1 Introduction

Magnetic resonance imaging (MRI) is one of the best established imaging modalities in preclinical research and clinical diagnosis, owing to the use of non-ionizing radiation, high resolution, superior soft-tissue contrast, and the non-invasive nature of the technique.\(^1\)\(^-\)\(^3\) Most MRI investigations in clinical settings use contrast agents – magnetic species administered to the patient prior to imaging – for contrast improvement.\(^4\) While gadolinium chelates are traditionally used to shorten the longitudinal relaxation time of water protons in tissues – thereby generating a brighter image (hyperintense MRI) – the nephrotoxicity of gadolinium-based contrast media, especially under the conditions to further improve image quality and contrast.\(^17\) Furthermore, these NPs allow enhanced tumor detection using susceptibility-weighted imaging.\(^11\) There are examples of nanocomposites functioning as bifunctional \(T_1 - T_2\) contrast agents.\(^12\) Surface-capped iron oxide nanoparticles have been shown to be very effective for MRI contrast enhancement.

From the theory of MRI, it is evident that the available signal, which is ultimately converted to an image, strongly depends on the static magnetic field strength \(B_0\).\(^2\) Most clinical MRI scanners operate at field strengths between 1.5 and 3 T. However, recent developments in magnet fabrication and shielding allowed 7–9.4 T scanners to be applied for whole-body imaging.\(^13\)\(^,\)\(^14\) The advantages of high-field MRI are manifold: higher signal-to-noise ratio, as well as improved spectral resolution for certain applications.\(^15\)\(^,\)\(^16\) Evidently, these improvements facilitate higher spatial and/or temporal resolution than previously possible with MRI. While the technological, physical and safety limitations of high-field and ultra-high-field MRI are still being investigated, the time is ripe for increased research into high-field contrast agents as their efficacy strongly depends on the magnetic field strength\(^17\) which could be used under these conditions to further improve image quality and contrast. Obviously, NP-based contrast agents are expected to play a major role in high-field MRI.\(^18\)\(^,\)\(^19\)

Given the number of potential factors that can affect the type of contrast \(\text{e.g.}, T_1, T_2, T_2^*, \text{or } T_1 - T_2\) and field-suitability (low, intermediate, high, ultra-high, or overlapping combinations...
magnetic core and the water molecules in organs being imaged attempts have been made to improve the contact between the were found to have iron oxide nanowhiskers coated with the surfactant Tween-80 the most widely cited examples, octapod Fe3O4 NPs with sub-enhancing the contrasting abilities of iron oxide NPs. In one of taken into consideration as a potential customization factor for ascorbate, citrate, other carboxylic acids, dopamine, ethyleneimine showed very high ratios. In a study by Hegmann and colleagues, Triton-X coated brick-shaped Fe3O4 NPs also showed very high \( r_2 \) values and \( r_2/r_1 \) ratios. One-dimensional nanostructures of iron oxide have also been known to exhibit tunable \( r_1 \) and \( r_2 \) values: ultra-thin iron oxide nanowhiskers coated with the surfactant Tween-80 were found to have \( r_1 = 6.13 \text{mM}^{-1} \text{s}^{-1} \) and \( r_2 = 11.15 \text{mM}^{-1} \text{s}^{-1} \) \(^{25} \) while 70 nm long Fe3O4 nanorods capped with poly-ethylenimine showed very high \( r_2 \) values of 608 \text{mM}^{-1} \text{s}^{-1}. ^{26} \n
Given that the proton spin–spin relaxation rate \( R_2 \) in the presence of NP contrast agents depends, among other things, on the thickness of the capping agent coating the NP surface, attempts have been made to improve the contact between the magnetic core and the water molecules in organs being imaged by selecting a small, hydrophilic NP-capping molecule. \(^{27} \) Small molecules that are expected to be bio-compatible, such as ascorbate, citrate, other carboxylic acids, \(^{27} \) glutathione, \(^{28} \) and dopamine, \(^{29} \) are being extensively studied to this end. Recently, we showed that maltol, an FDA-approved pharmaceutical additive that enhances the bioavailability of iron in vivo, is a suitable capping agent for obtaining efficient \( T_2 \) contrast agents at both clinical and high fields with demonstrated inertness to human cell lines. \(^{30} \n
It is to be noted that direct incorporation of a molecular (as opposed to a polymeric) capping agent on the NP surface during the formative stage may call for milder synthetic conditions to maintain the integrity of the capping agent, which may lead to reduced phase purity and magnetic behaviour of the ferrite nanocrystals formed. To avoid this, a two-stage synthesis is often used, initially generating a hydrophobic NP surface under harsher reaction conditions, which is then subjected to a post-synthetic ligand exchange regime, in order to render the NPs water soluble. \(^{31,32} \n
In this comparative study, poly(ethylene glycol) (PEG) was selected as a simple, well-studied, and biocompatible NP-stabilizing ligand, \(^{33,34} \) in order to divert attention from the ligand shell, and focus on the metal oxide core. While several individual reports on specific compositions of metal–ferrite NPs can be found in the relevant literature, there are only a few systematic studies where the composition is the primary variable under investigation. In a pioneering study by Cheon and coworkers, MFe2O4 NPs (M = Ni, Co, Fe, and Mn) were synthesized by a high-temperature reaction between the divalent metal chloride and iron(II) 2,4-pentadionate in the presence of oleic acid and oleylamine, followed by a surface ligand exchange and antibody conjugation. \(^{35} \) It was noted that the \( M \) and contrasting abilities of these NPs (at \( B_0 = 1.5 \text{T} \) increased as a function of the divalent metal in the following order: Mn > Fe > Co > Ni. Another significant study which explicitly investigates the high-field relaxivities of succimer-coated MFe2O4 NPs (M = Co, Mn, Fe) was performed by Brazel and colleagues in 2009; this study found CoFe2O4 NPs to be the best \( T_2 \) contrast agent of the three, with \( r_2/r_1 = 62.3. ^{36} \) In 2015, Bokias and colleagues encapsulated oleylamine-capped MnFe2O4, CoFe2O4, and NiFe2O4 NPs into the hydrophobic cores of spherical micellar structures formed by linear and comb-like co-polymers in an aqueous solution to generate colloidal superparticle structures; the \( r_2 \) relaxivity of these systems under 1.5 T and 3 T magnetic fields followed the order Co > Mn > Ni. Comb-type co-polymer enshrouded CoFe2O4 NPs showed the highest \( r_2 \) values (316.0 \text{mM}^{-1} \text{s}^{-1}). ^{37} \n
The present communication aims to augment these existing examples by providing a systematic study of the late 3d transition metal ferrite NPs via magnetometric and high-field relaxometric measurements. The spin–spin relaxation rate \( R_2 \) is given as \(^{38,39} \)

