The Effect of Surface Coating of Iron Oxide Nanoparticles on Magnetic Resonance Imaging Relaxivity

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Iron oxide nanoparticles (IONPs) with acceptable biocompatibility and size-dependent magnetic properties can be used as efficient contrast agents in magnetic resonance imaging (MRI). Herein, we have investigated the impact of particle size and surface coating on the proton relaxivity of IONPs, as well as engineering of small IONPs' surface coating as a strategy for achieving gadolinium-free contrast agents. Accordingly, polymer coating using poly(isobutylene-alt-maleic anhydride) (PMA) with overcoating of the original ligands was applied for providing colloidal stability to originally oleic acid–capped IONPs in aqueous solution. In case of replacement of the original ligand shell, the polymer had been modified with dopamine. Furthermore, the colloidal stability of the polymer-coated IONPs was evaluated in NaCl and bovine serum albumin (BSA) solutions. The results indicate that the polymer-coated IONPs which involved replacement of the original ligands exhibited considerably better colloidal stability and higher proton relaxivity in comparison to polymer-coated IONPs with maintained ligand shell. The highest $r_2/r_1$ we obtained was around 300.

Keywords: iron oxide nanoparticles, polymer coating, surface engineering, proton relaxivity, colloidal stability

INTRODUCTION

Magnetic iron oxide nanoparticles (IONPs) exhibit unique magnetic properties that make them attractive for different biomedical applications, including drug delivery (Karimi et al., 2016), magnetic resonance imaging (MRI) (Bruns et al., 2009; Kudr et al., 2017; Li et al., 2017; Smith and Gambhir, 2017; Woodard et al., 2018), magnetic particle imaging (Bauer et al., 2015), and magnetic hyperthermia (Laurent et al., 2011; Pardo et al., 2020). The magnetic properties of IONPs are influenced by the particle size, which arise from the magnetic domain structure (Tromsdorf et al., 2007; Li et al., 2017). Superparamagnetic iron oxide nanoparticles (SPIONs) are single-domain IONPs with a diameter of a few to a few tens nanometers that exhibit no remanent magnetization in the absence of an external magnetic field at room temperature. The superparamagnetic property of SPIONs, as well as their relatively good biocompatibility (Heine et al., 2014; Sheel et al., 2020), makes them the currently most used iron oxide–based T2 contrast agents (Kwon et al., 2018). Generally, large SPIONs provide T2 contrast due to the magnetic inhomogeneity induced by their strong magnetic moment. However, SPIONs-based T2 contrast agents generate dark signal in T2-weighted MRI that can mislead the clinical diagnosis (Zhao et al., 2013; Fernández-Barahona et al., 2020).
this respect, T₁ contrast agents are more desirable for high accurate resolution imaging (Wei et al., 2017; Li et al., 2019). T₁ contrast agents are commonly based on paramagnetic compounds with a large number of unpaired electrons. This includes, for example, Gd³⁺ and Mn²⁺ (Ni et al., 2017). T₁ contrast is induced by magnetic coupling interaction between the nucleic spins of the protons of water molecules and the electron spins of the contrast agents. Gadolinium complexes are widely used as T₁ contrast agents, despite the fact that free gadolinium ions, leached from gadolinium complexes, have shown a long-term toxicity including nephrogenic systemic fibrosis and Gd deposition in the brain (Khawaja et al., 2015; McDonald et al., 2015). Because of these limitations, development of alternatives may help to overcome the drawbacks of Gd-based T₁ contrast agents.

Some groups have suggested that the long-term biocompatibility of iron compared with gadolinium makes IONPs very attractive materials for T₁ contrast agents (Tromsdorf et al., 2009; Shen et al., 2017). The magnetic moment of IONPs rapidly decreases as their sizes decrease due to the reduction in volume magnetic anisotropy and spin canting effect (Morales et al., 1997; Jun et al., 2008). Since the paramagnetic properties of small IONPs are similar to Gd-based contrast agents, these nanoparticles can be utilized as T₁ contrast agents because of small magnetic moment and low toxicity (Bao et al., 2018). It however has to be pointed out that also for IONPs toxic effects exist (Joris et al., 2017; Feng et al., 2018; Patil et al., 2018), though to a much lesser extent than for chelated Gd. Toxicity may depend on various physicochemical properties such as size, shape, structure, concentration, surface modification, and solubility (Vanhecke et al., 2017; Feng et al., 2018; Patil et al., 2018; Vakili-Ghartavol et al., 2020).

Magnetic resonance imaging (MRI) contrast enhancement arises from the signal difference between water molecules residing in different environments that are under the effect of magnetic nanoparticles (NPs). The size of IONPs and their surface properties (thickness and chemical composition) (Wang et al., 2017), as also doping (Pardo et al., 2020), have influence on the contrast enhancement of SPIONs (Zhang et al., 2018). Consequently, understanding the relationships between the relaxivities of water protons under influence of magnetic NPs and intrinsic properties of these NPs can give decisive information for predicting the properties of engineered magnetic NPs. This may help enhancing their performance in MRI-based theranostic applications (Huang et al., 2012). Duan et al., for example, reported that the hydrophobic nature of the surface coating contributes to the relaxivity of MRI contrast agent (Duan et al., 2008).

