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Probing the Chemical Stability of Mixed Ferrites: Implications for MR Contrast Agent Design

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Abstract

Nanomaterials with mixed composition, in particular magnetic spinel ferrites, are emerging as efficient contrast agents for magnetic resonance imaging (MRI). Many factors, including size, composition, atomic structure, and surface properties are crucial in the design of such nanoparticle-based probes due to their influence on the magnetic properties. Silica-coated iron oxide (IO-SiO₂) and cobalt ferrite (CoIO-SiO₂) nanoparticles were synthesized using standard high temperature thermal decomposition and base-catalyzed water-in-oil microemulsion techniques. Under neutral aqueous conditions, it was found that 50–75% of the cobalt content in the CoIO-SiO₂ nanoparticles leached out of the core structure. Leaching caused a 7.2-fold increase in longitudinal relaxivity and an increase in the saturation magnetization from ~48 emu/g core to ~65 emu/g core. X-ray absorption fine structure studies confirmed that the atomic structure of the ferrite core was altered following leaching, while TEM and DLS confirmed that the morphology and size of the nanoparticle remained unchanged. The CoIO-SiO₂ nanoparticles converted from a partially inverted spinel cation arrangement (unleached state) to an inverse spinel arrangement (leached state). The control IO-SiO₂ nanoparticles remained stable with no change in structure and negligible changes in magnetic behavior. This detailed analysis highlights how important understanding the properties of nanomaterials is in the development of reliable agents for diagnostic and therapeutic applications.

Keywords

MRI; ferrite; nanoparticle; leaching; magnetic properties

Introduction

Magnetic spinel ferrites (M_xFe_{3-x}O₄, M = Fe, Co, Mn, Ni, or Zn) have emerged as versatile nanomaterials for use in numerous biological applications, such as thermal activation therapy, biosensing, drug delivery, and magnetic resonance imaging (MRI).^{1–7}

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SUPPORTING INFORMATION AVAILABLE. XRD spectra of Fe₃O₄ and CoFe₂O₄ nanoparticle cores, sample hydrodynamic size distributions obtained by DLS, quantitative data from SQUID measurements, additional 7 T MRI analysis and images, and additional XANES spectrum. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Superparamagnetism coupled with tunable surface properties render these structures particularly valuable as contrast agents for MR imaging. Among the ferrites, Fe_3O_4 (iron oxide, IO) nanoparticles have been the most widely used particle-based MRI contrast agents.^{8–13} However, advances in synthetic techniques that allow control over morphology and composition have made mixed metal ferrites, such as cobalt ferrite (CoFe_2O_4) and manganese ferrite (MnFe_2O_4), promising additions to the development of nanoparticle probes with tunable magnetism and MR contrast effects.^{2,14–16} Cobalt ferrite, in particular, has received substantial attention for its theranostic potential (MRI and thermal activation) due to its unique magnetic properties, such as large anisotropy energy, tunable coercivity, and high saturation magnetization.^{1,17–19}

Superparamagnetic (SPM) ferrite nanoparticles are powerful MRI contrast agents due to their large magnetic moments that result from the cooperativity of the individual spins when aligned in the presence of an external magnetic field.²⁰ This high magnetic susceptibility creates local magnetic field inhomogeneities, which appreciably shorten the T_2 and T_2^* relaxation times (dephasing) of nearby water protons and produces darker intensity in an MR image.²¹ Relaxivity is a measure of the effectiveness of a contrast agent at manipulating the relaxation times (T_1 and T_2) of proton spins.²² The higher the relaxivity, the more efficient the material is as an MRI contrast agent. Superconducting quantum interference device (SQUID) magnetometry is used to further assess the magnetic properties of SPM nanoparticles by measuring the magnetic susceptibility (χ) and saturation magnetization (M_s) of the material.

Optimization of magnetic properties, stability, and in vivo biocompatibility are at the forefront in the design of our nanoparticle MRI contrast agents.^{23–27} These properties depend on many factors, including particle size, crystallinity, composition, crystallographic structure (cation distribution), surface morphology, and surface coating.^{4,18,28–30} Particularly, the effects of size and composition on the magnetic properties of ferrite nanoparticles have been thoroughly investigated.^{14,31–33} It was determined that as the nanoparticle size increases, the magnetic properties (magnetization and relaxivity) are enhanced. Furthermore, the doping of magnetic atoms (e.g., Mn^{2+} , Co^{2+} , and Ni^{2+}) into the Fe_3O_4 structure leads to a decrease in the magnetic properties following the trend $\text{Mn} > \text{Fe} > \text{Co} > \text{Ni}$.