\[
R_2 = \frac{1}{T_2} = \frac{256\pi^2\gamma^4}{405} \frac{\kappa m a^2}{D(1 + \frac{L}{a})}
\]

(1)

where \( a \) is the magnetic radius core, \( L \) is the impermeable coating thickness, \( \gamma \) is the gyromagnetic ratio of the proton, \( m_s \) is the saturation magnetization of the NP sample, \( \kappa = V^*/C \) where \( V^* \) is the volume fraction of the magnetic core and \( C \) is the concentration of magnetic atoms, and \( D \) is the diffusion coefficient of water molecules. Looking in the literature, one often finds two or more variables being changed from study to study (e.g., morphology and state of aggregation, or nature of surface coating and size), making direct comparisons and benchmarking difficult. However, if the parameters are kept fairly constant (NP size, same ligand), then the effect of changing the divalent ion on the MRI properties of different MFe2O4 NPs can be studied in a consistent manner. In particular, looking at eqn (1), it then appears the saturation magnetization should be the determining factor – which is known to depend on the divalent ion in spinel ferrites – with higher magnetization expected to yield to higher \( r_2 \) values. We indeed find that at high fields (\( B_0 = 9.4 \text{T} \) the transverse relaxivity \( r_2 \) increases from Cu < Ni < Zn < Fe < Co < Mn while the ratio of the transverse to longitudinal relaxivities \( r_2/r_1 \) follows the Cu < Ni < Zn < Fe < Co < Mn trend.

2. Experimental section

2.1 Materials

The metal precursors – manganese(II), iron(II), cobalt(II), nickel(II), and zinc(II) 2,4-pentadionate, and copper(II) chloride
dihydrate – were purchased from Strem Chemicals, and used as received. Poly(ethylene glycol) \( (M_w = 400, \text{PEG}_{400}) \) and NaBH₄ (≥98%) were obtained from Sigma Aldrich. All reactions were carried out in clean oven-dried glassware, under an atmosphere of nitrogen.

2.2 Synthesis

PEGylated MFe₂O₄ NPs were generated using a highly modified reductive synthetic protocol.²¹ In a representative synthesis, Co(acac)₃ (171 mg, 0.67 mmol) and Fe(acac)₃ (472 mg, 1.33 mmol) were dissolved in 25 mL of PEG₄₀₀. Nitrogen was bubbled through the solution for 30 min. The solution was then taken in a three-necked flask fitted with a reflux condenser, heated with a J-KEM Scientific Gemini PID temperature controller to 170 °C and maintained at this temperature for 15 minutes. A separate solution of NaBH₄ (162 mg, 5 mmol) dispersed in ca. 5 mL of PEG₄₀₀ was vigorously stirred under nitrogen. The reaction flask was cooled from 170 to 100 °C, and the NaBH₄ dispersion was added all at once to the metal acetylacetonate solution at 100 °C, leading to brisk effervescence, and immediate darkening of color from orange to brown-black. This mixture was stirred at 170 °C for an additional 30 min, and then allowed to cool to 100 °C. A slight stoichiometric excess of water (approx. 5 mL) was added to the solution to quench unreacted NaBH₄. The system was then heated to 150 °C and maintained at that temperature for an additional 5 h, after which heating was removed, and the flask cooled to room temperature. The system was opened to air, and the black product was precipitated overnight from the reaction mixture with a large excess of ethyl acetate (approx. 200 mL). The mixture was centrifuged (6000 rpm, 10 min) and the supernatant decanted and discarded. This washing process was repeated thrice, via the addition of fresh portions (approx. 40 mL) of ethyl acetate, centrifugation, and removal of supernatant. Finally, the nanoparticles were air-dried at room temperature, and stored in tightly capped glass vials under air. The amounts of divalent metal precursor used in the other syntheses are presented in Table S1.³