Hydrophobic IONPs that are synthesized in organic solvents can exhibit improved sized distribution, crystallinity, and magnetic properties in comparison to iron oxide nanoparticles by aqueous phase methods (Lee and Hyeon, 2012; Wu et al., 2015). However, the effect of the coating and the ligand density on the T₁ and T₂ relaxation times is not fully understood yet.

The aim of this work is to prepare SPIONs stabilized with polymer by overcoating and replacement of the original ligand shell methods, in order to elucidate the influence of the polymer type and polymer coating on the corresponding longitudinal (r₁) and transverse (r₂) relaxivities.

MATERIALS AND METHODS

Materials. Oleic acid (OA, ≥93% technical grade), dopamine hydrochloride, poly(isobutylene-alt-maleic anhydride) (PMA) (average Mw: 6,000 Da), triethylamine, oleylamine, 1,2 hexadecanediol, benzyl ether, 1-octadecene, trioctylamine, dodecylamine dimethylformamide (DMF), and anhydrous sodium sulfate and iron (III) acetylacetonate were provided by Sigma. Ethanol (EtOH >96%), toluene (≥95% Sigma), and chloroform were obtained from Carl Roth. Poly(ethylene glycol) methyl ether (PEG, average Mw: 750 Da) was from Rapp Polymer. FeCl₃·6H₂O and sodium olate were purchased from Merck and TCI, respectively.

Column chromatography was performed using silica gel (60 Å) acquired from Fluka. The chemicals and solvents were used as received, unless otherwise specified. All the syntheses were carried out under N₂ atmosphere using MBraun LABmaster glovebox and standard Schlenk techniques.

Synthesis of Iron Oxide Nanoparticles

Iron Oxide Nanoparticles of 15 nm to 18 nm. IONPs were synthesized by thermal decomposition according to the method described by Hyeon and coworkers with minor modifications (Park et al., 2004). Briefly, to synthesize the iron oleate precursor, a 100 ml flask equipped with a Teflon-coated magnetic stir bar, 9.13 g sodium oleate (30 mmol), and hexane (3 ml) was added. The mixture was stirred, and then ethanol (20 ml) was added. All the solids dissolved by slow addition of distilled water (7.5 ml) according to the previously published protocol (Park et al., 2004). The reaction was heated to 40°C with stirring. At this point, the sodium oleate was completely dissolved. Then, a solution of 2.7 g of iron chloride (FeCl₃·6H₂O, 10 mmol) in 7.5 ml water was added to the reaction vessel. The resulting solution was heated under gentle reflux (65–70°C) and was kept at that temperature for 4 h. When the reaction was completed, the solution was transferred to a separation funnel. The upper red layer was washed with water and transferred to an Erlenmeyer flask containing anhydrous sodium sulfate (3 g) in order to remove residual water. Then, the solution was swirled and filtered with a hydrophobic filter (0.2 μm, Millipore #SLFG025N). The solution was concentrated on a rotary evaporator. After drying in high vacuum, the resulting product was a reddish brown viscous oil with a mass of around 9 g.

The synthesis of the IONPs relies on the reduction of iron oleate at high temperature. In a typical experiment, IONPs with a core diameter of 15 nm were prepared as follows: 3.6 g (4 mmol) of iron oleate and 0.57 g oleic acid were dissolved in 20 ml of 1-octadecene, in a three-neck round-bottom reaction vessel. The resulting solution was heated under gentle reflux at 100°C for 45 min, in order to remove volatile impurities and remaining traces of water in the iron oleate precursor. The mixture was degassed and dried through heating at 100°C for 45 min, in order to remove volatile impurities and remaining traces of water in the iron oleate precursor. The mixture was afterward heated to 320°C at a heating rate of around 3.3°C/min and was kept for 30 min under an inert atmosphere. A severe reaction occurred when the reaction temperature reached 320°C, and the initially transparent solution became turbid and brownish black. The
18 nm IONPs were obtained by thermal decomposition of iron oleate in the presence of oleic acid (0.57 g) in trietylamine (20 ml) at 360°C for 30 min. The resulting solution was allowed to cool down to room temperature by removing the heating mantle, and then, acetone was added to the solution to precipitate the IONPs. The IONPs were precipitated by centrifugation (3,500 rpm, 5 min). The supernatant was discarded, and the IONP precipitate was dispersed in toluene. The concentration of the IONPs in solution (i.e., toluene) was estimated by weighing and determining the mass of one IONP by transmission electron microscopy (TEM) analysis (Hühn et al., 2016). In the following, the concentration of IONPs was calculated by assuming that they are Fe₃O₄ spheres of 18 nm core diameter (d = 18 nm). The volume of one IONP is \( V_{\text{NP}} = \frac{4}{3} \pi \times \left(\frac{d}{2}\right)^3 = \frac{4}{3} \pi \times \frac{18 \times 10^{-7} \text{ cm}}{2}^3 = 3,050 \times 10^{-21} \text{ cm}^3 \). The density of the IONP cores was assumed as the bulk density of Fe₃O₄ which is 5.18 g/cm³ (Kurzhals et al., 2017; Patsula et al., 2019). The mass of each IONP is \( m_{\text{NP}} = \rho \times V_{\text{NP}} = \frac{5.18 \text{ g/cm}^3}{1,000} \times (3,050 \times 10^{-21} \text{ cm}^3) = 15,800 \times 10^{-21} \text{ g} \). The number of IONPs (N_{NP}) in solution of volume \( V_{\text{solution}} \) can be determined by dividing the total mass of dried IONPs (\( m_{\text{NP, total}} \)) originating for a solution of \( V_{\text{solution}} = 1 \text{ ml} \) by the mass of single nanoparticle \( m_{\text{NP}} = \frac{m_{\text{NP, total}}}{N_{NP}} \). The concentration of sample is then \( C_{\text{NP}} = \frac{N_{\text{NP}}}{V_{\text{solution}}} = \left(\frac{0.0019 \times 10^{18}}{6.022 \times 10^{23}}\right)/(1 \times 10^{-3} \text{ L}) = 3.15 \mu\text{M} \) with Avogadro’s constant \( N_A = 6.022 \times 10^{23} \text{ mol}^{-1} \).

