBs efficiency as CAs, but also to explore their fundamental magnetic properties. Indeed the NMR analysis allowed not only to evaluate important parameters such as the distance of minimum approach of the water molecules to the MNBs or the spin correlation time, but also to test the validity of the heuristic models commonly used to predict the MRI contrast efficiency for different spin topologies.

The synthesis of the magnetic nanobeads was performed in two different steps: (i) synthesis of iron oxide magnetic nanoparticles, and (ii) stabilization by polymer coating and dispersion in aqueous media at physiological pH.

It is important to remark that the different polymer coatings used in this work were designed in order to attend several requirements for biomedical application. The stability in physiological media over time and the biocompatibility are provided, for example, by an outer shell of hydrophilic polyethyleneglycol (PEG) chains.^{14,15} PEG is considered a highly biocompatible polymer and currently is recognized as safe (GRAS) by the FDA. This shell protects the SPIONs against adsorption of plasma proteins and non-specific uptake like macrophages¹⁶ and is well solvated by water molecules thus preventing the agglomeration of the nanoparticles.¹⁷ Actually, it has been shown that its capacity to scape recognition by the macrophages is not based on a total rejection of plasma proteins, but it is a selective adsorption of proteins forming the "protein corona" that play this role.¹⁸ PEG hydrophilic linear chains in sample A1 are directly attached to the iron oxide surface, which has been previously modified with acrylate acid, through a thioether (C–S–C) linkage. Samples A2 and B are both encapsulated in the hydrophobic part of amphiphilic poly(4-vinylpyridine) (P4VP) and PEG copolymers, but with a different architecture. In the second case the PEG chains are directly bonded as side chains to the P4VP backbone chain by N–C bonds between the pyridine groups and acrylate groups located at one of the ends of the PEG chains, while in the first case the coating is a block copolymer of linear P4VP chains and brush poly(PEG acrylate) chains formed by a polyacrylate backbone and PEG side chains linked to the acrylate groups by ester bonds. Moreover, the polymer coatings allow the incorporation of diverse functionalities such as fluorescent probes,¹⁹ anticancer drugs,²⁰ molecular fluorescence thermometers,²¹ radiochemical labels (^{111}In), and immunotherapy antibodies (Nimotuzumab). Due to the possible multifunctionalization these MNBs can be considered as a nanoplatform and their biocompatibility and potential application in biomedicine has been traced in previous works.^{21–24} The copolymer coating of sample B has also shown to be advantageous in terms of blood²⁵ and cell compatibility.²⁶

2. EXPERIMENTAL DETAILS

2.1. Synthesis

2.1.1. Synthesis of the Polymers

2.1.1.1. Synthesis of Methoxy-(polyethylene glycol)-Thiol (MPEG(350)-SH

2.1.1.1.1. Synthesis of Methoxy Polyethylene Glycol Tosylate (MPEG(350)-Ts). To a solution of MPEG(350)-OH (21 g, 60 mmol) and triethylamine (7.8 mL, 75 mmol) in dry acetonitrile (250 mL), tosyl chloride (14.44 g, 75 mmol) dissolved in acetonitrile (50 mL) was added dropwise under argon atmosphere. The mixture was stirred at a room temperature for 24 h.

Salts were filtered off and solvent evaporated under argon. The crude was dissolved in dichloromethane

(100 mL), washed with hydrochloric acid 6 N (60 mL) and distilled water (60 mL) dried over anhydrous magnesium sulfate and solvent evaporated under vacuum to yield 27.6 g (54 mmol) of the pure compound. Yield: 90%.

2.1.1.1.2. Synthesis of Methoxy Polyethylene Glycol Bromide (MPEG(350)-Br). A mixture of MPEG(350)-Ts (16.15 g, 30 mmol), lithium bromide (13.03 g, 150 mmol) in dry acetone (150 mL) was heated at reflux for 12 hours. The solvent was evaporated under vacuum and chloroform (150 mL) was added to the residue. The precipitated was filtered off and the solution was washed twice with water (75 mL) dried over magnesium sulphate and concentrated under vacuum to yield 12.7 of orange oil. Yield: 95%.

2.1.1.1.3. Synthesis of Methoxy-(polyethylene glycol)-Thioacetate (MPEG(350)-SAc). A mixture of MPEG(350)-Br (5.60 g, 12.5 mmol), potassium thioacetate (1.75 g, 15 mmol) and ethanol (62.5 mL) was heated at 80 °C for 6 hours. The solution was allowed to cool to room temperature, salts filtered off and solvent evaporated under vacuum. The residue was dissolved in dichloromethane (60 mL), washed twice with water (30 mL), dried over anhydrous magnesium sulfate and solvent evaporated under vacuum yielding 8.20 g (20 mmol) of an orange oil. Yield: 95%.

2.1.1.1.4. Synthesis of Methoxy-(polyethylene glycol)-Thiol (MPEG(350)-SH). A mixture of MPEG(350)-SAc (4.4 g, 10 mmol) and hydrochloric acid 1 M (50 mL) was heated under reflux for 2 hours and then stirred at room temperature overnight under argon atmosphere. The reaction mixture was extracted twice times with chloroform (3 × 25 mL). The combined organic layers was washed twice with distilled water (2 × 25 mL) dried over magnesium sulfate and solvent evaporated under vacuum to yield 3.4 g (8.5 mmol) of an orange oil. Yield 85%.

2.1.1.3. Synthesis of Carboxyl End-Capped Polyethylene Glycol Monoacrylate COOH-PEGA(1000). PEGA(1000) (10 mmol), succinic anhydride (15 mmol), and 4-dimethylaminopyridine (0.5 mmol) were dissolved in 50 mL of dry CH₂Cl₂ under argon atmosphere. The reaction was carried out at room temperature for 48 h. The reaction mixture was filtered and concentrated under vacuum. Product was precipitated three times in cold diethyl ether from tetrahydrofuran and then dried under vacuum.

2.1.1.4. Synthesis of Methyl Fluorescein Poly(ethylene glycol) Acrylate (MeFluPEGA(200)). To a suspension of MeFluOH (3.46 g, 10 mmol) in anhydrous tetrahydrofuran (50 mL), triphenylphosphine (2.89 g, 11 mmol) and PEGA (200) (2.75 g, 11 mmol) previously purified, were added under argon atmosphere. The reaction was cooled in an ice-water bath and then diisopropyl-azodicarboxylate (2.3 mL, 11 mmol) were added dropwise. After the addition was complete, the mixture was stirred at room temperature for three days. Then 0.25 mL of water was added to quench the reaction. Afterwards, it was filtered and the solvent was evaporated under vacuum. The crude residue was

dissolved in 50 mL dichloromethane. The organic phase was washed successively with three fractions of 25 mL of potassium carbonate 1 M, 25 mL of a saturated solution of sodium chloride, dried over anhydrous magnesium sulfate and concentrated under vacuum. The product was purified twice by precipitation in excess of cold diethyl ether from a THF solution. Finally, the pure compound was obtained by flash column chromatography on silica gel increasing the solvent polarity from ethyl acetate to ethyl acetate/methanol (9.5:0.5). Yield 45%.