An extensive amount of research has been focused on learning how changes in structure, cation distribution, and surface properties affect the magnetic properties of a material.^{34–38} Bulk ferrites adopt a spinel arrangement of metal cations with cubic symmetry that contains 32 oxygen atoms, 16 trivalent metal ions, and 8 divalent metal ions.^{18,39} In a normal spinel arrangement, the divalent cations occupy tetrahedral (T_d) crystallographic sites and the trivalent cations reside in octahedral (O_h) sites. Magnetic ferrites (e.g., Fe_3O_4 , CoFe_2O_4 , and MnFe_2O_4) are expected to have an inverse spinel arrangement, where the 16 trivalent cations are split between the T_d and O_h sites with the 8 divalent cations occupying the remaining O_h sites. The distribution of the cations among the two types of crystallographic sites depends on the radii, electronic structure, and valence of the metal ions in the ferrite.^{34,38,40} Bulk Fe_3O_4 is known to have a pure inverse spinel arrangement; however, it has been found that mixed ferrites, such as CoFe_2O_4 and MnFe_2O_4 , often have a partially inverted spinel structure (in between normal and inverse).³⁴ On the nanoscale, the preparation conditions, namely the $\text{M}^{2+}:\text{Fe}^{2+}$ ratio in the precursor solution and temperature, influence the resulting cation distribution (core structure) and magnetic properties of the nanoparticle product.^{36,40}

X-ray diffraction (XRD), Mössbauer spectroscopy, and x-ray absorption fine structure (XAFS) are each useful methods for obtaining structural information. However, XAFS

techniques, which include x-ray absorption near edge structure (XANES) and extended x-ray absorption fine structure (EXAFS), are highly sensitive for studying the atomic structure of multicomponent, dilute, and disordered systems on the nanoscale.^{34,41} XANES provides information on the site symmetry and oxidation state of the absorbing atom in the material. EXAFS reveals the atomic coordination around the absorbing atom, including information on bond distances and coordination numbers.

The surface of the nanoparticle core can have an important effect on the structure and magnetic properties of the material. When decreasing particle size into the nano-regime, the properties of the material are altered as the surface area becomes very large in comparison to the bulk state. The electron spins on the surface of a magnetic material are canted (or disordered) due to reduced spin-spin exchange near the surface.² In bulk material, the canted surface is minimal compared to the large volume, but the effect of the canting is a great deal more prominent for nanomaterials having high surface area-to-volume ratios. Due to this large surface area, more metal cations are located near the surface.⁴⁰ Changes in cation distribution of the metal ions will inevitably alter the morphology of the surface layer and, in turn, the magnetic properties of the nanoparticle. The role of surface morphology on the properties of the material is further complicated by the coating surrounding the magnetic core. Ferrite nanoparticles require surfactants or coatings to prevent aggregation when in aqueous media and to afford biocompatible, stable MR contrast agents. These coatings are typically organic ligands, small-molecule charge stabilizers, polymers, and inorganic materials.^{3,4,21,28,32,42} The nature of the coating will affect water access to the magnetic core, thereby influencing relaxivity and efficacy of the agent.

In the design and evaluation of mixed ferrite nanoparticles as viable MR probes, the chemical stability is one of the most critical parameters to consider. The nanoparticle and its properties need to remain constant in order to have an effective probe. There is some literature precedence for the release of metal ions from ferrite nanoparticles, specifically CoFe_2O_4 . Soler et al. studied the influence of surface passivation on the aging of CoFe_2O_4 in highly acidic media ($\text{pH} < 2$).⁴³ In all the aged samples, dissolution of CoFe_2O_4 was observed (up to 50%), affecting the structure, cation distribution, and magnetization of the nanoparticles. Baldi et al. demonstrated the impact of surface coating on the leaching of cobalt ions from CoFe_2O_4 nanoparticles.⁴⁴ They found that the nature of the surface coating influenced the extent of cobalt release at neutral pH. While these studies confirmed the leaching of metal ions from CoFe_2O_4 nanoparticles, the experimental conditions were not biologically relevant or the effect of leaching on the structural and magnetic properties were not investigated.

Here, we present a detailed analysis of the chemical stability of silica-coated CoFe_2O_4 core-shell nanoparticles in solution for use as MRI contrast agents. We describe the leaching process of metal ions from the core and its direct impact on the structural and magnetic integrity of the nanoparticles. Typically, structural and magnetic investigations of ferrites have been performed on powder samples and the changes in chemical composition are deliberate and imposed by the synthetic conditions.^{34,36,37,40,45–48} We find that significant changes in chemical composition of these CoFe_2O_4 nanoparticles occur *spontaneously* during dialysis in aqueous solution without the use of harsh reagents or pH conditions. The leaching of metal ions alters the crystal structure of the nanoparticle core, significantly affecting the magnetic properties. The magnetism and structure of the nanoparticles in this study were characterized by magnetometry, relaxometry, and x-ray absorption fine structure (XAFS) analysis and compared to control Fe_3O_4 nanoparticles. Such detailed, localized analysis of nanocomposites is imperative to understanding their properties and behavior to ultimately develop reliable carriers for diagnostic and therapeutic applications.