Iron Oxide Nanoparticles of 6 nm. IONPs with a core diameter of around 6 nm were synthesized via the procedure reported by Sun et al. (Sun et al., 2004), Fe(acac)₃ (2 mmol), oleic acid (6 mmol), benzyl ether (20 ml), oleylamine (6 mmol), and 1,2-hexadecanediol (10 mmol) were mixed and magnetically stirred under the flow of nitrogen. The mixture was heated to 200°C for 2 h and then was refluxed (300°C) for 1 h under nitrogen atmosphere. A black-brown hexane dispersion of 6 nm IONPs was produced. After that, the heating was switched off and the black-brown mixture was allowed to cool to room temperature. Finally, the product was precipitated with ethanol (40 ml) and collected by centrifugation (5,000 rpm, 10 min). Then, IONPs were redispersed in a mixture of hexane with 1% (v/v) oleic acid and oleylamine.

We note that, in the present work, synthesis was reproduced from previous publications, in which key characterization of these IONPs is also provided (Park et al., 2004; Sun et al., 2004). The Fe₃O₄ structure of the IONPs has not been explicitly verified in the present work, though there are different methods for this (Komadel and Stucki, 1988; Corrias et al., 2009), but it is based on the findings in the original reports about the syntheses. Some additional basic characterization (XRD, FTIR, and VSM) is provided in the Supporting Information.

**Polymer Coating**

**Polymer Coating Involving Overcoating of the Original Ligand Shell.** The IONPs were transferred into aqueous solution with overcoating them using the polymer dodecylamine–modified poly(isobutylene-alt-maleic anhydride) as described previously (Hühn et al., 2016). The amphiphilic polymer comprises a backbone of poly(isobutylene-alt-maleic anhydride), and hydrophobic side chains in the form of dodecylamine were linked to the anhydride rings through formation of amide bonds. In the amphiphilic polymer PMA–DDA used in the present work, 75% of its maleic anhydride rings had been reacted with dodecylamine and 25% of its anhydride rings were left unreacted (Hühn et al., 2016). The leftover anhydride rings of the hydrophobic backbone open up under alkaline conditions, yielding negatively charged carboxylic groups that make the IONPs soluble in aqueous solutions. Briefly, polymer coating of the IONPs was performed by dissolving a desirable amount of polymer monomers per surface area of IONP. In all of the samples, we added 3,000 monomers of poly(isobutylene-alt-maleic anhydride) modified with dodecylamine (PMA–DDA) dissolved in chloroform per 1 nm² of effective surface area of the IONPs (Yang et al., 2017). The concentrations of the IONPs CNP samples were calculated (Hühn et al., 2016) as described above. Then, the volume of polymer solution (\( V_p \)) for efficient polymer coating of the IONPs was determined as described in previous work (Hühn et al., 2013; Hühn et al., 2016; Zhu et al., 2019):

\[
V_p = \frac{C_p \times \pi \times d_{\text{ligand}}^2 \times C_{\text{NP}} \times V_{\text{NP}}}{R_p/\text{area}},
\]

where \( C_p \) and \( R_p/\text{area} \) are the monomer concentration and the ratio of polymer units per nm² of effective surface area, respectively (Hühn et al., 2016). CNP and VNP are the concentration and the volume of the IONP solution. The effective diameter of IONPs (\( d_{\text{eff}} \)) includes the diameter of the iron oxide cores from TEM images and twice the hydrophobic stabilizing ligand shell: \( d_{\text{eff}} = d_l + 2d_{\text{ligand}} \) and we assumed \( d_{\text{ligand}} = 1 \text{ nm} \). Here, we mixed IONPs with \( C_p = 0.05 \text{ M} \) monomer concentration and \( R_p/\text{area} = 100 \text{ nm}^{-2} \). After addition of the polymer in a round flask, the solvent was slowly removed using a low-pressure system under heating to 40°C in order to force the polymer to wrap around the IONPs. Then, again chloroform was added and the drying process was repeated. The IONP powder was then dissolved in sodium borate buffer at pH 12. Here, hydrolysis of the remaining maleic anhydride left two carboxylic groups per newly opened anhydride ring. Then, the solution was filtered using a 0.22 μm syringe filter. Afterward, the IONPs were precipitated by centrifugation and the supernatant was discarded, and the IONPs were redispersed in Milli-Q water. This procedure was repeated in order to remove residual empty polymer micelles (Fernández-Argüelles et al., 2007). After purification, the IONPs were redispersed and kept in Milli-Q water.