2.1.1.5. Synthesis of the Block Copolymer. Macroinitiator P4VP-Cl and the amphiphilic block copolymer, P4VP-b-P(MPEGA-co-PEGMA), was prepared using atom transfer radical polymerization (ATRP) according to Piñol et al.¹² P4VP-b-P(MPEGA-co-PEGMA) consists of a poly(4vinylpyridine) (P4VP) block and a water-soluble and biocompatible block of poly((methoxy poly(ethylene glycol)acrylate) (MPEGA, Mn: 480 Da) and poly(ethylene glycol)methacrylate) (PEGMA, Mn: 390 Da determined by NMR after purification). The use of bifunctional PEGMA allows the functionalization of the polymer with carboxylic groups to obtain P4VP-b-P(MPEGA-co-carboxylic PEGMA) through post polymerization modification of the terminal hydroxyl group of the pendant PEGMA chains with succinic anhydride as for the preparation COOH-PEGA (1000). These carboxylic groups provide sites for the anchoring of biomolecules as antibodies or drugs as cis-platin derivatives to the nanoparticle surface so they can be directed to specific targets.

2.1.1.5.1. Synthesis of the Poly(4-vinyl pyridine) Macroinitiator P4VP-Cl. In a typical synthesis a 250 mL Schlenk flask with a magnetic stir bar was charged with CuCl (233.5 mg, 2.37 mmol), CuCl₂ (135.9 g, 1.01 mmol) and TPMA (0.98 g, 3.38 mmol). The flask was degassed by three vacuum-argon cycles and 18 mL of 2-propanol previously deoxygenated by passing argon during 30 minute were introduced via cannula. After stirring, deoxygenated 4-VP (18 mL, 167 mmol) was added under argon atmosphere. Immediately, the mixture was frozen in liquid nitrogen and degassed by 3 freeze-pump-thaw cycles. The flask was immersed in an oil bath thermostated at 40 °C and deoxygenated methyl 2-chloropropionate (0.375 mL, 3.38 mmol) were added into the flask using a syringe purged with argon. After 5 h of reaction the flask was open to the atmosphere and the mixture was cooled to room temperature. The mixture was diluted with a small amount of dichloromethane and poured into a large amount of cold diethyl ether. The polymer was dissolved in 150 mL of dichloromethane, 3 g of Dowex® 50WX2-10 ion-exchange resin were added and the mixture was stirred for 90 minutes at room temperature. After stirring, the mixture was filtered to remove the resin beads. The obtained polymer solution was washed twice with 75 mL of distilled water until a colorless aqueous phase was observed, once with 75 mL of brine, dried over anhydrous

magnesium sulfate and concentrated under vacuum. The polymer was purified twice by dissolving in a small volume of dichloromethane and precipitation in cold diethyl ether. Finally the purified polymer, precipitated as a white powder was collected by filtration and dried under vacuum at 30 °C for three days. Yield (5.89 g, 34%).

2.1.1.5.2. Synthesis of the P4VP-*b*-P(MPEGA-co-PEGMA) Block Copolymer. In a typical synthesis a 50 mL Schlenk flask with a magnetic stir bar was charged with P4VP-Cl (1.84 g, 0.50 mmol), MPEGMA (3.5 mL, 8 mmol), PEGMA (0.72 mL, 2 mmol, Mn: 394 Da) Me6-Tren (134 μ L, 0.50 mmol) and 16 mL of a mixture of H₂O:2-propanol (1:1). The mixture was frozen in liquid nitrogen and degassed by three freeze-pump-thaw cycles. Then the flask was filled with argon and CuCl (34.65 mg, 0.35 mmol) and CuCl₂ (20.17 mg, 0.15 mmol) was added while the mixture was still frozen. The flask was sealed again; air was purged by three short vacuum-argon and then immersed in an oil bath thermostated at 60 °C. After 3 hours the flask was open to the atmosphere and mixture was cooled to room temperature. Reaction mixture was poured into a large volume of cold diethyl ether. The crude product was dissolved in 50 mL of dichloromethane, and the organic phase was washed twice with 25 mL of distilled water and 25 mL of brine and dried over magnesium sulfate. 0.35 g of DOWEX® Marathon MSC (H) ion-exchange resin was added to the polymer solution and the mixture was stirred for 1 hour minutes at room temperature. After stirring the mixture filtered to remove the resin beads, and concentrate under vacuum. The block copolymer was dissolved in methanol and filtered through a pad of silicagel, concentrated under vacuum, purified twice by dissolving in dichloromethane and precipitation in a large volume of cold ether and finally dried by freeze drying. 2 g of polymer was obtained.

2.1.1.5.3. Synthesis of the P4VP-*b*-P(MPEGA-co-carboxylic PEGMA) Block Copolymer. To a solution of P4VP-*b*-P(MPEGA-co-PEGMA) (1.9 g, 1 mmol (-OH)) in dry tetrahydrofuran (20 mL), 4-(Dimethylamino)pyridine (12.21 mg, 0.1 mmol) and succinic anhydride (202.18 mg, 2 mmol) were added under argon atmosphere. The mixture was stirred at room temperature for 24 hours, salts were filtered off and solvent evaporated under vacuum. Polymer was purified twice by dissolving in small volume of dichloromethane and precipitation in a large volume of cold ether and finally dried by freeze drying. 1.67 g (Yield: 84%) of polymer was obtained.

2.1.2. Synthesis of the Ferrofluids

2.1.2.1. Synthesis of γ -Fe₂O₃ NPs. Iron oxide nanoparticles were prepared by a procedure published elsewhere.²³ FeCl₃ (83.2 g 0.513 mol, Aldrich) and FeCl₂·4H₂O (51.4 g 0.253 mol, Aldrich) were dissolved in 0.165 M HCl (400 mL) and the aqueous solution deoxygenated by bubbling argon for 30 min. Then, concentrated aqueous

NH₃ was added dropwise under mechanical stirring until pH 9.2. The resulting solution was stirred for additional 30 min, and the iron oxide precipitate separated by magnetic decantation and washed six times by re-dispersion in water and magnetic decantation. The precipitate (10 g) was treated with 3 M HNO₃ for 2 h, filtered, and redispersed by sonication in 0.01 M HCl solution (500 mL) to obtain an acidic ferrofluid suspension. The suspension was filtered through 0.45 and 0.22 μ m nitrocellulose filters (Millipore). The γ -Fe₂O₃ concentration in the final ferrofluid determined by ICP-OES was 8.6 g/L.