2.3 Transmission electron microscopy (TEM)

TEM imaging was performed with a Hitachi H7650 microscope operated at 100 kV. Powdered samples were dispersed in methanol and drop-cast onto a carbon-coated copper grid (Ted Pella) and le methanol and drop-cast onto a carbon-coated copper grid (Ted Pella) and stored in tightly capped glass vials under air. The amounts of divalent metal precursor used in the other syntheses are presented in Table S1.

2.4 Powder X-ray diffraction (PXRD)

A Bruker D8 ECO Advance powder diffractometer (Cu Kα = 1.5406 Å, 40 kV, 40 mA) was used for phase identification. The sample was suspended in ether and smeared on a zero-background silicon sample plate (Si, P-type, B-doped; MTI Corp.). Intensity was measured in the 2θ = 25–75° range.

2.5 Fourier-transform infrared spectroscopy

Infrared spectra of nanoparticles in a KBr disk were collected on a Nicolet Nexus 470 spectrometer.

2.6 Energy dispersive X-ray spectroscopy (EDXS)

A Zeiss SigmaVP field-emission scanning electron microscope operated at 20 kV equipped with an Oxford INCA EDXS unit was used to determine the composition of the nanoparticles. Samples were prepared by dropping ca. 5 mg of powder onto double-sided adhesive conductive carbon tape, which was fixed on standard aluminum stubs.

2.7 Zeta potential measurement

Zeta potentials of PEGylated MFe₂O₄ NPs in aqueous solutions were measured at pH 7 using disposable folded capillary zeta cells in a Malvern Zetasizer Nano ZS dynamic light scattering system. For time-dependent measurements, the cuvette remained undisturbed in the instrument for the requisite length of time.

2.8 Superconducting quantum interference device (SQUID) magnetometry

Magnetic properties of the samples were measured using a Quantum Design XL-7S MPMS. Samples were prepared by placing a weighed quantity of powdered sample into a gelatin capsule, which was inserted in a clear, diamagnetic plastic straw. \( M(\mu_0H) \) measurements were conducted at temperatures of 1.9 K and 300 K in a maximal field strength of 4 T. For measurements at the physiological temperature, taken to be 310 K, the maximum field strength was extended to 7 T to mimic the conditions of high-field MRI. Zero-field-cooled (ZFC) magnetization of the sample was measured between temperatures of 1.9 and 300 K in a field of 10 mT, after the sample had been cooled in the absence of a magnetic field. Field-cooled (FC) measurements were done as for the ZFC measurement, however the sample was cooled from 300 K in the presence of a 10 mT magnetic field.

2.9 9.4 T MRI experiments

\( T_1 \) and \( T_2 \) relaxation measurements and phantom images were obtained using a 9.4 T/21 cm magnet (Magnex, UK) and a Bruker console (Bruker, Germany). Standard 5 mm diameter NMR tubes were used for experiments. A transmit/receive radio frequency (RF) volume birdcage coil was applied. A single slice multi-echo pulse sequence was used for \( T_2 \) measurements with the following parameters: repetition time \( (T_{E}) = 7.5 \text{s} \), 1 average, matrix size 128 × 128, field of view (FOV) 3 cm × 3 cm, slice thickness 2 mm, 64 echoes 4 ms apart. The \( T_2 \) relaxation time was calculated using a single exponential fitting of the echo train (Bruker, Germany). For \( T_1 \) measurements the TRUE FISP method was used with the following parameters: slice thickness 2 mm, FOV 3 × 3 cm, 1 average, matrix size 128 × 128, echo time \( (T_R) = 1.5 \) ms, \( T_E = 1 \) s.

The relaxivity values \( (r_1, r_2) \) were determined from relaxation times measured at different concentrations from the following equation:

\[
T_i^{-1} = T_{0_i}^{-1} + r_iC
\]
where $T_1$ are the observed relaxation times in the presence of magnetic nanoparticles, $T_{1\text{H}_2O}$ is the relaxation time of pure water, and $C$ is the concentration of magnetic ions in the PEGylated MFe$_2$O$_4$ NPs. The subscripts indicate longitudinal ($i = 1$) or transverse ($i = 2$) relaxivities and relaxation times, respectively.