**Polymer Coating Involving Replacement of the Original Ligand Shell.** The polymer coating was carried out by following a previously reported procedure (Wang et al., 2014). Briefly, to achieve dopamine functionalized PMA, the polymer synthesis was carried out by dissolving 0.385 g of PMA in 10 ml of DMF in a 50 ml three-necked round bottom flask. The solution was purged with nitrogen, and then, the temperature was raised to 70°C. Then, a mixture of amino–PEG (H₂N–PEG–OMe, 0.995 g) and dopamine hydrochloride (0.237 g) activated with triethylamine (resulting in free amine dopamine) was added dropwise to the solution, and the mixture was left to react...
overnight at 70°C. To collect the polymer, DMF was removed under vacuum and then dissolved in chloroform. The solution was purified by silica gel column chromatography and eluted with chloroform. Afterward, the solvent evaporated and a gel-like yellow oil was collected as the resultant product. The polymer coating was carried out as follows: First, 5 mg of precipitated IONPs was dispersed in 0.5 ml THF. To this mixture, 1 ml of THF containing 0.25 mg of dopamine-PMA–PEG was added. Subsequently, the mixture was sealed and stirred overnight at 50°C under nitrogen atmosphere. Then, an excess of hexane was added to precipitate the sample and was centrifuged. The supernatant was discarded and the precipitate was dried. The final black pellet was dispersed easily in Milli-Q water by sonication. The aqueous solution was filtered with a 0.22 µm syringe filter, and the IONPs were precipitated by centrifugation. Unbound excess ligands were discarded with the supernatant and the IONPS were redispersed and kept in Milli-Q water. Both coating procedures are summarized in Figure 1.

Characterization of Iron Oxide Nanoparticles

All samples were characterized by dynamic light scattering (DLS; Nanosizer, Malvern) and inductively coupled plasma mass spectroscopy (ICP-MS; Agilent 7700 series ICP-MS). The morphology and size distribution of the IONPs were examined with a transmission electron microscope (TEM, JEOL 1400 plus 100 kV and LEO 912 AB, 120 kV). TEM samples were prepared by dropping a dilute solution of IONPs on carbon-coated copper grids and letting the solvent evaporate. The thickness of the organic shell was determined by TEM with uranyl acetate negative staining (Yang et al., 2017). Gel electrophoresis analysis was performed on a Bio-Rad system using 2% agarose gel. Electrophoresis was carried out for 60 min at 100 V.

Relaxivity Measurements. The magnetic resonance (MR) relaxivity profile was evaluated in phantoms and the solutions of the IONPs at different concentrations (cFe = 0.035, 0.07, 0.14, 0.28, and 0.56 mM equivalent Fe content) by using a MRI scanner with 3 T field strength (Siemens). The longitudinal relaxation times (T<sub>1</sub>) were determined using an inversion recovery pulse sequence (repetition time (T<sub>R</sub>) = 100, 200, 500, 750 and 1,000 ms and fixed echo time (T<sub>E</sub>) = 12 ms. The T<sub>1</sub> relaxation time of each sample was determined based on the equation I ∼ M<sub>0</sub> (1 - 2·exp (-t/T<sub>1</sub>)) to fit the magnitude of the MRI signals at different inversion times.

A multiple spin echo was used to simultaneously collect data points at different echo times (T<sub>E</sub> = 6–180 ms with an increment of 6 ms) for the T<sub>2</sub> measurements. A nonlinear monoexponential equation I ∼M<sub>0</sub>·exp (-T<sub>E</sub>/T<sub>2</sub>) was used to determine the T<sub>2</sub> relaxation time of each IONP sample (Ahmad et al., 2011). Finally, r<sub>1</sub> and r<sub>2</sub> values were calculated according to the linear relationship of longitudinal and transverse relaxation rates vs. iron concentration of IONPs (Hobson et al., 2019; Ahmadpoor et al., 2020).

RESULTS

Iron Oxide Nanoparticles Synthesis and Polymer Coating

IONPs can be prepared by aqueous and nonaqueous methods. Aqueous methods such as coprecipitation usually produce IONPs
with low crystallinity and a broad size distribution (Ali et al., 2016). In contrast, nonaqueous methods such as thermal decomposition may generate better monodispersed IONPs with high crystallinity. In the present work, three different sizes of IONPs capped with oleic acid were synthesized by the thermal decomposition method. For this purpose, a modified protocol of the previously reported procedure was used. Figure 2 depicts TEM images of the uniform spherical IONPs, which demonstrate a narrow size distribution. The inorganic (core) diameter of the IONPs ($d_c$) was derived from the TEM images using the software Image J, resulting in core diameters $d_c$ of the three different samples of $6.15 \pm 0.98$, $14.6 \pm 1.5$, and $17.6 \pm 0.91$ nm.

The XRD pattern of IONPs (6 nm) (Supplementary Figure SI-1) demonstrates sharp diffraction peaks that are consistent with the magnetite phase (JCPDF #19–0629). Also, the FTIR spectra of IONPs (Supplementary Figure SI-3) show the strong characteristic band at $574$ cm$^{-1}$ with a shoulder at $630$ cm$^{-1}$, which are related to the vibrations of Fe–O from octahedral and tetrahedral sites of magnetite (Muthukumaran and Philip, 2016). The peaks from $1,408$ to $1,586$ cm$^{-1}$ are due to the vibrations of COO from adsorbed oleic acid over magnetite. The peaks at $2,874$ and $2,915$ cm$^{-1}$ are assigned to the stretching modes of CH$_2$ and CH$_3$ groups of oleic acid (Li et al., 2010). Supplementary Figure SI-2 shows the magnetic behavior of IONPs at room temperature. The saturation magnetization value obtained from the M-H curve under an applied magnetic field of 80 kOe is about 35 emu/g, and the IONPs exhibit superparamagnetic properties.