2.1.2.2. Synthesis of Sample A1. Sample A1 was synthesized according to a general strategy for nanoparticle stabilization and functionalization of nanoparticles at physiological pH at room temperature. In a first stage, γ -Fe₂O₃ NP surface is modified by addition of sodium acrylate, a Michael acceptor. Then, the surface is coated with hydrophilic linear PEG chains by 1,4-addition of PEG thiol terminal groups to the α,β -unsaturated acrylate groups on the NP surface. A typical procedure could be as follows: Sodium acrylate (acNa, 96.95 mg, 1 mmol) is dissolved in 10 mL of the γ -Fe₂O₃ NPs ferrofluid. After stirring for 10 min, MPEG(350)-SH (360 mg, 1 mmol) is added to the colloidal dispersion, and the pH is raised to 7.4. The dispersion is sonicated 4 min, heated at 50 °C for 12 h, sonicated again and filtered through 0.45 and 0.22 mm nitrocellulose filters (Millipore). Finally, the NPs are purified by separation in a magnetic column (MACS® Miltenyi Biotec), washed with water and collected in 10 mL of water, to obtain a pure stable dispersion of γ -Fe₂O₃@acNa@MPEG-SH NPs. The γ -Fe₂O₃ concentration in the final ferrofluid determined by ICP-OES was 2.7 g/L.

2.1.2.3. Synthesis of Sample A2. The coating polymer in this sample consist on a pH responsive block of poly(4vinylpyridine) (P4VP) block and a water-soluble and biocompatible poly(ethylene glycol) P(MPEGA-co-carboxyl PEGMA) block with a brush like architecture. At pH between 2 and 4.8 the pyridine groups of the P4VP block (pK_a = 4.8) are partially protonated, and both of the blocks are hydrophilic. But above this pH value the pyridine groups are fully deprotonated and hydrophobic, and therefore the block copolymer has an amphiphilic character. Consequently, the copolymer undergoes a self-assembly process that ends up in a highly efficient encapsulation of γ -Fe₂O₃ NPs in the interior of the hydrophobic nucleus. The procedure has been previously described in Ref. [1]. Briefly, 50 mg of block copolymer (P4VP-*b*-P(MPEGA-co-carboxyl PEGMA) are dissolved in 10 ml of γ -Fe₂O₃ ferrofluid, then the pH is raised to 7.4 by slow additions of 1 M NaOH solution, and the dispersion is sonicated and filtered through 0.45 and 0.22 μ m nitrocellulose filters (Millipore). Finally, the polymer coated γ -Fe₂O₃ NPs are purified by separation in a magnetic column (MACS® Miltenyi Biotec), washed with MES

(0.1 M) and collected in 10 mL of 0.1 M MES (2-(N-morpholino)ethanesulfonic acid). The $\gamma\text{-Fe}_2\text{O}_3$ concentration in the final ferrofluid determined by ICP-OES was 3.0 g/L.

2.1.2.4. Synthesis of Sample B. Contrary to samples A1 and A2, the $\gamma\text{-Fe}_2\text{O}_3$ NPs in sample B were not precipitated in solution but inside a P4VP polymer matrix. In this case, the $\gamma\text{-Fe}_2\text{O}_3$ NPs do not form aggregates; instead they precipitate as individual NPs uniformly distributed within the polymer matrix. This methodology is highly versatile (feasible) as the size of the ($\gamma\text{-Fe}_2\text{O}_3$) cores can be easily tuned by using different Fe(II)/Fe(III) and Fe/P4VP ratios. In a second step, the iron oxide-polymer composite is redispersed in water at pH = 3, in which the pyridine groups are partially protonated and hydrophilic, as explained above. Then the composite nanobeads are further coated with a hydrophilic poly(ethylene glycol) (PEG) shell that provides long-term stability and biocompatibility. The PEG macromonomers are grafted to the P4VP polymer chain by a Michael addition reaction between the double bond of the acrylate groups located at the end of the PEG chains and the nucleophilic nitrogen of the pyridine that becomes quaternized (40% of the total pyridine groups). After the reaction with PEG, the pH is increased to physiological causing a total deprotonation of the pyridine rings, and consequently a highly packing of the hydrophobic P4VP block entrapping the magnetic cores inside. This strategy allows an easy multifunctionalization in one step by using a mixture of poly(ethylene glycol) acrylates that might carry diverse functionalities at the free end of the PEG chain. The procedure is carried out in two stages as described in Refs. [27–29].

P4VP (Aldrich, 60 kD) (0.5 g) were dissolved in a mixture of water:acetone (1:1) (25 mL). FeBr_3 1 M in 0.05 M HBr (2.51 mL) and FeBr_2 1 M in 0.05 M HBr (2.26 mL) previously filtered through 0.45 and a 0.22 μm nitrocellulose filters (Millipore) were then added to the polymer solution under stirring. A film of iron-polymer precursor was obtained by evaporation of the solution in a Petri dish. The precursor film was treated with NaOH 1 M (45 mL) for 90 min, washed with water, and dried in open air to obtain a polymer composite (0.9 g).

$\gamma\text{-Fe}_2\text{O}_3$ /P4VP nanocomposite (0.30 g, 1.90 mmol of pyridine groups) was dispersed in an acidic solution at approx. pH 3. The resulting acidic ferrofluid was filtered through 0.45 and a 0.22 μm nitrocellulose filters (Millipore), mixed with a mixture of PEGA (200) (0.16 mL, 0.72 mmol), COOH-PEGA(1000) (20 mg, 1.75×10^{-2} mmol) and MeFlu-PEGA(1000) (20 mg, 3.37×10^{-2} mmol) dissolved in ethanol/water (1:1) (2 mL) and heated to 70 °C during 24 h. Then, the pH was adjusted to 7 by slow addition of a 0.5 M NaOH solution. Then the dispersion was filtered again through 0.45 and a 0.22 μm nitrocellulose filters (Millipore). Finally, the ferrofluid was purified by separation in a magnetic column

(MACS® Miltenyi Biotec), washed with distilled water, and collected in distilled water. The $\gamma\text{-Fe}_2\text{O}_3$ concentration in the final ferrofluid determined by ICP-OES was 1.2 g/L.

2.2. Chemico-Physical Characterization

Iron content in the magnetic fluid samples was determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) in a plasma 40 ICP Perkin-Elmer spectrometer. Samples for ICP-OES were prepared by freeze drying the MNB suspensions and digestion of the solids in concentrated HNO_3 overnight. Dynamic light scattering (DLS) and zeta potential measurements of the ferrofluids were performed on a Malvern Zetasizer NS (Malvern Instruments Ltd., Worcestershire, UK) using a He-Ne laser with a 633 nm wavelength with a detector angle of 173° at 25 °C. The measurements were performed after 1:20 dilution in ultra-pure water, like in NMR measurements, and were repeated at least twice (see SI).

Transmission electron microscopy (TEM) observations were carried out with a JEOL 2000-FXII microscope equipped with an Energy Dispersive X-ray (EDX) Microanalysis Detector INCA 200 X-Sight (Oxford Instruments). Samples for TEM analysis were prepared by dip coating on carbon coated copper grids. ED studies were performed to determine the crystalline nature of the iron oxide nanoparticles. Cryo-TEM observations were performed on a FEI Tecnai F30 microscope. Samples for cryo-TEM experiments were prepared by plunge-freezing of a grid previously immersed in the ferrofluid and partially dried in a liquid ethane bath.