### 3 Results and discussion

#### 3.1 Synthesis

We report a novel synthesis of mixed-metal ferrites in neat PEG (Fig. 1). As may be expected from their PEG coatings, the NPs were highly soluble in water. This synthesis has the advantage of simplicity, using simple reactants and a single-pot protocol. The chemistry is general, and worked with all metal precursors used, yielding phase-pure samples (*vide infra*). The exception was CuFe$_2$O$_4$, which is also seen to contain elemental Cu in addition to CuFe$_2$O$_4$ (identified in Fig. 2 with asterisks). CuFe$_2$O$_4$ NPs synthesized from Cu(acac)$_2$ showed prominent impurity peaks showing the presence of copper and iron oxides (not shown), which is documented. The presence of secondary phases was minimized (but not eliminated) by using CuCl$_2$-2H$_2$O as a copper source, as recommended by Ros and colleagues. The size distributions are not quite monodisperse, with ratios of the standard deviation over the mean of approx. 20% (by this metric, a value smaller than 10% would be required for a sample to be deemed monodisperse). It is anticipated that size can be controlled in these systems by varying the ratio of reducing agent to metal precursor.

This synthesis was inspired by a reductive synthesis of Fe$_3$O$_4$ NPs. In the initial report, Yathindranath et al. describe a reductive step to form the divalent Fe$^{2+}$ cations necessary for the formation of the spinel structure. In our case, no such reductive step is required as the divalent cation is directly supplied. Nonetheless, the addition of NaBH$_4$ triggers the nucleation of MFe$_2$O$_4$ NPs. We surmise NaBH$_4$ reacts with adventitious water or end groups of the PEG polymer chains to form hydroxyl ions, which form intermediate metal hydroxides (Fe(OH)$_3$ and M(OH)$_2$) that condense and dehydrate to form the oxide, regenerating water. No NPs were obtained in a control experiment setting which omitted the addition of NaBH$_4$ to the reaction mixture.

#### 3.2 Structural characterization

PXRD measurements were performed to determine the phase of MFe$_2$O$_4$ NPs. Fig. 2 shows typical XRD patterns of the various MFe$_2$O$_4$ nanoparticles, along with the corresponding standard line patterns for each ferrite. The XRD analysis revealed that all the samples are spinel cubic in structure (space group: $Fd\overline{3}m$), exhibiting six prominent peaks. A slight shift in peak positions for the substituted spinel NPs with respect to unsubstituted Fe$_3$O$_4$ NPs could be noted; this change is well-recorded in literature, and is in agreement with the ionic radii of the divalent cations, indicating a successful incorporation of divalent cations into the lattice. In general, the powder X-ray diffractograms of all the MFe$_2$O$_4$ NPs are in excellent agreement with their theoretical counterparts, as can be seen from Fig. 2, with superimposed powder patterns obtained from standard JCPDS data for each system. For CuFe$_2$O$_4$, the peaks attributed to metallic Cu are marked with asterisks.

**Fig. 1** Synthetic scheme for the one-pot synthesis of superparamagnetic, PEG$_{4000}$-coated MFe$_2$O$_4$ (M = Mn, Fe, Co, Ni, Cu, Zn) NPs with sizes ranging from ca. 6 to 10 nm.

**Fig. 2** Powder X-ray diffractograms of the six PEGylated metal ferrites, with superimposed powder patterns obtained from standard JCPDS data for each system. For CuFe$_2$O$_4$, the peaks attributed to metallic Cu are marked with asterisks.
comparable size distributions. The reasonable size monodispersity is believed to be controlled by the nucleation and growth of the NP seeds generated upon the rapid addition of NaBH₄ to the precursor in PEG medium at high temperatures.

Fig. 4 shows a representative FT-IR spectrum of PEGylated MnFe₂O₄ NPs superimposed on the spectrum of neat PEG. It is seen that there are several bands that appear in both the spectra, some of which have been highlighted in the figure: (i) the characteristic absorption band at 950 cm⁻¹, associated with the –CH out-of-plane bending vibrations of PEG; (ii) the absorption band at 1240 cm⁻¹, assigned to C–H twisting in PEG; (iii) the C–O–C ether stretching absorption band at 1094 cm⁻¹; (iv) the bands at ∼2800–3000 cm⁻¹, corresponding to –CH symmetric and asymmetric stretching vibrations; (v) the band around 2910 cm⁻¹, corresponding to –CH₂ stretching vibrations. The C–O–C, –CH₂ and –CH peaks confirm the tethering of PEG onto the MFe₂O₄ NP surfaces.⁷ The FT-IR spectra of the other PEGylated MFe₂O₄ NPs are shown in Fig. S2.†