Materials and Methods

All chemicals and reagents were purchased from Sigma Aldrich (St. Louis, MO) and used without further purification. XAFS reference compounds of the highest purity available were purchased from Alfa Aesar (Ward Hill, MA) and used as received. Regenerated cellulose 12–14,000 molecular weight cutoff (MWCO) Spectra/Por® 4 membrane tubing was purchased from Spectrum Laboratories, Inc. (Rancho Dominguez, CA). Amicon Ultra-4 100,000 MWCO centrifugal filter units were purchased from Millipore (Billerica, MA). Elemental ICP standards for Fe and Co were purchased from Sigma Aldrich (St. Louis, MO). Multi-element internal standard (10 µg/mL Bi, Ho, In, Li, Sc, Tb, and Y in 2% nitric acid) was purchased from Spex Certiprep Inc. (Metuchen, NJ). Water was purified using a Millipore Milli-Q Synthesis water system (Billerica, MA).

Nanoparticle Synthesis: Core Formation

CoFe₂O₄ cores were prepared using a previously published procedure.¹ Briefly, iron(III) acetylacetonate (4 mmol) and cobalt(III) acetylacetonate (2 mmol) were dissolved in 40 mL benzyl ether. The surfactants dodecylamine (12 mmol), lauric acid (12 mmol) and 1,2-hexadecanediol (20 mmol) were added to reduce and stabilize the nanoparticle surface. The solution was heated to 200 °C for 30 min under a nitrogen atmosphere and then raised to 280 °C for 30 min. The nanoparticles were precipitated with ethanol followed by magnetic separation. Fe₃O₄ cores were synthesized by the same procedure using an iron(III) acetylacetonate precursor (2 mmol), 6 mmol dodecylamine, 6 mmol lauric acid and 10 mmol 1,2-hexadecanediol in 20 mL benzyl ether.

Nanoparticle Synthesis: Silica Shell Formation

Silica (SiO₂) was used to coat the core by a base-catalyzed water-in-oil microemulsion approach. Igepal CO-520 (1 mL) was mixed with anhydrous cyclohexane (20 mL) and stirred for 10 min. The nanoparticle cores were dispersed in cyclohexane at a concentration of 1 mg/mL and slowly added to the Igepal CO-520/cyclohexane emulsion. An aqueous solution of 30% NH₄OH (0.140 mL) was added dropwise and stirred for 15 min, followed by the addition of tetraethylorthosilicate (TEOS, 0.170 mL). The mixture was stirred for 48 h before the addition of ethanol to precipitate the core-shell nanoparticles. The precipitate was collected by centrifugation and the nanoparticles were washed by redispersing in ethanol. The nanoparticles were washed at least three times to remove excess surfactant. The final product was dispersed directly in water.

Metal Ion Leaching Experiments

Nanoparticles (0.5 mL of ~1 mg/mL) were leached by extensive dialysis against approximately 20 L of water for 2–3 days using 12–14,000 MWCO regenerated cellulose membranes. After dialysis, the leached nanoparticles were concentrated by size filtration using Amicon Ultra-4 100,000 MWCO centrifugal filter devices. The nanoparticles were washed three times with water in the centrifugal filter units. The resulting leached nanoparticle stock solutions were adjusted with water to a final volume of 0.5 mL and brief ultrasonication was used to fully disperse the nanoparticles.

Transmission Electron Microscopy (TEM)

TEM images were obtained at room temperature on a Hitachi HF-8100 instrument operated at 200 kV using the imaging mode (Pleasanton, CA). The nanoparticle diameters were determined by statistical averaging using a digital micrograph. All TEM samples were prepared by directly transferring and evaporating the nanoparticle suspension onto a standard copper grid coated with a carbon film under ambient conditions.

X-ray Diffraction (XRD)

XRD measurements were performed on a powder sample of CoFe_2O_4 core structures using a Rigaku goniometer. The instrument was equipped with a long, fine-focus copper x-ray tube ($\lambda = 0.15406$ nm) and operated at 40 kV and 20 mA with 1° divergent and antiscattering slits coupled with 0.1 mm receiving slits. The scan was performed with 2 θ steps of 0.05 deg/step and collection time was 2 sec for each point. The XRD patterns for Fe_3O_4 cores were collected on a Scintag x-ray machine with similar settings (except for a step size of 0.1 deg/step). XRD analysis was performed in the J.B. Cohen X-Ray Diffraction Facility at Northwestern University (Evanston, IL).