Freeze dried samples were characterized by Fourier transform infrared spectroscopy (FTIR) and Thermogravimetric analysis (TGA) (see SI). FTIR spectra were recorded at room temperature in a Perkin Elmer Spectrum 100 FT-IR spectrometer equipped with a Universal ATR sampling accessory with 4 cm^{-1} resolution within 4000–380 cm^{-1} . Measurements were performed using a simultaneous DTA-TGA unit SDT-2960 (TA Instruments) at a heating rate of 10 °C/min under air atmosphere.

The magnetic properties of these ferrofluids were studied by means of dc magnetization (i.e., measurements in static magnetic field) at room temperature with applied field up to 9 T, and ac magnetic susceptibility measurements (i.e., measurements in oscillating magnetic field of 0.3 mT) as a function of temperature (5–300 K) and frequency (1–10000 Hz range for A1 and A2 and 1–1000 Hz range for B) in MPMS-XL SQUID magnetometers from Quantum Design (Quantum Design, San Diego, CA). Zero Field Cooled/Field Cooled (ZFC/FC) curves were obtained by measuring the temperature dependence of the magnetization applying a probe magnetic field (5 mT), after cooling the sample in the presence (FC) or in the absence (ZFC) of the field.

The local spin dynamics and the MRI contrast efficiency were assessed by means of ^1H nuclear magnetic resonance (NMR) relaxometric characterization.

The NMR-dispersion profiles were performed at room temperature by measuring the longitudinal and the transverse nuclear relaxation times T_1 and T_2 in the frequency range $10 \text{ kHz} \leq \nu \leq 170 \text{ MHz}$. The NMR signal detection and generation was obtained by a Smartracer Stellar relaxometer (for $10 \text{ kHz} \leq \nu \leq 9.5 \text{ MHz}$) which makes use of the fast-field-cycling technique and a Stellar Spinmaster and an Apollo-Tecmag Fourier transform-nuclear magnetic resonance (FT-NMR) spectrometers (for $\nu \geq 9.5 \text{ MHz}$).

3. RESULTS AND DISCUSSION

3.1. Composition, Particle Size and Structure of the Ferrofluids

According to ICP-OES results, the iron oxide concentration in the ferrofluids was 8.2, 2.7, 3.0 and 1.2 mg/mL for the uncoated, A1, A2 and B samples respectively. TGA and DTA analysis were used to determine the relative amount and thermal stability of the polymer coating. Sample A1 undergoes a mass loss of 5% between 220 °C and 300 °C, which can be assigned to degradation of the acrylate-PEG coating, and it is consistent with a thin polymer layer as observed by FTIR and DLS. In sample A2, the calculated mass of block copolymer was around 24%. The polymer degradation occurs in two steps. A first weight loss step corresponding to PEG acrylate degradation occurs between 200 °C to 320 °C. A second one occurs between 320 °C and 420 °C and can be assigned to degradation of the P4VP polymer backbone according to the literature.³⁰ Curve for sample B1 showed a three-step polymer degradation process corresponding to a relative polymer content of about 70%.

Electron Diffraction (ED) patterns of A1, A2 and B samples shown in Figure 2, are consistent with a spinel crystal structure. Previous studies by EELS and analysis of Fe(II) demonstrated the absence of magnetite and that the samples are constituted by maghemite ($\gamma\text{-Fe}_2\text{O}_3$).²⁸

TEM and CryoTEM experiments have been used for the morphological characterization of the magnetic iron-oxide nanoparticles of the beads and to measure the size

of single nanoparticles (by TEM, after water evaporation) and of agglomerates once they are dispersed in the ferrofluid (b CryoTEM after the solutions were frozen). The polymer coating is essentially not visible by TEM since it does not give enough contrast on the substrate and the procedure used for the preparation of the TEM specimens alters irreversibly the samples, causing the nanoparticles aggregation. Nevertheless, as said above, it is possible to identify the single magnetic particles and determine their size by TEM images (see Fig. 1). The average sizes with the corresponding errors are reported in Table I.

Since the samples A1 and A2 are synthesized from the same stock iron oxide ferrofluid, they have the same size distribution. TEM images showed rounded nanoparticles with a consistent size dispersion peaked at mean size $D_p(\text{SD}) = 10.4(2.1) \text{ nm}$ ($N = 200$). CryoTEM images of sample A1 (Fig. 1(a)) showed that the nanoparticles form clusters of 1–7 nanoparticles aggregated in a random packing manner with a mean size $D_a(\text{SD}) = 23.0(8.1) \text{ nm}$ ($N = 210$). These experimental results are also valid for the sample A2 as the clustering process takes place during the synthesis of the iron-oxide ferrofluid, before the coating reaction. The aggregates appear well separated in the Cryo-TEM images and, considering also the average hydrodynamic sizes measured for both samples from DLS measurements (see below), we can assume that each nanobead is constituted by a single aggregate. The log-normal standard deviation of the cluster size distribution and its average estimation from the log-normal fit are also reported in Table I. Again, we can assume that the aggregates are well separated, being the coating dimension lower than the inter-aggregate distance. Iron oxide nanoparticles in sample B have a spherical shape and are organized in a grape-like structure (Fig. 1(b)). Cryo-TEM images show that the nanoparticles in this sample are embedded in the polymer P4VP block without aggregation as some spacing between the magnetic cores is visible (Fig. 1(a)). An area with a weak contrast can be discerned around the particles, corresponding to the hydrophobic P4VP. The presence of the PEG residues forming the

Figure 2. Electron diffraction patterns of samples A1, A2 and B. Characteristic reflections of an inverse spinel structure are visible, in particular referring to A2 the labels specify the indexing of the pattern peaks: a is (220), b is (311), c is (400), d is (422), e is (511) and f is (440).

Table I. Results from TEM, Cryo-TEM and DLS experiments. From left: Average core size d_{NP} and polydispersity P_{NP} of the iron-oxide nanoparticles from TEM measurements; average diameter d_C of the clusters of iron-oxide nanoparticles from Cryo-TEM measurements; average cluster size $d_{C,ln}$ and polydispersity P_{ln} according to the log-normal distribution fitting function; average hydrodynamic diameter d_H and half width at half maximum (HWHM) from DLS measurements. PDI is the polydispersity index from DLS measurements.

Sample	TEM		Cryo-TEM			DLS		
	d_{NP} (nm)	P_{NP}	d_C (nm)	$d_{C,ln}$ (nm)	P_{ln}	d_H (nm)	HWHM (nm)	PDI
A1	10.4 ± 0.2	0.20	23 ± 1	21.8 ± 1.0	0.35	24.2	8	0.21
A2	10.4 ± 0.2	0.20	23 ± 1	21.8 ± 1.0	0.35	34.6	11.1	0.21
B	11.4 ± 0.3	0.22	–	–	–	93.0	25	0.14

hydrophilic shell of the nanobead is not exposed due to the low contrast between the polymers and the vitrified water matrix. Cryo-TEM images show the presence of 10–20 nanoparticles inside each bead. The calculated average number of nanoparticles per bead is 15.