3.3 Magnetic characterization

The magnetic properties of the samples were investigated by SQUID magnetometry. For all the magnetic measurements presented here, samples were powdered, and thus the measured magnetic properties represent the average values for an ensemble of randomly oriented nanoparticles. Magnetization vs. magnetic field strength loops were recorded at 310, 300 and 1.9 K (310 K: Fig. 5; 300 and 1.9 K: Fig. S3†) for all the metal ferrite samples. In the $M(H)$ curves, hysteresis is observed at 1.9 K, which is characteristic of ferromagnetic behavior, while anhysteretic loops are seen at the physiological temperature, 310 K. The magnetization of the samples was also examined under FC and ZFC conditions. Combined FC–ZFC plots for PEGylated MFe₂O₄ NPs are found in Fig. S4.† All NPs showed blocking temperatures ($T_B$, indicating the transition from low-temperature, ferromagnetic NPs to freely fluctuating superparamagnetic NPs at higher temperatures) below room temperature. For all samples, the behavior is typical of superparamagnetism, which is expected for nanoscaled magnetic NPs.⁶ The exception is CoFe₂O₄, whose $T_B$ is slightly below room temperature. Upon closer inspection, the room-
temperature $M(H)$ loop for CoFe$_2$O$_4$ shows a very weak hysteresis. This is consistent with a sample with the majority of NPs in the superparamagnetic state, with a minor component of blocked NPs. The magnetic properties are compiled in Tables 1 and S2.$^\dagger$

In general, the $M_s$ values of our PEGylated MFe$_2$O$_4$ NPs were lower than their bulk counterparts: for example, at 300 K, MnFe$_2$O$_4$–PEG$_{400}$ NPs showed a saturated magnetization $M_s$ of 35.9 emu g$^{-1}$, which is considerably smaller than the theoretical value of bulk MnFe$_2$O$_4$ material (110 emu g$^{-1}$), but very close to the $M_s$ shown by tetraethylene glycol coated 7 nm MnFe$_2$O$_4$ NPs synthesized by Yang et al.$^4$ The reduced saturation magnetization of NPs is attributed to the existence of a magnetically 'dead' surface layer for each particle in which magnetic moments do not contribute to the magnetization.$^4$ Given that our CuFe$_2$O$_4$ NPs had a non-negligible amount of copper contaminant, it is difficult to compare its magnetic properties with the other uncontaminated PEGylated MFe$_2$O$_4$ NPs, especially since elemental copper is orders of magnitude less magnetic than CuFe$_2$O$_4$. A brief discussion on the observed magnetic properties of individual NPs vis-a-vis literature is found in the ESI.$^\dagger$

From the $M$ vs. $H$ curve of CoFe$_2$O$_4$ NPs at 1.9 K, it can be clearly seen that they exhibit an anomalous hysteresis behaviour. These broadened hysteresis loops are often seen in nanocomposite systems with strongly coupled soft and hard magnets; most notably in CoFe$_2$O$_4$ thin films.$^5,5^1$ Ros and co-workers noted a similar broadened hysteresis loop for their $\sim$7 nm CoFe$_2$O$_4$ NPs, which they attributed to the presence of a strain inhomogeneity from the surface to the core of the NP, leading to the coexistence of soft and hard magnetic behavior within a single particle.$^4$ The $T_B$ for these NPs, at $\sim$280 K, is also significantly greater than that of the other ferrite NPs; this is expected for CoFe$_2$O$_4$ NPs.$^5,5^2$ The Co$^{2+}$ ion – with a $d^7$ configuration – imparts a high magnetocrystalline anisotropy from

Table 1  Summary of magnetometric measurements on PEGylated MFe$_2$O$_4$ NPs

| Sample     | $M_s$, 310 K (emu g$^{-1}$) | $T_B$ (K) |
|------------|----------------------------|-----------|
| MnFe$_2$O$_4$ | 33.7                        | 47        |
| Fe$_3$O$_4$  | 34.4                        | 150       |
| CoFe$_2$O$_4$| 33.3                        | 280       |
| NiFe$_2$O$_4$| 28.4                        | 150       |
| CuFe$_2$O$_4$| 23.2                        | 61        |
| ZnFe$_2$O$_4$| 16.0                        | 32        |

Fig. 7  (a) $T_2$-weighted image of Fe$_3$O$_4$ samples in water at various [Fe] concentrations (b) $T_2$-weighted image of MFe$_2$O$_4$ samples in water.

Fig. 6  (a) $\zeta$-Potentials of MFe$_2$O$_4$–PEG$_{400}$ NPs in water (pH 7). (b) Temporal evolution of the $\zeta$-potential of Fe$_3$O$_4$–PEG$_{400}$ and MnFe$_2$O$_4$–PEG$_{400}$ NPs in water.