Dynamic Light Scattering (DLS)

Hydrodynamic size was determined using DLS on a Malvern Instruments Zetasizer Nano Series Nano-ZS with Dispersion Technology Software 5.03 (Worcestershire, United Kingdom). Samples were measured in aqueous solution using polystyrene cuvettes with 10 mm optical pathway at 25 °C. Each sample was measured at a concentration of 0.25 mM Fe. Fifteen scans were performed per sample and the results were averaged. The data were obtained as lognormal distribution plots. Intensity-, volume-, and number-weighted size distributions were analyzed, however only number-weighted values are reported.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Element concentrations were determined using a Thermo Electron Corporation XSeries^{II} ICP-MS with Thermo PlasmaLab software (Waltham, MA). The instrument was operated in collision cell technology (CCT) mode. Samples for ICP analysis were digested in 70% TraceSELECT® nitric acid at 65 °C for ~3 h and diluted to 3% (v/v) nitric acid. Fe and Co standards were prepared in 3% (v/v) nitric acid with concentrations of 0.5, 1, 5, 15, 50, 125, and 250 ng/mL. A multi-element internal standard (containing 10 µg/mL Bi, Ho, In, Li, Sc, Tb, and Y) was added to each standard and sample to yield a final concentration of 5 ng/mL. Isotopes ^{57}Fe and ^{59}Co were used for determining element concentration. Isotopes ^{45}Sc , ^{89}Y , ^{115}In , and ^{165}Ho were used as internal standards.

Magnetic susceptibility

Magnetic susceptibility measurements were carried out on a Quantum Design MPMS5 Superconducting Quantum Interference Device (SQUID) magnetometer (San Diego, CA). Samples were dispersed in water and frozen under a nitrogen environment. The magnetization (M), as a function of applied magnetic field (H), was acquired at 260 K over fields between -50 kOe and 50 kOe. From the hysteresis loops (M vs. H plots), coercivity, remanence and saturation magnetization (M_s) were obtained. M_s was determined by plotting M vs. $1/H$ at high fields and extrapolating to $1/H = 0$. All SQUID data were corrected for diamagnetism by subtracting the diamagnetic contribution at high fields near magnetic saturation.

Relaxometry

Longitudinal (T_1) and transverse (T_2) relaxation times were measured at 60 MHz and 37 °C on a Bruker mq60 NMR Analyzer equipped with The Minispec V2.51 Rev.00/NT software (Billerica, MA). Each sample was diluted with water to make 5 concentrations (0.0625-1 mM Fe). An inversion-recovery pulse sequence was used to measure the T_1 relaxation times, while a spin echo pulse sequence was used to measure transverse T_2 relaxation times. Initial and final pulse separations were adjusted to obtain the best monoexponential curve fit. Relaxation times were determined using monoexponential curve fitting of 10 data points (4 averages for each point). The relaxivity was determined by taking the slope of a plot of $1/T_n$ ($n = 1, 2$) versus concentration (mM Fe).

MR phantom imaging and analysis at 7 T

7 T MR imaging and T_1/T_2 measurements were performed on a Bruker PharmaScan 7 T horizontal, wide bore (89 mm) imager (Bruker, Billerica, MA, USA) fitted with shielded gradient coils at 25 °C. For solution phantom images, 25 μ L of solutions containing leached or unleached CoFe nanoparticles (0.25, 0.125, 0.0625, and 0.03125 mM Fe) in water were each added to a flame-sealed 9" inch Pasteur pipet. The bottom of the flame-sealed pipet was then scored with a glass scribe and broken into small capillaries, tops wrapped in Parafilm® to avoid sample evaporation, and then imaged in a transmit/receive 23-mm mouse-head coil.

Spin lattice relaxation times (T_1) were measured using a rapid acquisition rapid echo (RARE) T_1 -map pulse sequence, with static TE (11 ms) and variable TR (117, 200, 300, 500, 750, 1000, 1500, 3000, 6000, 8000, 10000, and 15000 ms) values. Imaging parameters were as follows: field of view (FOV) = 25×25 mm², matrix size (MTX) = 256×256 , number of axial slices = 4, slice thickness (SI) = 1.0 mm, and averages (NEX) = 3. T_1 analysis was carried out using the image sequence analysis tool in Paravision 5.0 pl3 software (Bruker, Billerica, MA) with mono-exponential curve-fitting of image intensities of selected regions of interest (ROIs) for each axial slice.