Dynamic light scattering (DLS) was performed to determine the hydrodynamic size of the samples. DLS observations show monomodal size distribution for all the samples. Hydrodynamic size distribution of the original precursors was also determined for comparative purposes for samples A1 and A2.

The DLS size distribution of sample A1 shows a peak at ~24 nm, a value in very good agreement with the average cluster size observed in cryo-TEM images. After PEG modification the average hydrodynamic size of sample A1 is very similar to that observed for the aggregates by DLS and TEM. This result is in good agreement with data obtained in the TGA analysis and FTIR studies indicating a very thin polymer coating.

Surface charge was also determined, as it represents an important factor for stability and have profound effect in biodistribution and toxicology of the nanomaterials. The uncoated SPIONs are positively charged with Z-potential average values of +40 mV. After surface modification with acrylate, the surface charge becomes highly negative with average values of –40 mV. This value decreases to –30 mV after coating with mercapto poly(ethylene glycol) polymer. In the case of sample A2, the average hydrodynamic size increased from 24 to 35 nm after coating with the block copolymer, and the Z-potential becomes negative with average values of –25 mV due to the carboxylates groups at the end of a part of the PEG chains.

Sample B show a single population with an average hydrodynamic diameter of 93 nm in DLS observations (such higher value due the presence of the polymeric matrix), which is in accordance with the synthetic procedure, and a positive Z-potential of +30 mV in pure water. The positive charge is associated with the cationic character of the quaternized pyridines present in the polymer coating in higher percentage than PEG residues with terminal carboxyl groups. This surface charge is reduced to

nearly zero in buffers with a high ionic strength phosphate buffered saline (PBS) due to a screening effect.

As a final note of peculiar importance, it should be observed that according to the previous results A1 and A2 can be classified as USPIO (ultra small particle of iron oxide) particles while sample B can be classified as SPIO (small particle of iron oxide).

3.2. DC Magnetic Measurements

Figures 3(a)–(c) shows ZFC/FC DC magnetization curves as a function of temperature at 50 Oe on powder samples. ZFC curves display maxima at temperature $T = 212$ K for A1, $T = 248$ K for A2 and $T > 300$ K for B respectively. In the ideal case of a monodisperse non interacting nanoparticle, in the ZFC/FC DC curves the system performs a transition between the so-called blocked and unblocked regimes in a really narrow temperature range. This temperature transition represents the threshold passage from a superparamagnetic to a blocked magnetization behaviors and it is normally considered as the blocking temperature of the system (T_b). Namely above T_b the thermal energy is enough to overcome the characteristics anisotropy energy barrier E_A of the nanoparticle (E_A is proportional to K_A , the anisotropy constant, and V , the volume of nanoparticles). Real samples always present a size dispersion that implies a different energy barrier and therefore a different T_b for each size fraction.

In order to study the energy barrier distributions of the investigated samples we evaluated from ZFC/FC measurements the blocking temperature distribution as proposed by Chantrell et al. and Bruvera et al.³¹ assuming $f(T_b) \propto -d(FC - ZFC)/dT$ which is plotted as a function of T in Figures 3(d)–(f). It should be remarked that this formula is valid for non-interacting nanoparticles, a condition approximately verified by our samples.

Indeed, since $k_B T_b$ is the minimal thermal energy necessary to overcome the anisotropy barrier, there exists a direct correlation of $f(T_b)$ to the distribution of energy barriers. Interestingly the distributions of T_b present two peaks for all the samples: a narrow peak (T_b^G) at low temperature, which can be possibly attributed to the dynamics of the surface spins (see the discussion below), and a broader peak (T_b^L) at higher temperature correlated to the dynamics of the nanoparticle core magnetization. To describe the temperature dependence of $f(T_b)$, we used a model (Eq. (1)) that considers a sum of Gaussian and Log-normal functions

$$y = y_0 + \frac{A e^{(-4 \ln(2)(x-x_c)^2)/w^2}}{w \sqrt{\pi/(4 \ln(2))}} + \frac{B}{s x \sqrt{2\pi}} e^{-[\ln(x/x_b)]^2/(2s^2)} \quad (1)$$

where A is the amplitude, x_c is the center, w is the FWHM (the Full Width at Half Maximum) of the Normal distribution and B is the area, s is the standard deviation and x_b is the mean of the Log-normal distribution; y_0 was fixed to 0, while the other parameters were free to vary.

Figure 3. ZFC/FC curves of sample A1(a), A2(b), B(c) and the corresponding blocking temperature distributions (d–f), calculated as described in the text. Red lines represent the fit of the $f(T_b)$ curves obtained by means of (1).

The parameters extracted from the fit of $f(T_b)$ by means of Eq. (1) are reported in Table II and the red lines in Figures 3(d)–(f) represent the best fit curves.

The peak temperature of the Gaussian distribution (i.e., T_b^G) of B and its relative position with respect to the same feature in A1 and A2 sample, induced us to attribute this contribution (Normal distribution) to the surface spins in all 3 samples. Due to the clusters spin topology, the surface spins of the NPs composing A1 and A2 can be considered more strongly coupled (e.g., via exchange coupling) than in the polymer bead of sample B (where the NPs are well separated) thus leading to a slightly higher surface blocking temperature in the former case.

The Log-normal contribution to $f(T_b)$ is ascribable to the energy barrier distributions due to the size distribution of our samples, see Table I. To evaluate the peak position, corresponding to the so-called blocking temperature of the system, we considered the mode of the Log-normal distribution, obtaining an increase of the peak temperature from B to A1 to A2. The difference in T_b^L of B and A samples resides once again in the spin topology. In fact,

the lower the temperature of T_b^L peak, the smaller is the average magnetic volume of the nanoconstruct that originates it. Taking into account that the sample B presents single interacting NPs and sample A1 and A2 are agglomeration of 1–7 NPs, it's easy to predict $T_b^L(B) < T_b^L(A1)$, $T_b^L(A2)$ as experimentally revealed. The magnetic behavior of samples A1 and A2 can be ascribed to the different coatings that can affect the iron ions valence. Sample A1 was indeed coated first by an acrylate layer, that coordinates the iron ions with a carboxylic group, and then by polyethyleneglycol chains with a terminal thiol group, MPEG350-SH. The sulfur of the MPEG (350)-SH molecule probably reduces iron from Fe^{3+} to Fe^{2+} , thus producing wustite (FeO) from $\gamma-Fe_2O_3$. As a consequence of the antiferromagnetism of wustite, the magnetization inside the nanoparticle decreases, leading to a lower value of the anisotropy energy barrier. Information obtained from the Infrared spectroscopy (SI) also sustains this explanation. The FTIR spectra of iron oxide nanoparticles before coating in both A1 and A2 samples showed bands at 550 and 629 cm^{-1} that are associated to vacancy ordering in maghemite crystal structure.^{31,32} However the band at 629 cm^{-1} disappears in sample A1 after polymer coating. Another observation that endorses this explanation is the color change observed in the sample A1 once the MPEG(350)-SH was added on the coating reaction. The initial reddish-brown color of the suspension turned brownish-black that is characteristic of Fe^{2+} (wustite). This color variation did not occur during the synthesis of the sample A2, having a light brown color, that is constituted almost totally by $\gamma-Fe_2O_3$.