Two strategies have been applied for surface modification of hydrophobic magnetic IONPs to render them colloidally stable.

**Figure 2** | TEM bright field images of IONPs dried on a grid from a suspension of IONPs in hexane and their corresponding histogram, plotted as the number of NPs (N) that have a core diameter of $d_c$. (A) IONPs with $d_c = (6.5 \pm 0.98)$ nm; the scale bar corresponds to 40 nm. (B) IONPs with $d_c = (14.6 \pm 1.5)$ nm; the scale bar corresponds to 200 nm. (C) IONPs with $d_c = (17.6 \pm 0.91)$ nm; the scale bar corresponds to 50 nm.
in aqueous media. The IONPs were hereby coated with polymers, either maintaining or replacing the original ligand shell. An overview of the synthesis strategy is illustrated in Figure 1. PMA was selected as a model polymer. Maleic anhydride groups are highly reactive and can be modified with amine containing functional molecules. This nucleophilic addition was carried out without needing any additional reagents, which simplifies the purification steps and characterization of the prepared product (Wang et al., 2015). For polymer coating involving replacement of the original ligands, the polymer was modified with dopamine (Wang et al., 2014). The ligands of dopamine–PMA–PEG were characterized by H-NMR spectroscopy. The presence of methoxy groups, PEG moieties, and catechol groups in the modified PMA was confirmed by H-NMR analysis. A multiple peak at δ = 6.32–6.78 ppm, a sharp peak at around 3.3 ppm, and a broad peak at 0.9 ppm are attributed to the catechol protons of dopamine, PEG, and the methyl group of the modified PMA, respectively (Figure 3).

The hydrodynamic diameters (d_h) of the different IONPs with d_c = 18 nm were determined with DLS. The "hydro"dynamic diameter of the initial IONPs before the polymer coating was determined to be d_h = 24.06 nm in toluene. The corresponding hydrodynamic diameters after the polymer coating were d_h = 28.97 nm and 30.80 nm for the PMA–DDA and dopamine–PMA–PEG-coated IONPs; see Table 1. The DLS data indicate that after the polymer coating, the IONPs had maintained their uniform size with a low polydispersity index (Table 1). A negative zeta potential of around ζ = -38.4 and -12.6 mV was determined in water for the PMA–DDA and dopamine–PMA–PEG-coated IONPs, respectively, using laser Doppler anemometry (Hühn et al., 2016).

The PMA–DDA polymer-coated IONPs were also investigated with agarose gel electrophoresis (Figure 5). Here, the electrophoretic mobility of the IONPs with different core diameters d_c was investigated. As these IONPs have the same surface chemistry, retardation in electrophoretic mobility is associated with increased diameter, which can be seen in Figure 5 (Pellegrino et al., 2007). The narrow bands on the polymer-coated IONPs on the gel show that these NPs have homogenous size and charge distribution (Pellegrino et al., 2004). Gel electrophoresis is an excellent method to probe colloidal stability of IONPs (Pellegrino et al., 2007). The buffer has high ionic strength, and by the applied electric field, the IONPs are pulled through the pores of the agarose gel, which removes loosely bound ligands and thus leads to agglomeration. The data shown in Figure 5 clearly indicate high colloidal stability of the polymer-coated IONPs. Due to their low charge (i.e., zeta potential), the dopamine–PMA-PEG-coated IONPs were not probed by gel electrophoresis.
Colloidal Stability in Physiological Environments

In various biological applications, NPs are expected to be exposed to salt- and protein-containing media (Pfeiffer et al., 2014). We thus examined the colloidal stability of aqueous dispersions of PMA–DDA and dopamine–PMA–PEG-coated IONPs in sodium chloride and bovine serum albumin (BSA) containing media. Measurements of the effective hydrodynamic diameter were used as a tool to probe the colloidal stability of IONPs (Hühn et al., 2016). Loss in colloidal stability hereby is indicated by agglomeration and thus increased effective hydrodynamic diameters. As aggregation is time dependent, the hydrodynamic diameter of IONPs was measured immediately after exposing the IONPs to NaCl/BSA, and then measurements were repeated after 24 h incubation time at room temperature. DLS histograms of the hydrodynamic diameter are shown in Figure 4.

**TABLE 1** The hydrodynamic diameter \(d_h\) of 18 nm IONPs as measured by DLS before and after polymer coating. Values were obtained from the intensity distribution \(d_{h,I}\), the number distribution \(d_{h,N}\), and the Z-average \(d_{h,Z}\). PDI refers to the polydispersity index. Also the mean value of the zeta potential \(\zeta\) is provided.

| Sample                  | Solvent     | \(d_{h,I}\) [nm] | \(d_{h,N}\) [nm] | \(d_{h,Z}\) [nm] | PDI   | \(\zeta\) [mV] |
|------------------------|-------------|-------------------|-------------------|------------------|-------|---------------|
| IONP                   | Toluene     | 31.15             | 24.06             | 29.91            | 0.01  | —             |
| IONP@PMA–DDA           | Water       | 38.69             | 28.97             | 36.77            | 0.29  | −38.4         |
| IONP@dopamine–PMA–PEG  | Water       | 59.22             | 30.80             | 59.74            | 0.22  | −12.6         |