Spin-spin relaxation times (T_2) were measured using a multi-slice multi-echo (MSME) T_2 -map pulse sequence, with static TR (6000 ms) and 32 fitted echoes in 11 ms intervals (11, 22, ... 352 ms). Imaging parameters were as follows: field of view (FOV) = 25×25 mm², matrix size (MTX) = 256×256 , number of axial slices = 4, slice thickness = 1.0 mm, and averages (NEX) = 3. T_2 analysis was carried out using the image sequence analysis tool in Paravision 5.0 pl3 software (Bruker, Billerica, MA) with mono-exponential curve-fitting of image intensities of selected regions of interest (ROIs) for each axial slice.

X-ray Absorption Fine Structure (XAFS)

XAFS experiments were carried out at the bending magnet beamline (5-BM-D) operated by DND-CAT at the Advanced Photon Source of Argonne National Laboratory (Argonne, IL, USA). The beamline uses a Si(111) monochromator for energy selection. The energy resolution at the Fe K edge (7112 eV) is ~1.0 eV. During the x-ray absorption measurements, the synchrotron storage ring was operated in the "top-up" mode with the electron beam current kept at ~100 mA. The x-ray beam size in the experimental station is selected by two sets of Huber slits that are 2×8 mm². The x-ray energies are calibrated by using Fe and Co standards for the measurements carried out at each K absorption edge, respectively. In addition to these, a series of oxide powder samples, Fe₃O₄, γ -Fe₂O₃, CoFe₂O₄, CoO, and Co₃O₄ were measured in transmission mode at room temperature.

The incident x-ray intensity was monitored by an ion chamber (Oxford Danfysik). A 13-element Ge solid state detector (Canberra) was used to collect the fluorescence emissions of Fe and Co $K\alpha$ to obtain x-ray absorption near edge structure (XANES) and extended x-ray absorption fine structure (EXAFS) spectra at room temperature. The samples were oriented 45° to the incident x-ray and to the 13-element detector. The digital x-ray process electronics (DXP2X) from the x-ray instrument associate (XIA) are used for x-ray pulse processing which provides precise dead-time correction within a given experimental condition. Typically, 5 to 10 energy scans were averaged to obtain sufficiently good data statistics. The XANES spectra allow determination of chemical states of the absorbing atoms and the symmetry of the nearest coordination. The EXAFS spectra reveal the inter-atomic distances (R), the root mean squares of the bond distance spread (or the Debye-Waller factor, σ^2) and the coordination numbers (N) around each absorbing atom.⁴¹

The x-ray absorption data analyses were carried out using the ATHENA software package.⁴⁹ The EXAFS phases and amplitudes were all generated using the FEFF8 code that is embedded in the ATHENA package. The intrinsic loss parameter (S_0^2) for various absorbing atoms, which is taken as a constant (independent of photoelectron kinetic energies), was determined using the reference materials. The EXAFS data of Fe_3O_4 and its crystallographic structure were used to extract the S_0^2 value for the Fe *K* edge data. Fe_3O_4 belongs to the cubic space group $\text{Fd}\bar{3}\text{m}$ with lattice constant $a = 8.391 \text{ \AA}$ and $\alpha = 90^\circ$. The EXAFS data for Co_3O_4 and its crystallographic structure were used to extract the S_0^2 value for the Co *K* edge data. Co_3O_4 belongs to the cubic space group $\text{Fd}\bar{3}\text{m}$ with lattice constant $a = 8.084 \text{ \AA}$ and $\alpha = 90^\circ$.

Reference compounds were used in powder form. Samples were ground to make a fine powder, spread on a piece of Scotch® tape to a suitable, highly uniform density and the tape was layered to increase the optical thickness. Nanoparticle samples were measured as aqueous solutions. The stable solutions were loaded into a polypropylene sample holder sealed with Kapton tape.

Results and Discussion

Ferrite cores, Fe_3O_4 (IO) and CoFe_2O_4 (CoIO), were synthesized using a high-temperature solution decomposition method followed by silica (SiO_2) coating using a base-catalyzed water-in-oil microemulsion approach to produce aqueous dispersed nanoparticles (IO- SiO_2 and CoIO- SiO_2) (Scheme 1). Silica was chosen based on its hydrophilicity and established chemistry for surface modification.^{50–52} The uniform shape and monodispersity of the nanoparticles was confirmed by TEM analysis (Figure 1). The average size of the cores is $7 \pm 1 \text{ nm}$ with SiO_2 shell thickness of approximately 10 nm. In solution, the nanoparticles have hydrodynamic sizes of $70 \pm 9 \text{ nm}$ for IO- SiO_2 and $52 \pm 19 \text{ nm}$ for CoIO- SiO_2 . The SiO_2 -coated nanoparticles exhibit core-shell morphology with long-term colloidal stability in aqueous media. XRD measurements verified the formation of the ferrite phase for both nanoparticle compositions (Figure S1).