Table II. Fitting results by means of Eq. (1). From left: Center of Gaussian distribution, full width at half maximum of Gaussian distribution, mode of log-normal distribution and relative log-normal standard deviations.

Sample	Gaussian T_b^G (K)	FWHM	Log-normal T_b^L (mode) (K)	σ_L (K)
A1	16.1 ± 0.3	28.4 ± 0.9	86.7 ± 6.3	85.5 ± 6.3
A2	15.3 ± 0.3	41.0 ± 3.0	101.1 ± 7.0	98.2 ± 8.5
B	11.9 ± 0.4	21.4 ± 0.9	67.7 ± 7.5	251.7 ± 13.5

Figure 4. Magnetization M as a function of the applied magnetic field H at $T = 300$ K. Insets show the saturation magnetization M_s for sample A1, A2 and B.

The hysteresis curves (i.e., magnetization vs. field) recorded at $T = 300$ K confirm that all the systems are superparamagnetic and unblocked at room temperature. As shown in Figure 4, the magnetization of A2 and B samples do not saturate in the field range of measurements therefore the estimation of the saturation magnetizations was done by assuming the empiric law $M = M_s + a/H + b/H^2$ valid in the high field branch. The calculated saturation magnetizations are specified in the inset of the graphs in Figure 4.

The M versus H measurements further confirm the conclusions about the magnetization values of A1 and A2 outlined above. In fact the saturation is reached at lower field in A1 sample, in agreement with the higher T_b^L of sample A2 with respect to the one of A1, (see the analysis of ZFC/FC curves performed in the previous paragraph).

3.3. AC Magnetic Measurements

The dynamics of the spin blocking process was studied in more detail by AC susceptibility measurements, which allows also to estimate the influence of dipolar interactions. Figure 5(a) shows the in-phase (χ' , solid symbols) and out-of-phase (χ'' , empty symbols) parts of the magnetic susceptibility of A1, A2 and B samples as a function of temperature in the frequency range $1 \text{ Hz} < f < 10000 \text{ Hz}$ at a field of amplitude $H_{AC} = 0.3 \text{ mT}$, in the temperature range $5\text{--}300 \text{ K}$. A maximum at fixed f and H_{AC} in the energy absorption (χ'') is expected when T is varied, at a temperature $T = T_{max}$ such that the correlation time τ_c of the spin dynamics of the system matches the

frequency of the external stimulus ω_m (i.e., $\omega_m \tau_c \sim 1$). The observation of such maxima was not possible, especially for sample A1 and A2, due to a phase transition of the solvent that occurs around $T^* = 260 \text{ K}$ causing a narrow step in the raw data. Sample B showed a very broad maximum whose position in temperature however is difficult to individuate.

The real (χ') and imaginary (χ'') part of magnetic susceptibility were also measured at fixed temperature $T = 300 \text{ K}$ as a function of frequency (Fig. 5(b)). It is possible to note that χ'' (blue points) exhibits a maximum at different positions for the samples A2 ($\nu_{max} = 4000 \text{ Hz}$) and B ($\nu_{max} = 800 \text{ Hz}$), while it increases monotonically for the sample A1. The χ'' maximum position seems to be correlated to the size of the samples: it shifts toward lower frequencies with the increase of the full particle size. $\chi''(f)$ measurements were carried out both in colloidal suspension and in gelified agar-agar samples to constrain the bead rotation in order to study the origin of the maximum. The maximum for $\chi''(f)$ was not longer observed in the gel samples. As a consequence it was possible to attribute the origin of the maximum in $\chi''(f)$ to the Brownian rotation of the beads: indeed the Brownian rotational diffusion is the dominant relaxation time and the position (in frequency) of the peak in $\chi''(f)$ is inversely proportional to the hydrodynamic size of the nanoparticle. Considering the expression of rotational Brownian relaxation time $\tau_B = (3\eta V_H)/(k_B T)$ (where we used for η the water dynamic viscosity at $T = 300 \text{ K}$) and the average hydrodynamic diameter d_H of our MNBs obtained by DLS (see

Figure 5. (a) χ_{ac} measurements as a function of temperature. Solid circles represent the in-phase AC susceptibility χ' , open circles represent the out-of-phase AC susceptibility χ'' . (b) χ_{ac} measurements as a function of frequency at $T = 300$ K. Red points represent the in-phase AC susceptibility χ' , blue points represents the out-of-phase AC susceptibility χ'' .

Table I), it is possible to estimate the following Brownian times: $\tau_B = 5.4 \times 10^{-6}$ s for A1, $\tau_B = 1.6 \times 10^{-5}$ s for A2 and $\tau_B = 3.1 \times 10^{-4}$ s for B. These values lead to frequency values higher than the ones deduced from the maximum of $\chi''(f)$ curves. The difference could be due to the contribution of the Néel relaxation. Moreover, it should be taken into account that the nanoparticles are multicore and, as a consequence, the corresponding correlation times could be more complicate than the simple ones normally calculated on the basis of the Brown and Neel relaxation processes of single particles. By concluding, to interpret correctly the ac susceptibility versus frequency measurements (see for instance Ref. [33]), a more detailed theoretical approach has to be developed, a scientific issue outside the aims of the present experimental paper. As an independent example of peculiar $\chi''(f)$ results found in compounds constituted not simply by single particle cores, Chen et al. reported a shift in the peak of $\chi''(f)$ to lower frequencies upon binding a protein to their MNPs which

they associated with the R_H increase, corresponding to the size of the biomolecule.³³

3.4. Relaxivity Properties

^1H Nuclear Magnetic Resonance measurements were performed at room temperature as a function of the strength of the external magnetic field in order to evaluate the efficiency of MNBs to enhance the nuclear relaxation rate of protons of the aqueous media other than to study the fundamental mechanisms beyond the nuclear relaxation induced by our samples. The nuclear longitudinal (r_1) relaxivity curves of samples A1, A2 and B are shown in Figure 6(a). It is worth noticing that, even if for superparamagnetic compounds is expected a low efficiency in longitudinally relaxing the water protons, the r_1 values of the investigated samples are comparable to the ones of paramagnetic Gadolinim-based compounds³⁷ (i.e., T1 MRI contrast agents). The flatness in the low frequency region

Figure 6. Longitudinal relaxivities r_1 (a) and transverse relaxivities r_2 (b) for samples A1, A2 and B.

and the absence of a maximum in the NMRD curves at intermediate frequencies is indicative for the high magnetic anisotropy of these samples.³⁸

Even though sample A1 and A2 have the same magnetic core, their efficiency in relaxing the longitudinal part of the nuclear magnetization of the solvent is different (see Fig. 6(a)). The different values of r_1 are explained by the different value of M_S (the longitudinal relaxivity r_1 is predicted³⁹ to increase with M_S) and to the different coating thickness, which influences the distance of minimum approach $d_{\min}$ of solvent protons. Indeed the relaxation efficiency is proportional to the strength of the hyperfine dipole–dipole interaction ($\propto 1/d^3$) among the hydrogen nuclei of the bulk water and the electrons of this MNB. In this sense the thinner and hydrophilic coating of A1 sample allows the water molecules to get closer to the MNPs thus relaxing faster. Conversely the amphiphilic character of a part of the P4VP-b-P (PMEGA-co-PEGMA) coating contributes to partially repulse the water molecules, if compared to A1 coating, decreasing the r_1 values.