Fig. 8  (a) Relaxometric plot for MnFe$_2$O$_4$–PEG$_{400}$ NPs in water. (b) Compiled relaxivities ($r_1$ below the break with hatched bars, $r_2$ above the break). (c) Compiled $r_2/r_1$ ratios.
the presence of spin–orbit coupling. The $M_s$ values recorded were 52.8 emu g$^{-1}$ at 1.9 K and 33.7 emu g$^{-1}$ at 300 K.

### 3.4 Zeta potentials

In order to better understand the physiological stability of MFe$_{2}$O$_{4}$–PEG$_{400}$ NPs, zeta potentials ($\zeta$) were measured post-sonication in distilled water at pH ~ 7. The recorded values are compiled in Fig. 6a, and are in accordance with literature precedents, such as the work of Feng et al., who found $\zeta = -21$ mV for their (methoxy polyethylene glycol)-coated Fe$_3$O$_4$ NPs.$^{53}$ It is expected that $\zeta$ values for these systems might become even higher in physiological buffer owing to protein corona formation and the associated electrosteric repulsion.$^{34,55}$

A time-dependent study of the evolution of $\zeta$ values for PEGylated Fe$_3$O$_4$ and MnFe$_2$O$_4$ NPs (Fig. 6b) didn’t show any drastic changes in the $\zeta$-potential values of these systems over a period of 24 h or more.

### 3.5 Magnetic resonance imaging

The dispersed solutions of MFe$_{2}$O$_{4}$–PEG$_{400}$ NPs in water were used in a relaxometric study to determine the $T_1$ and $T_2$ relaxation times, and evaluate their potential as MRI contrast agents. Representative images are show in Fig. 7. As can be seen, contrast increases with concentration (Fig. 7a), while the various MFe$_2$O$_4$ species showed distinct contrast. A sample relaxometric plot for PEGylated MnFe$_2$O$_4$ NPs is shown in Fig. 8, with the relaxation rates $R_1 (= T_1^{-1})$ and $R_2 (= T_2^{-1})$ plotted as a function of the concentration of the magnetic ions in solution.

These same measurements for the other samples are shown in Fig. S5.$^\dagger$ The effectiveness of NPs as contrast agent is measured in the form of relaxivity, which represents the reciprocal of the relaxation time per unit concentration of magnetic ions (cf. eqn (2)).

This is one of the few systematic investigations of the high-field MRI capabilities in a series of MFe$_2$O$_4$, spanning over six compositions. Of the six MFe$_2$O$_4$–PEG$_{400}$ NPs, MnFe$_2$O$_4$–PEG$_{400}$ NPs are found to be the most effective $T_2$ contrast agents in aqueous media, with $r_1 = 0.69 \pm 0.02$ mM$^{-1}$ s$^{-1}$, $r_2 = 118 \pm 20$ mM$^{-1}$ s$^{-1}$, and $r_2$/$r_1 = 173$. Table 2 lists the $r_1$, $r_2$, and $r_2$/$r_1$ values of the other PEGylated MFe$_2$O$_4$ NPs. CuFe$_2$O$_4$ NPs, laced with elemental copper NPs, show modest contrast-enhancing capabilities which must be considered a lower limit. Interestingly, they show the highest $r_1$ of the series; as such, further

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**Table 2** Comparison of NP-based high-field ($B_0 = 7$ T) contrast agents