Exposing aqueous solutions of the CoIO- SiO_2 nanoparticles (0.33–0.50 Co/Fe ratio) to dialysis produced a *significant* change in the core composition. Elemental analysis of the nanoparticles revealed that 50–75% of the Co ions consistently leach out of the ferrite structure. The core composition reaches equilibrium at approximately 12–16% Co (0.12–0.16 Co/Fe ratio). The high degree of leaching associated with these nanoparticles is surprising given that silica has been shown to be a robust coating for metal-based materials by protecting against degradation and preventing release of toxic metallic species.^{53–55} TEM and DLS analysis showed that the size and distribution of the nanoparticles did not change following the leaching process (Figure 1, Figure S2).

The magnetic susceptibility of the nanoparticles was measured using SQUID magnetometry. Isothermal hysteresis loops were collected at 260 K (Figure 2). The superparamagnetic nature of the nanoparticles at this temperature is reflected in the negligible coercivity and remanence (Figure 2, Table S1). CoIO- SiO_2 nanoparticles exhibited a large increase in saturation magnetization (M_s) between the unleached and leached states (48.4 emu/g core and 64.9 emu/g core, respectively). The M_s of control IO- SiO_2 nanoparticles was 56.9 emu/g of the core.

Considering the influence of leaching on the core magnetization of CoIO- SiO_2 , the relaxivity of the nanoparticles was evaluated at 60 MHz and 37 °C to determine if there was an analogous effect. Prior to leaching, CoIO- SiO_2 and IO- SiO_2 nanoparticles exhibit similar spin-lattice relaxivity (r_1) values ($3.0 \text{ mM}^{-1}\text{s}^{-1} \text{ Fe}$ and $2.7 \text{ mM}^{-1}\text{s}^{-1} \text{ Fe}$, respectively).

However, CoIO-SiO₂ nanoparticles have a higher spin-spin relaxivity (r_2) than IO-SiO₂ (113 mM⁻¹s⁻¹ Fe vs. 82.2 mM⁻¹s⁻¹ Fe). Following leaching, a substantial increase in r_1 is observed for CoIO-SiO₂ (21.5 mM⁻¹s⁻¹ Fe), representing a 7.2-fold improvement. IO-SiO₂ nanoparticles were dialyzed as a control and showed a 1.5-fold higher r_1 post-leaching (4.1 mM⁻¹s⁻¹ Fe). With respect to r_2 , there is only a 1.2-fold increase post-leaching for both CoIO-SiO₂ and IO-SiO₂ (142 mM⁻¹s⁻¹ Fe and 97.6 mM⁻¹s⁻¹ Fe, respectively). In comparison, Ferumoxytol (a commercial nanoparticle) has a relaxivity of 17.5 mM⁻¹s⁻¹ Fe for r_1 and 63.3 mM⁻¹s⁻¹ Fe for r_2 .

The effect of leaching on the relaxivity of CoIO-SiO₂ is partly attributed to the change in M_s . The enhancement in the M_s of the leached nanoparticles, and subsequent increase in relaxivity, renders these nanoparticles more effective MR agents. The contrast enhancement afforded by leaching of CoIO-SiO₂ was detectable by MR imaging at 7 T (298 MHz). The overall magnitude of r_1 decreases at this higher field strength; however, the leached nanoparticles clearly show brighter contrast in T_1 -weighted images compared to the unleached counterparts (Figure 3, Supporting Information). The leached and unleached nanoparticles were not as distinguishable in the T_2 -weighted images.

To determine if the observed changes in magnetic properties following leaching can be correlated to structural changes in the nanoparticle, XAFS was employed. Figure 4a shows the Fe *K* edge XANES of IO-SiO₂ and CoIO-SiO₂ compared to Fe-based reference compounds. The nanoparticles clearly resemble the CoFe₂O₄ and Fe₃O₄ reference oxides. The cation arrangement of these reference bulk oxides is inverse spinel, where Fe³⁺ ions occupy all the tetrahedral (T_d) crystallographic sites and half of the octahedral (O_h) sites, and Fe²⁺ (or Co²⁺) ions occupy the remaining O_h sites. The pre-edge peak at ~7112 eV is due to the $1s \rightarrow 3d$ transition that is only allowed in the absence of inversion symmetry, as in the case of T_d site occupancy. The similarity in the XANES spectra of the nanoparticles to the references Fe₃O₄ and CoFe₂O₄ support that the Fe atoms in IO-SiO₂ and CoIO-SiO₂ favor the inverse spinel arrangement. Importantly, no apparent structural changes in the local environment of the Fe atoms were observed in the nanoparticles following leaching (see Figure S5).