**FIGURE 4** TEM images of IONPs after negative staining with uranyl acetate. (A) IONP cores \((d_c = 17.6 \pm 0.91)\) plus an organic shell of PMA–DDA, resulting in \(d_{cs} = 29.65 \pm 1.35\); the scale bar corresponds to 200 nm. (B) IONP cores \((d_c = 17.6 \pm 0.91)\) plus an organic shell of dopamine–PMA–PEG, which resulted in \(d_{cs} = 29.85 \pm 1.28\); the scale bar is 100 nm.
Diameters upon exposure to NaCl/BSA are shown in Figure 6. As colloidal stability of the polymer-coated IONPs was already demonstrated with gel electrophoresis (Figure 5), here the DLS studies are limited to the bigger IONPs of $d_c = 18$ nm, as bigger IONPs are more sensitive to agglomeration in general than smaller IONPs. Salt (here in the form of NaCl) in the solution screens the electric charge on the surface of the NPs, thus decreasing the effective surface charge density, resulting in colloidal instability and aggregation of the NPs, as can be seen for the PMA–DDA-coated IONPs. As PEG can contribute steric stabilization, the dopamine–PMA–PEG-coated IONPs are less affected by the addition of salt, which is in good agreement with previous studies (Caballero-Díaz et al., 2013). To probe protein adsorption (Vilanova et al., 2016) on the surface of the polymer-coated IONPs, we used serum albumin (bovine: BSA) as a model protein, because serum albumin is the most abundant protein in blood serum (Hühn et al., 2013). While the protein corona depends on the details of the surface chemistry (Guerrini et al., 2018), here we wanted to probe only colloidal stability. The data of Figure 6 demonstrate that up to high BSA concentrations, there was no protein-induced agglomeration of the IONPs (apart from the PMA–DDA-coated IONPs at the maximum concentration). Due to limits in the resolution of measuring the hydrodynamic diameter of the NPs with DLS, the formation of the protein corona could not be observed as possible with other techniques (Carril et al., 2017), but the data indicate that, in particular, the dopamine–PMA–PEG-coated IONPs are colloidal stable under physiological conditions.

**Relaxivity Measurements**

To investigate the MR performance of the polymer-coated IONPs, we carried out longitude relaxivity ($r_1$) and transverse relaxivity ($r_2$) measurements. Relaxation times $T_1$ and relaxivities $r_i$ are related by
where \( T_1 \) are the observed relaxation times in the presence of the contrast agent, \( T_{1,0} \) is the relaxation time of pure water protons, and \( C_{Fe} \) is the concentration of the MRI contrast agent, in this case, iron (Banerjee et al., 2017). The small IONPs significantly affect the spin–lattice (\( T_1 \)) relaxation due to the high number of metal ions on the surface and the spins canting, while the spin–spin (\( T_2 \)) relaxation is related to proton dephasing by local field inhomogeneity (Ni et al., 2017). The data clearly show a dependence of proton relaxivity on particle size (Figure 7). This size dependence is believed to arise from surface spin anisotropy, due to the larger surface area-to-volume ratio for smaller IONPs (Smolensky et al., 2013). Since the larger IONPs have more effective magnetic relaxation of the water protons around the NPs, they show higher transverse relaxivity \( r_2 \). In addition to the intrinsic material and size-dependent properties of IONPs, the surface coating is also an important factor for \( T_2 \) relaxivity (Ni et al., 2017). According to the quantum mechanical outer-sphere theory, the \( T_2 \) relaxivity is described by

\[
\frac{1}{T_2} = \left( \frac{256 \cdot \pi^2 \cdot \frac{r^2}{405}}{V'} \cdot \frac{V}{D \cdot (1 + \frac{1}{2})} \right) + \frac{r^2 \cdot Ms^2}{D \cdot (1 + \frac{1}{2})},
\]

where \( V' \) and \( Ms \) are the volume fraction and the magnetic saturation of the magnetic NPs, respectively, \( r \) is the proton gyromagnetic ratio, \( r \) is the radius of the nanoparticles, \( D \) is the diffusion coefficient of water, and \( L \) is the thickness of an impermeable surface shell on the iron oxide core (Ni et al., 2017). From our experimental data, we can determine \( r = d_c/2 \) and \( L = (d_s - d_c)/2 \). The dependence of \( r_2 \) from \( d_c \) (i.e., \( r \)) follows the tendency as given by the formula above: the higher \( d_c \), the higher \( r_2 \).

The dependence from the surface is harder to discuss, in particular, as it is not known to what degree the polymer shells are impermeable to water. The hydrophilic coating on the surface of the IONPs improves their diffusion and should also partly hinder water molecules to reach the surface of the cores, resulting in faster \( T_2 \) relaxation (Zhang et al., 2018). The relaxivity results in Figure 7 show that dopamine–PMA–PEG-coated IONPs have higher \( r_2 \) relaxivity than PMA–DDA-coated IONPs with similar iron oxide core sizes \( d_c \). As mentioned before, at the core size of \( d_c = 18 \text{nm} \), the TEM images show no significant difference between the diameters \( d_s \) of IONPs coated with dopamine–PMA–PEG and PMA–DDA. These results indicate that the increase in \( T_2 \) relaxivity can be attributed to the increased volume of diffusion of water surrounding each NP to the outer sphere due to the highly hydrophilic nature of PEG in the dopamine–PMA–PEG coating (Duan et al., 2008; Zhang et al., 2018). On the other hand, the catechol groups of dopamine are rich in \( \pi \)-electrons which influence the magnetic field inhomogeneity around the polymer-coated IONPs and accelerate the \( r_2 \) relaxivity of water molecules (Zeng et al., 2014).