| Sample | Label | Core | Coating | Size (nm) | $B_0$ (T) | $r_1$ (mM$^{-1}$ s$^{-1}$) | $r_2$ (mM$^{-1}$ s$^{-1}$) | $r_2/r_1$ | Reference |
|--------|-------|------|---------|-----------|-----------|-----------------|-----------------|-----------|----------|
| 1 Gd$_3$O$_3$ | PEG | 1.3 | 11.7 | 10.4 | 17.2 | 1.7 | Faucher et al.$^{57}$ |
| 2 Gd$_2$O$_3$ | DEG | 4.6 | 7 | 4.4 | 28.9 | 6.6 | Bridot et al.$^{58}$ |
| 3 CoFe$_2$O$_4$@ZnO | Chitosan | 11.6 | 9.4 | — | 31.8 | — | Venkatesha et al.$^{59}$ |
| 4 Mn$_{0.25}$Fe$_{2.75}$O$_4$ | PEG$_{4000}$ | 6.5 | 9 | — | 32.7 | — | Vamvakidis et al.$^{60}$ |
| 5 CuFe$_2$O$_4$ | PEG | 8.6 | 9.4 | 1.21(16) | 34(2) | 28(4) | This work |
| 6 NiFe$_2$O$_4$ | PEG | 6.1 | 9.4 | 0.78(1) | 36(4) | 46(5) | This work |
| 7 Zn | ZnFe$_2$O$_4$ | 5.9 | 9.4 | 0.60(2) | 49(5) | 82(9) | This work |
| 8 ZnFe$_2$O$_4$ | Chitosan-liposome | ≤200 | 9.4 | — | 54 | — | Hoque et al.$^{61}$ |
| 9 Fe | Fe$_3$O$_4$ | HDA-G2 | 10 | 7 | — | 60 | — | Zhao et al.$^{23}$ |
| 10 CoFe$_2$O$_4$ | Chitosan | 8.3 | 9.4 | — | 60.9 | — | Venkatesha et al.$^{59}$ |
| 11 Mn$_{0.33}$Fe$_{2.67}$O$_4$ (1 : 1) | 1,2 PG + TEG | 6.0 | 9 | — | 64.5 | — | Vamvakidis et al.$^{60}$ |
| 12 ZnFe$_2$O$_4$ | PEG | 5 | 9.4 | — | 68 | — | Hoque et al.$^{61}$ |
| 13 Fe$_3$O$_4$ | PEG | 10.4 | 9.4 | 0.80(7) | 75(12) | 94(17) | This work |
| 14 ZnFe$_2$O$_4$ | Chitosan | 4.8 | 9.4 | — | 76 | — | Hoque et al.$^{61}$ |
| 15 NiFe$_2$O$_4$ | Chitosan | 3 | 9.4 | 0.348 | 89 | 256 | This work |
| 16 CoFe$_2$O$_4$ | PEG | 5.8 | 9.4 | 0.45(4) | 99(14) | 219(37) | This work |
| 17 Mn$_{0.25}$Fe$_{2.75}$O$_4$ | PMAO–PEG | 20.3 | 9.4 | 0.33 | 101 | 306 | Das et al.$^{18}$ |
| 18 Fe$_3$O$_4$ | PEG | 6.4 | 9.4 | 0.69(2) | 118(20) | 171(30) | This work |
| 19 Fe$_3$O$_4$ | HDA-G2 | 16 | 7 | — | 126 | — | Zhao et al.$^{23}$ |
| 20 NaHoF$_4$ | PMAO–PEG | 17 | 9.4 | 0.17 | 130.6 | 781 | Zhang et al.$^{19}$ |
| 21 Dy$_3$O$_7$ | Dextran | 70 | 7 | — | 190 | — | Norek et al.$^{53}$ |
| 22 Fe$_3$O$_4$ | Malrol | 10.1 | 9.4 | 2.11 | 191 | 90.5 | Clements et al.$^{30}$ |
| 23 NiFe$_2$O$_4$ | DMSA | 9.0 | 11.7 | — | 200 | — | Menelaou et al.$^{54}$ |
| 24 NaDyF$_4$ | PMAO–PEG | 25 × 35 | 9.4 | 0.50 | 204.4 | 410 | Zhang et al.$^{19}$ |
| 25 Fe$_3$O$_4$ octapods | HDA-G2 | 20 | 7 | — | 209 | — | Zhao et al.$^{23}$ |
| 26 Mn$_{0.25}$Fe$_{2.75}$O$_4$ | Succimer | 7.6 | 9.4 | 18.6 | 227.6 | 12.2 | Kim et al.$^{36}$ |
| 27 Fe$_3$O$_4$ | Succimer | 6.0 | 9.4 | 11.1 | 255.9 | 23.1 | Kim et al.$^{36}$ |
| 28 NiFe$_2$O$_4$ | CTAB | 9.0 | 11.7 | — | 278.9 | — | Menelaou et al.$^{54}$ |
| 29 CoFe$_2$O$_4$ | Succimer | 8.0 | 9.4 | 6.3 | 392.5 | 62.3 | Kim et al.$^{36}$ |
| 30 Fe$_3$O$_4$ brick-like | Succimer | 64.0 | 7 | 4.3 | 599 | 139 | Worden et al.$^{24}$ |
| — Gd$_3$F$_4$ | Succimer | 30–50 | 9.4 | 1.44 | — | — | Ju et al.$^{65}$ |

$^a$ Refers to Fig. 9. $^b$ DEG: diethylene glycol; PAA: poly(acrylic acid); CTAB: cetyltrimethylammonium bromide; PMAO: poly[maleic anhydride-alt-1-octadecene]; HDA-G2: conjugates of dendritic molecules and 1-hexadecylamine; 1,2-PG: 1,2-propylene glycol; TEG: tetraethylene glycol.
investigation of this species for T₁-weighted MRI may be warranted. We note that to the best of our knowledge, this is only report of CuFe₂O₄ NPs used for high-field MRI.

It is evident that MnFe₂O₄, CoFe₂O₄, and Fe₂O₃ NPs are better T₂ contrast agents compared to NiFe₂O₄, CuFe₂O₄, and ZnFe₂O₄. We generally find that r₂ increases with increased saturation magnetization (Fig. S6†), while the r₁ values are tightly clustered, with no clear trend with respect to magnetization. As may then be expected, the r₂/r₁ ratios track the trend seen for r₂.