Figure 4b compares the Co *K* edge XANES spectra of unleached and leached CoIO-SiO₂ nanoparticles with reference oxides CoO, Co₃O₄, and CoFe₂O₄. The XANES spectrum of the unleached CoIO-SiO₂ is distinctly different from the other samples, and the existence of a pre-edge peak, though weak, signifies that some of the Co ions are situated in T_d sites. Further, the Co *K* edge data for the unleached sample resembles the Fe *K* edge spectrum (Figure 4a), indicating that the unleached CoIO-SiO₂ nanoparticles have a partially inverted spinel structure (between normal and inverse cation arrangements). For the Co content observed in the unleached nanoparticles (Co/Fe ratio = ~0.5), this arrangement is consistent with literature.³⁷

Upon leaching, changes occur in the local Co structure of the nanoparticles as indicated by the differences in the XANES spectra (Figure 4b). The near edge structure around the Co ions is similar to that in bulk CoFe₂O₄. The negligible pre-edge peak of the leached nanoparticles indicates the Co ions occupy slightly distorted O_h sites. Therefore, following leaching, the decrease in Co content (50–75%) results in a completely inverted spinel arrangement where Co occupies only O_h sites (Figure 4c). The T_d sites are not structurally stable for the Co (2+) ions to reside in and are thus removed by leaching. Based on the extent of cobalt leaching, it is probable that a fraction of the leached ions came from O_h -coordinated sites.

The EXAFS data corroborates the findings from the XANES analysis. As shown in Figure 5a, the EXAFS oscillations measured after the Fe *K* edge for the unleached CoIO-SiO₂ are well-aligned with bulk CoFe₂O₄. The same is true for leached nanoparticles (data not shown). Interestingly, the EXAFS oscillations measured past the Co *K* edge for the unleached CoIO-SiO₂ have greater similarity to the Fe *K* edge EXAFS than to the Co *K* edge of bulk CoFe₂O₄, supporting occupancy of some Co ions in *T_d* sites. Fourier transforms of the EXAFS data validate that the average local structure around the Co ions closely resembles that of the Fe ions (Figure 5c, solid lines). In the leached nanoparticles, the Co *K* edge EXAFS and Fourier transform are essentially identical to that of the CoFe₂O₄ reference, confirming *O_h* occupancy of the Co ions in the inverse spinel arrangement (Figure 5b,d).

Quantitative analysis of the EXAFS data using the theoretical phases and amplitudes based on the inverse spinel Fe₃O₄ accurately simulate both Fe and Co *K* edge EXAFS spectra for the unleached CoIO-SiO₂ sample (Figure 5c, dashed lines). The Fe-O bond distances are 1.85 Å (*T_d* site) and 1.99 Å (*O_h* site), comparing favorably to 1.86 Å and 2.03 Å found in the bulk Fe₃O₄ reference measured in this work. In the unleached nanoparticles, the Co-O bond distances are indistinguishable for *T_d* and *O_h* sites (1.94 Å and 1.95 Å, respectively). For the leached sample, the theoretical simulation yields a Co-O bond distance of 2.04 Å for the nanoparticles versus 2.03 Å for bulk CoFe₂O₄ (Figure 5d, dashed line). The shorter Co-O bond distances in the unleached nanoparticles are consistent with a partially inverted structure, whereas the leached nanoparticles are representative of a pure inverse arrangement.^{40,56}

The structural changes in cation distribution and degree of inversion resulting from leaching account for the changes in magnetic behavior observed here. According to the Néel model, magnetic ferrites, such as Fe₃O₄ and CoFe₂O₄, are ferrimagnetic and the alignment of their dipoles is governed by superexchange interaction.¹⁸ The cations in *T_d* sites form one sublattice with their spins oriented in one direction, while the cations in *O_h* sites form a second sublattice with oppositely oriented spins. In these inverted spinels, the trivalent atoms in the *T_d* sublattice cancel those in the *O_h* sublattice, and the net magnetic moment is governed by the nature of the divalent atoms in the *O_h* sites. A partially inverted arrangement complicates the situation since divalent atoms are present in both types of crystallographic sites. Here, the increased *M_s* after leaching is due in part to the removal of the *T_d* site Co²⁺ that had previously compensated magnetically for some of the Co²⁺ in the *O_h* sublattice.