The NMR dispersion curve of sample B shows both r_1 and r_2 lower values with respect to the other two samples. Again this occurrence is related to the spin topology of B, probably the main responsible for the lower efficiency in relaxing the water protons of the solvent. In particular we expect that the water protons “see” the magnetic core of sample A1 and A2 as a single cluster with average volume equal to the sum of the volumes of beads. The nanoparticles composing sample B are instead seen as clusters of separated beads thus leading to lower relaxivity values.

As already explained above, the analysis of the transverse relaxivity r_2 allows evaluating the T_2 -contrast efficiency of the samples in MR images. As Figure 6(b) shows, all samples display high r_2 values for $\nu > 10$ MHz ($r_2(\text{A1}) \sim 280 \text{ s}^{-1} \text{mM}^{-1}$; $r_2(\text{A2}) \sim 200 \text{ s}^{-1} \text{mM}^{-1}$ and $r_2(\text{B}) \sim 130 \text{ s}^{-1} \text{mM}^{-1}$) well above the commercial product Endorem® ($r_2(\text{EN}) = 100 \text{ s}^{-1} \text{mM}^{-1}$). Besides, the different transversal relaxation efficiencies of the A samples reflect again the different characteristics of the MNBs. In fact the higher r_2 of A1 is explained by the higher local dipolar field felt by the dispersant protons due to the closer distance between spins and the magnetic bead. The interesting increase of r_2 values as a function of the frequency for A2 evidences the higher magnetization with respect to A1, as revealed by magnetic measurements, that allows to partially offset the lower efficiency caused by the different coating. On the other hand the lower r_2 values of B over the whole frequency range reflect its completely different spin topology, which clearly does not favor a so high NMR efficiency (as happens to the other two MNBs).

The NMR data analysis of single USPIO is usually performed by means of the well-known heuristic model proposed by Roch et al. in 1999.³⁹ The model is able to describe the longitudinal relaxation of the water proton induced by low interacting particles with a maximum diameter of 20 nm. In this sense all of the investigated

sample are out of the threshold of the Roch model. Indeed, even if the clusters composing sample A1 and A2 could be considered as single particles, their dimensions are > 20 nm. On the other hand, the ordered matrix in which are embedded the NPs of sample B put also this sample outside the validity of the model (i.e., single particle). In view of the above considerations the data analysis of the NMR-D curves of MNBs needs the development of suitable models able to describe the relaxivity of clusters or matrix of NPs, discussed in literature not only in the past^{40–42} but also recently.¹³

4. CONCLUSIONS

Three iron oxide-based (maghemite) magnetic nanoparticles belonging to two completely different spin topology classes were investigated by means of DC and AC magnetometry and ¹H-NMR dispersion relaxivity profiles. In this study we aim at exploring both fundamental properties and applicative perspectives as contrast agents in MRI. To this goal, the structural properties were assessed and their superparamagnetic character established, by means of a wide morphological and magnetic characterization. From DC magnetization curves analysis we singled out two different energy distribution barriers for the magnetization reversal corresponding to a “high temperature” ($T > 60$ K) log-normal peak T_b^L and to a “low temperature” ($T < 20$ K) Gaussian peak T_b^G . The T_b^G values are very similar for all our samples and we suggest that they are due to the blocking temperature of the surface spins, whose dynamics is faster than the bulk spins. The latter ones block at T_b^L , whose value increases with increasing the NPs clusters dimensions, in qualitative agreement with the Neel model of superparamagnetic NPs.

It is worth to notice that, even though T_b^G is here ascribed to a thermal activation mechanism, it could in principle originate from a spin-glass transition of the disordered surface layer. However, from the magnetic data we have no precise indication that we are in presence of such transition, an occurrence that requires anyway a detailed separate investigation. Additionally, we exclude that the maximum occurring at T_b^G could be due to the presence of a transition of surface spins shell from paramagnetic to antiferromagnetic or ferromagnetic state because of the small dimensions of the shell in all investigated samples.

From AC susceptibility measurements versus frequency at $T = 300$ K in solution and in gel, the presence just in solution of the absorption peak in the imaginary part of χ_{ac} in samples A2 and B allowed to conclude that the dominant correlation time at high temperature in the range frequency $10 < f < 10000$ Hz is the Brownian one τ_B . On the other hand the experimental results obtained from relaxivities measurements reveal a clear dependence of the dynamics of the relaxation on the spin topology of our systems as shown by the different efficiency (r_2 values) in NMR-D

profiles. Moreover the high values of the transverse relaxivity r_2 (up to $280 \text{ s}^{-1}\text{mM}^{-1}$ for A1 sample) are well above the typical values of commercial iron oxide-based T_2 -CAs. Due to these evidences and considering the coating biocompatibility, we can conclude that all the MNBs of this research work can be considered very promising T_2 -CAs for MRI application and allow to give suggestions on the effect of the spin topology in the nuclear relaxation mechanism. Lastly the interesting high r_1 values of all the samples open the possible further use of our systems as T_1 and T_2 -CAs at the same time.