While the r₁ and r₂ values of our MFe₂O₄–PEG₄₀₀ NPs are within range with examples in literature (Fig. 9), we believe further improvements are possible by, e.g., templating directional growth to form morphological variations like octapods,²³ bricks,²⁴ or whiskers;²⁵ or subjecting them to controlled aggregation and/or self-assembly, which are known to lead to higher contrasting abilities.²⁶

Most contrast agents were investigated with clinical MRI scanners (1.5–3 T). Well-know examples include the commercially available Ferridex® and Combidex®. Ferridex® has r₂ = 120 mM⁻¹ s⁻¹ at a magnetic field of 1.5 T, while the r₂/r₁ increases from 10.1 to 22 when increasing the magnetic field from 1.5 to 3.0 T.³⁰ Similarly, Combidex® has r₂ = 65 mM⁻¹ s⁻¹ at a magnetic field of 1.5 T, with r₂/r₁ = 6.³⁰

There are few reports of transition metal ferrite NP contrast agents under high-field conditions; this makes a direct one-to-one comparison with our MFe₂O₄–PEG₄₀₀ NPs at 9.4 T difficult. This difficulty is exacerbated by significant dependencies of magnetic relaxivities on the magnetic field strength,⁶⁶ the nature of the solvent (i.e., water vs. plasma vs. buffer), the surface coating,⁶⁵,⁶⁶ aggregation,⁶⁶ and magnetic dynamics.⁶⁷

A non-exhaustive compilation of relaxivities of various NP contrast agents used at high fields (B₀ > 7 T) is presented in Table 2 and Fig. 9. The nanoparticles in this table can be roughly classified as lanthanide oxides,⁵⁷,⁶⁵,⁶⁶ and fluorides,⁵⁷ sodium lanthanide fluorides NaLnF₄ (Ln = Ho, Dy)¹⁸,¹⁹ and spinel ferrites.²³,²⁴,¹⁶,¹⁰,⁶⁸–⁶¹ Upon quick inspection of Fig. 9, it is apparent there is no clear segregation of NP type – or even specific composition – with regards to r₂. As an example, our MnFe₂O₄ (Mn) and CoFe₂O₄ (Co) NPs are fairly similar in both r₁ and r₂ to NaHoF₄ (13)¹⁹ and NaDyF₄ (11)¹⁹ (see Fig. 9b). The advantage of the NaLnF₄ NPs is their paramagnetic nature; unlike superparamagnetic (i.e. electromagnetically coupled spins), they do not saturate in fields up to 9 T and above. However, their magnetization is substantially lower (Mₛ ≈ 12 emu g⁻¹ at 7 T for 13 (ref. 19)). In this specific comparison, the two effects seem to balance each other. Another striking example is the comparison of Fe₂O₃–PEG₄₀₀ (Fe) with maltol-coated Fe₂O₄ NP (15).³⁰ Both have comparable sizes (10.4 vs. 10.1 nm), yet 15 has a r₂ two and a half times that of Fe, despite a lower saturation magnetization (26.8 emu g⁻¹). This discrepancy is attributed to the proximity of water molecules to the magnetic core in 15 due to the small capping ligand.

A compilation of r₂/r₁ ratios is found in Fig. S7.† As can be seen, the ratios found here compare favourably with state-of-the-art. CoFe₂O₄ and MnFe₂O₄ in particular have high values, showing their promise for use as T₂ high-field contrast agents.

4 Conclusion

In this study, PEGylated sub-15 nm MFe₂O₄ (M = Mn, Fe, Co, Ni, Cu, Zn) NPs were synthesized following a novel protocol, with the capping agent (PEG₄₀₀, a liquid polymer) itself serving as the reaction medium. The NPs were found to be small, fairly monodisperse, and superparamagnetic at room temperature. These NPs were systematically evaluated for high field (B₀ = 9.4 T) MRI in vitro. Of the six MFe₂O₄–PEG₄₀₀ NP compositions studied, MnFe₂O₄, CoFe₂O₄, and Fe₂O₄ NPs displayed superior T₂ contrasting abilities in MRI, with reasonably high r₂ values (∼118 mM⁻¹ s⁻¹ for MnFe₂O₄, ∼98 mM⁻¹ s⁻¹ for CoFe₂O₄, and ∼75 mM⁻¹ s⁻¹ for Fe₂O₄) and r₂/r₁ ratios (∼171 for MnFe₂O₄, ∼219 for CoFe₂O₄, and ∼94 for Fe₂O₄). Given the
well-documented biocompatibility of PEG, these transition metal ferrite NP systems deserve to be better investigated as potential contrast agents for dark-field MRI.

Author contributions

ST and AB designed the project, AB and EP carried out the synthetic work and physical characterization, BB and BT oversaw MRI measurements, AB, ST and BT contributed to the writing of the manuscript.

Conflict of interest

There are no conflicts of interest to declare.

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