The leaching of Co ions creates vacancies in the nanoparticle core structure. As evident from the relaxometry data, this has a more substantial effect on *r*₁ as compared to *r*₂. For SPM nanoparticle contrast agents, outer-sphere interactions (diffusing bulk water in the local environment) are important in the *T*₁ relaxation process, and changes in surface morphology can influence the interactions of bulk water with the nanoparticle, strongly affecting relaxivity.^{22,57} The vacancies created during the leaching process may distort the overall spinel structure, altering not only the magnetic properties but the surface properties of the nanoparticle as well.^{40,58} It is likely that the metal cations nearest the core surface are removed by leaching, which results in an increase in the surface area available to interact with water molecules. Because the porous silica coating remains intact (Figure 1), we speculate that the large enhancement in *r*₁ is from these vacancies in the core yielding greater accessibility of water to the surface.

Conclusions

The importance of investigating the chemical stability of nanoparticle composites was illustrated by a comprehensive characterization of the magnetic and structural properties of SiO₂-coated ferrite core-shell nanoparticles. Core-shell nanoparticles were successfully synthesized and exhibited long-term colloidal stability. The nanoparticles presented here were synthesized and purified with methods commonly employed in the making of nanoparticle-based MR contrast agents. It was observed, however, that spontaneous leaching of Co ions from the nanoparticle core occurred under aqueous conditions without the addition of other reactants or changes in pH. We have shown that leaching directly affects the saturation magnetization and relaxivity of CoIO-SiO₂ nanoparticles due to changes in local structure and cation distribution of the nanoparticle core. The leaching of cobalt ions resulted in an improvement in saturation magnetization and longitudinal relaxivity of the CoIO-SiO₂ nanoparticles. While a favorable effect, the significant differences in the magnetic behavior of the unleached and leached nanoparticles demonstrate how essential it is to carefully characterize such nanomaterials in solution. Further investigation is necessary to fully understand the driving force and mechanism of the leaching process. Additionally, there is still much that is unknown about nanomaterials and how they behave in vivo, which should not be further complicated by a lack of understanding of the properties of the agent itself. If ferrites and other nanomaterials of mixed composition are to become viable, efficient MRI contrast agents, then their chemical composition and magnetic properties must be maintained prior to biological studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

TEM images of IO-SiO₂ (left), unleached CoIO-SiO₂ (center), and leached CoIO-SiO₂ (right) nanoparticles. Note the uniform coating around the nanoparticle core, which is retained following the leaching process.

Figure 2. Hysteresis loops at 260 K measured using SQUID magnetometry. The curves show the superparamagnetic behavior and M_s of the core-shell nanoparticles: IO-SiO₂ (blue), unleached CoIO-SiO₂ (black), and leached CoIO-SiO₂ (red).

Figure 3.

MR images of solution phantoms of unleached and leached CoIO-SiO₂ nanoparticles at different iron concentrations (0.031 mM Fe to 0.25 mM Fe) at 7 T and 25 °C. T_1 -weighted (RARE- T_1 map) and T_2 -weighted (MSME- T_2 map) images were acquired using the following parameters: FOV = 25 × 25 mm², matrix = 256 × 256, axial slices = 4, slice thickness = 1.0 mm, averages = 3.

Figure 4.

Fe K edge (a) and Co K edge (b) XANES spectra for core-shell nanoparticles (IO-SiO_2 , unleached and leached CoIO-SiO_2) and reference oxides (Fe_2O_3 , Fe_3O_4 , CoFe_2O_4 , CoO , and Co_3O_4). (c) Crystallographic model of lattice changes imposed by leaching. Structural analysis revealed that the leaching process removed T_d -coordinated Co ions from the nanoparticle core, resulting in a transition from a partially inverted structure (unleached) to an inverse spinel structure (leached).

Figure 5.

EXAFS spectra (a, b) and Fourier transforms (c, d) of the unleached (red) and leached (green) CoIO-SiO_2 nanoparticles. The EXAFS spectra of the reference CoFe_2O_4 (blue) are presented for comparison. The dashed lines are the fitting results obtained using the phases and amplitudes generated by the FEFF code based the inverse spinel Fe_3O_4 structure. The unleached data are well described by a mixed occupancy model [red dashes in (c)], while the leached data are fitted by an O_h -occupancy model [green dashes in (d)].

Scheme 1.

Synthesis of SiO_2 -coated iron oxide (IO-SiO_2) and cobalt ferrite (CoIO-SiO_2) core-shell nanoparticles. The red coating represents organic-capping ligands (oleylamine and oleic acid) on the core surface.