When IONPs are smaller than \( d_c = 10 \text{nm} \), they show dual \( T_1/T_2 \) imaging, in which \( T_1 \) enhancement refers to surface effects on the magnetization and water/ion metal center interaction on the surface of NPs (Bao et al., 2018; Thapa et al., 2018). According to the inner-outer sphere theory, the exposure of surface iron ions contributes to the \( T_1 \) behavior of small IONPs. \( T_1 \) relaxation of IONPs arises from direct contact of water protons with the iron (Bao et al., 2018). Therefore, the \( T_1 \) signal is very sensitive to surface-capping molecules and their packing density on the surface of the IONPs. Both the exposure of iron ion centers on the surface of NPs and the water accessibility affects the \( T_1 \) performance of IONPs (Peng et al., 2016; Bao et al., 2018; Xiao et al., 2018).

Our results reveal that, at similar core sizes \( d_c \), the \( r_1 \) relaxivity of polymer-coated IONPs is dependent on the nature of polymer coating (Figure 7). In particular, the small IONPs (\( d_s = 6 \text{nm} \)) coated with dopamine–PMA–PEG show higher \( r_1 \) relaxivity than the ones coated with PMA–DDA (Table 2). Dopamine serves as a strong anchor...
relaxivity measurements demonstrated high r\textsubscript{1} and r\textsubscript{2} stability in NaCl-containing solutions. Furthermore, proton (Dotarem\textsuperscript{®}) as a positive contrast agent with 2.7 mM wrapped within the polymer layer. Encapsulation of the IONPs is amphiphilic PMA ligand on the surface of IONPs and provide hydrophilic dispersion of dopamine are replaced with oleic acid as the original hydrophobic ex Gibbs poor, F., Delavari, H., and Shojaosadati, S. A. (2020). Porous versus dense—effect of silica coating on contrast enhancement of iron carbide nanoparticles in T\textsubscript{2} -weighted magnetic resonance imaging. ChemistrySelect 5 (3), 1135–1139. doi:10.1002/slct.201902548

| Coating         | d\textsubscript{c} [nm] | r\textsubscript{1} (mM\textsuperscript{−1}\textperiodcentered s\textsuperscript{−1}) | r\textsubscript{2} (mM\textsuperscript{−1}\textperiodcentered s\textsuperscript{−1}) | r\textsubscript{2}/r\textsubscript{1} |
|-----------------|----------------------|---------------------------------|---------------------------------|------------------|
| PMA–DDA         | 6                    | 3.09                            | 48.3                            | 15.63            |
| PMA–DDA         | 15                   | 0.72                            | 135                             | 150              |
| PMA–DDA         | 18                   | 0.44                            | 144                             | 306.8            |
| Dopamine–PMA–PEG| 6                    | 4.7                             | 174.9                           | 62.46            |
| Dopamine–PMA–PEG| 15                   | 2.8                             | 183                             | 76.25            |
| Dopamine–PMA–PEG| 18                   | 2.4                             | 183                             | 76.25            |
| Gd-DOTA         | —                    | 2.72                            | 3.1                             | 1.14             |

In summary, IONPs of 6, 15, and 18 nm core diameter with a narrow size distribution were synthesized through thermal decomposition, followed by polymer coating with/without replacement of the original ligand shell. Polymer coating of IONPs with dopamine–PMA–PEG exhibited higher colloidal stability in NaCl-containing solutions. Furthermore, proton relaxivity measurements demonstrated high r\textsubscript{1} and r\textsubscript{2} relaxivities for 6 and 18 nm dopamine–PMA–PEG-coated IONPs, respectively. Considering the higher colloidal stability and enhancement of negative and positive contrast of dopamine–PMA–PEG-coated IONPs with increase in size, this polymer could be assumed as an efficient candidate for polymer coating of IONPs as T\textsubscript{2} and T\textsubscript{1} contrast agents. Thereupon, the present investigation of r\textsubscript{1} and r\textsubscript{2} relaxivities of IONPs with different surface coatings and similar shell sizes helps toward a reasonable understanding of surface impacts in obtaining high-performance NPs as MRI contrast agents. While here the focus is given on the aspect of the influence of the surface coating on relaxivity, future studies would also have to take into account other effects of the surface coating, such as different biocompatibilities and biodistributions.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

**AUTHOR CONTRIBUTIONS**

Conceptualization: FA, NF, and WP; formal analysis: FA; funding acquisition: WP; investigation: FA and AM; resources: WP and SS; supervision: NF; writing original draft: FA; reviewing and editing: FA, AM, NF, WP, and SS.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnano.2021.644734/full#supplementary-material.