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References and Notes

- J. Gupta, A. Prakash, M. K. Jaiswal, A. Agarrwal, and D. Bahadur, *J. Magn. Magn. Mater.* (2017), In press, <https://doi.org/10.1016/j.jmmm.2017.05.084>.
- F. Canfarotta and S. A. Piletsky, *Adv. Healthcare Mater.* 3, 160 (2014).
- S. Dutz and R. Hergt, *Nanotechnology* 25, 452001 (2014).
- M. Zahraei, M. Marciello, A. Lazaro-Carrillo, A. Villanueva, F. Herranz, M. Talelli, R. Costo, A. Monshi, D. Shahbazi-Gahrouei, M. Amirnasr, B. Behdadfar, and M. P. Morales, *Nanotechnology* 27, 255702 (2016).
- C. Janko, J. Zaloga, M. Pottler, S. Durr, D. Eberbeck, R. Tietze, S. Lyrer, and C. Alexiou, *J. Magn. Magn. Mater.* 431, 281 (2017).
- J. W. M. Bulte and D. L. Kraitchman, *NMR Biomed.* 17, 484 (2004).
- C. Blanco-Andujar, A. Walter, G. Cotin, C. Bordeianu, D. Mertz, D. Felder-Flesch, and S. Begin-Colin, *Nanomedicine* 11, 1889 (2016).
- P. Arosio, J. Thevenot, T. Orlando, F. Orsini, M. Corti, M. Mariani, L. Bordonali, C. Innocenti, C. Sangregorio, H. Oliveira, S. Lecommandoux, A. Lascialfari, and O. Sandre, *J. Mater. Chem. B* 1, 5317 (2013).
- M. S. Seehra (ed.), *Magnetic Spinels-Synthesis, Properties and Applications*, Intech (2017), Chap. 1, pp. 3–29, ISBN 978-953-51-2974-5.
- S. Mørup and Mössbauer, *Hyperfine Interact.* 60, 959 (1990).
- D. A. Garanin and H. Kachkachi, *Phys. Rev. Lett.* 90, 65504 (2003).
- M. Basini, T. Orlando, P. Arosio, M. F. Casula, D. Espa, S. Murgia, C. Sangregorio, C. Innocenti, and A. Lascialfari, *J. Chem. Phys.* 146, 034703 (2017).
- S. Hak, P. E. Goa, S. Stenmark, F. Bjerkholt, and O. Haraldseth, *Magnetic Resonance in Medicine* 74, 858 (2015).
- J. Kolosnjaj-Tabi, L. Lartigue, Y. Javed, N. Luciani, T. Pellegrino, C. Wilhelm, D. Alloyeau, and F. Gazeau, *Nano Today* 11, 280 (2016).
- M. Azhdarzadeh, A. A. Saei, S. Sharifi, M. J. Hajipour, A. M. Alkilany, M. Sharifzadeh, F. Ramazani, S. Laurent, A. Mashaghi, and M. Mahmoudi, *Nanomedicine* 10, 2931 (2015).
- G. Chen, I. Roy, C. Yang, and P. N. Prasad, *Chem. Rev.* 116, 2826 (2016).
- S. Laurent, D. Forge, M. Port, A. Roch, C. Robic, L. V. Elst, and R. N. Muller, *Chem. Rev.* 108, 2064 (2008).
- C. Xu and S. Sun, *Dalton Trans* 29, 5583 (2009).
- C. Sun, K. Du, C. Fang, N. Bhattarai, O. Veisich, F. Kievit, Z. Stephen, D. Lee, R. G. Ellenbogen, B. Ratner, and M. Zhang, *ACS Nano* 4, 2402 (2010).
- S. Schöttler, G. Becker, S. Winzen, T. Steinbach, K. Mohr, K. Landfester, V. Mailänder, and F. R. Wurm, *Nature Nanotechnol.* 11, 372 (2016).
- R. Bustamante, A. Millán, R. Piñol, F. Palacio, J. Carrey, M. Respaud, R. Fernandez-Pacheco, and N. J. O. Silva, *Phys. Rev. B* 88, 184406 (2013).
- P. Díez, M. González-Muñoz, M. González-González, R. M. Dégano, R. Jara-Acevedo, S. Sánchez-Paradinas, R. Piñol, J. L. Murillo, G. Lou, F. Palacio, M. J. Almendral, A. Millán, E. Rodríguez-Fernández, J. J. Criado, N. Ibarrola, and M. Fuentes, *Nanoscale* 9, 9960 (2017).
- R. Piñol, C. D. S. Brites, R. Bustamante, A. Martínez, N. J. O. Silva, J. L. Murillo, R. Cases, J. Carrey, C. Estepa, C. Sosa, F. Palacio, L. D. Carlos, and A. Millán, *ACS Nano* 9, 3134 (2015).
- H. Amiri, R. Bustamante, A. Millán, N. J. O. Silva, R. Piñol, L. Gabilondo, F. Palacio, P. Arosio, M. Corti, and A. Lascialfari, *Magn. Reson. Med.* 66, 1715 (2011).
- L. M. A. Ali, R. Piñol, R. Villa-Bellosta, L. Gabilondo, A. Millán, F. Palacio, and V. Sorribas, *Toxicology In Vitro* 29, 962 (2015).
- R. Piñol, C. D. S. Brites, R. Bustamante, A. Martínez, N. J. O. Silva, J. L. Murillo, R. Cases, J. Carrey, C. Estepa, C. Sosa, F. Palacio, L. D. Carlos, and A. Millán, *ACS Nano* 9, 3134 (2015).
- J. Park, K. An, Y. Hwang, J.-G. Park, H.-J. Noh, J.-Y. Kim, J.-H. Park, N.-M. Hwang, and T. Hyeon, *Nat. Mater.* 3, 891 (2004).
- R. Piñol, A. Millán, F. Palacio, and L. Gabilondo, Spanish Patent no. P201031493 (2010).
- A. Millán, F. Palacio, A. Falqui, E. Snoeck, V. Serin, A. Bhattacharjee, V. Ksenofontov, P. Gütlisch, and I. Gilbert, *Acta Mater.* 55, 2201 (2007).
- A. Millán, F. Palacio, G. Ibarz, and E. Natividad, Patent No. ES2308901B1, Spain (2007).
- L. M. A. Ali, M. Gutierrez, R. Cornudella, J. A. Moreno, R. Pinol, L. Gabilondo, A. Millán, and F. Palacio, *J. Biomed. Nanotechnol.* 9, 1272 (2013).
- (a) R. W. Chantrell, M. El-Hilo, and K. O’Grady, *IEEE Transaction on Magnetism* 27, 3570 (1991); (b) I. J. Bruvera, P. Mendoza Zelis, M. Pilar Calatayud, G. F. Goya, and F. H. Sanchez, *J. Appl. Phys.* 118, 184304 (2015).
- C. Pecharroman, T. González-Carreño, and J. E. Iglesias, *Phys. Chem. Miner.* 22, 21 (1995).
- F. Ludwig, C. Balceris, C. Jonasson, and C. Johansson, *IEEE Trans. Magn.* 53 (2017).
- A. Ercuta and M. Chirita, *J. Cryst. Growth* 380, 182 (2013).
- S. H. Chung, A. Hoffmann, S. D. Bader, C. Liu, B. Kay, L. Makowski, and L. Chen, *Appl. Phys. Lett.* 85, 2971 (2004).
- P. Caravan, J. J. Ellison, T. J. McMurtry, and R. B. Lauffer, *Chem. Rev.* 99, 2293 (1999).
- L. Bordonali, T. Kalaivani, K. Sabareesh, C. Innocenti, E. Fantechi, C. Sangregorio, M. F. Casula, L. Lartigue, J. Larionova, Y. Guari, M. Corti, P. Arosio, and A. Lascialfari, *J. Phys.: Condens. Matter* 25, 066008 (2013).
- A. Roch, R. N. Muller, and P. Gillis, *J. Chem. Phys.* 110, 5403 (1999).
- Q. L. Vuong, J. F. Berret, J. Fresnais, Y. Gossuin, and O. Sandre, *Adv. Healthcare Mater.* 1, 502 (2012).
- Q. L. Vuong, P. Gillis, A. Roch, and Y. Gossuin, *Wires Nanomed. Nanobiotechnol.* e1468 (2017).
- Y. Gossuin, T. Orlando, M. Basini, D. Henrard, A. Lascialfari, C. Mattea, S. Stapf, and Q. L. Vuong, *Nanotechnology* 27, 155706 (2016).

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