Ali, A., Zafar, H., Zia, M., Ul Haq, I., Pfall, A. R., Ali, J. S., et al. (2016). Synthesis, characterization, applications, and challenges of iron oxide nanoparticles. *Nanotechnol. Sci. Appl.* 9, 49. doi:10.2147/NSA.S99886

Banerjee, A., Blasiak, B., Pasquier, E., Tomanek, B., and Trudel, S. (2017). Synthesis, characterization, and evaluation of PEGylated first-row transition metal ferrite nanoparticles as T\textsubscript{2} contrast agents for high-field MRI. *RSC Adv.* 7 (61), 38125–38134. doi:10.1039/c7ra05495e

Bao, Y., Sherwood, J. A., and Sun, Z. (2018). Magnetic iron oxide nanoparticles as T\textsubscript{1} contrast agents for magnetic resonance imaging. *J. Mater. Chem. C* 6 (6), 1280–1290. doi:10.1039/c7tc05854c

**REFERENCES**

Ahmadpoor, F., Delavari, H., and Shojaosadati, S. A. (2020). Porous versus dense—effect of silica coating on contrast enhancement of iron carbide nanoparticles in T\textsubscript{2} -weighted magnetic resonance imaging. *ChemistrySelect* 5 (3), 1135–1139. doi:10.1002/slct.201902548

Ahmadpoor, F., Delavari, H., and Shojaosadati, S. A. (2020). Porous versus dense—effect of silica coating on contrast enhancement of iron carbide nanoparticles in T\textsubscript{2} -weighted magnetic resonance imaging. *ChemistrySelect* 5 (3), 1135–1139. doi:10.1002/slct.201902548

Ahmadpoor, F., Delavari, H., and Shojaosadati, S. A. (2020). Porous versus dense—effect of silica coating on contrast enhancement of iron carbide nanoparticles in T\textsubscript{2} -weighted magnetic resonance imaging. *ChemistrySelect* 5 (3), 1135–1139. doi:10.1002/slct.201902548
Ahmadpoor et al. Surface Coating of Iron Oxide Nanoparticles

Patsula, V., Horák, D., Kučka, J., Macková, H., Lobaz, V., Francová, P., et al. (2019). Synthesis and modification of uniform PEG-neridonate-modified magnetic nanoparticles determines prolonged blood circulation and biodistribution in a mouse preclinical model. *Sci. Rep.* 9 (4), 10765. doi:10.1038/s41598-019-47262-w

Pellegrino, T., Manna, L., Kudera, S., Liedl, T., Koktysh, D., Rogach, A. L., et al. (2004). Hydrophobic nanocrystals coated with an amphiphilic polymer shell: a general route to water soluble nanocrystals. *Nano Lett.* 4 (4), 703–707. doi:10.1021/nl035172

Pellegrino, T., Sperling, R. A., Alivisatos, A. P., and Parak, W. J. (2007). Gel

Ahmadpoor et al. Surface Coating of Iron Oxide Nanoparticles

Pellegrino, T., Sperling, R. A., Alivisatos, A. P., and Parak, W. J. (2007). Gel

Ahmadpoor et al. Surface Coating of Iron Oxide Nanoparticles

Pellegrino, T., Sperling, R. A., Alivisatos, A. P., and Parak, W. J. (2007). Gel

Ahmadpoor et al. Surface Coating of Iron Oxide Nanoparticles

Pellegrino, T., Sperling, R. A., Alivisatos, A. P., and Parak, W. J. (2007). Gel

Ahmadpoor et al. Surface Coating of Iron Oxide Nanoparticles

Pellegrino, T., Sperling, R. A., Alivisatos, A. P., and Parak, W. J. (2007). Gel

Ahmadpoor et al. Surface Coating of Iron Oxide Nanoparticles

Pellegrino, T., Sperling, R. A., Alivisatos, A. P., and Parak, W. J. (2007). Gel

Ahmadpoor et al. Surface Coating of Iron Oxide Nanoparticles

Pellegrino, T., Sperling, R. A., Alivisatos, A. P., and Parak, W. J. (2007). Gel

Ahmadpoor et al. Surface Coating of Iron Oxide Nanoparticles

Pellegrino, T., Sperling, R. A., Alivisatos, A. P., and Parak, W. J. (2007). Gel

Ahmadpoor et al. Surface Coating of Iron Oxide Nanoparticles

Pellegrino, T., Sperling, R. A., Alivisatos, A. P., and Parak, W. J. (2007). Gel

Ahmadpoor et al. Surface Coating of Iron Oxide Nanoparticles

Pellegrino, T., Sperling, R. A., Alivisatos, A. P., and Parak, W. J. (2007). Gel

Ahmadpoor et al. Surface Coating of Iron Oxide Nanoparticles

Pellegrino, T., Sperling, R. A., Alivisatos, A. P., and Parak, W. J. (2007). Gel

Ahmadpoor et al. Surface Coating of Iron Oxide Nanoparticles

Pellegrino, T., Sperling, R. A., Alivisatos, A. P., and Parak, W. J. (2007). Gel

Ahmadpoor et al. Surface Coating of Iron Oxide Nanoparticles

Pellegrino, T., Sperling, R. A., Alivisatos, A. P., and Parak, W. J. (2007). Gel

Ahmadpoor et al. Surface Coating of Iron Oxide Nanoparticles

Pellegrino, T., Sperling, R. A., Alivisatos, A. P., and Parak, W. J. (2007). Gel

Ahmadpoor et al. Surface Coating of Iron Oxide Nanoparticles

Pellegrino, T., Sperling, R. A., Alivisatos, A. P., and Parak, W. J. (2007). Gel