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Superparamagnetic nanosystems based on iron oxide nanoparticles for biomedical imaging

Magnetic iron oxide nanoparticles and their dispersion in various mediums are of wide interest for their biomedical applications and physicochemical properties. MFe_2O_4 or $MOFe_2O_3$ (where $M = Co, Li, Ni$ or Mn , for example) can be molecularly engineered to provide a wide range of magnetic properties. In this article, we survey the literature, integrating the results of our work to give a rational view on the synthesis, physicochemical properties and applications of MFe_2O_4 , especially for MRI. However, retrieving detailed biological information on a subcellular level is difficult, owing to the limited resolution and low sensitivity of the MRI technique. Thus, this article also concentrates on the development of a magnetic iron oxide nanoparticles/quantum dot hybrids, as a dual-mode magnetic-fluorescent probe. The synthesis and physicochemical properties of the magnetic iron oxide nanoparticles/quantum dot hybrids and, especially, its application as an MRI-fluorescent probe, will also be described.

KEYWORDS: dual-mode probe ■ iron oxide nanoparticle ■ magnetic-fluorescent ■ MFe_2O_4 ■ quantum dot

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Nanoparticles (NPs) have prompted increasing attention from researchers from different disciplines during the past decade. The term 'nanoparticle' is generally used to specify particles with a size range between 1 and 100 nm, where the physical, chemical and biological properties are significantly enhanced or absolutely different from those of the corresponding bulk materials [1]. Therefore, many industries and manufacturers are now introducing NP systems in their mainstream products so as to exploit these new capabilities.

The design of this new kind of material focuses on the control of properties of NPs, including chemical (ratio between compositions, hydrophilicity/hydrophobicity and surface charge) and/or structural (crystalline/amorphous, size and morphology) characteristics. With the booming development of supermolecular, biomolecular and dendrimer chemistries, engineering substances on the nano- and submicron-scale dimensions has been the focus of interest in recent research. The emerging disciplines of nanoengineering, nanoelectronics and nanobioelectronics require suitably sized functional NPs as building blocks, to construct complex architectures and devices [2–5].

Magnetic iron oxide NPs (MIONPs) are widely studied for their potential applications in several fields, especially in biomedicine for MRI [6,7], cell labeling [8,9] and drug delivery [10,11]. Of special interest are their magnetic properties in which the difference between a

massive or bulk material and a nanoscale one is especially pronounced. Furthermore, it has been reported that the magnetization and the magnetic anisotropy of MIONPs were much greater than those of their bulk analogs, and the difference in Curie and Néel temperatures between MIONPs and the corresponding microscopic phases could be as large as hundreds of degrees [12]. The magnetic properties of MIONPs are determined by many factors, including the chemical composition, the type and degree of defectiveness of the crystal lattice, the particle's size and shape, the morphology (for NPs with inhomogeneous structures), and the interaction of the particle with the matrix and surrounding particles. A wide variety of MIONPs have been synthesized with different processes. They differ in hydrodynamic particle sizes, varying between 10 and 200 nm [13]. However, the other properties cannot be well controlled during the synthesis of the MIONPs nearly equal in size and chemical composition, so the magnetic properties of MIONPs with the same type may be markedly different. Moreover, MIONPs have been found to possess a number of unusual properties, including giant magnetoresistance and abnormally high magnetocaloric effect.

Among the different kinds of MIONPs, magnetite (Fe_3O_4) is the most widely studied for its high saturation magnetization (M_s) [14–16]. However, the Fe^{2+} ions in octahedral sites of the inverse spinel structure (B-sites) are very apt to be oxidized to Fe^{3+} ions in air atmosphere,

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which is not desirable for the applications [17]. To solve this problem, one option is to coat the Fe_3O_4 NPs with a protective shell, using, for example, polymers (including synthetic ones, such as poly[ethylene glycol], and natural ones, such as dextran or starch), albumin or silicones [18–21]. The other solution is to use stable divalent metal ions instead of Fe^{2+} to synthesize MFe_2O_4 or MOFe_2O_3 compounds (where, for example, $\text{M} = \text{Co}, \text{Li}, \text{Ni}$ or Mn), which show a perfect resistance to oxidation. The cubic spinel structured MFe_2O_4 represents a well known and important class of iron oxide materials, in which oxygen forms a face-centered cubic (fcc) close packing, and M^{2+} and half of Fe^{3+} ions occupy the octahedral interstitial sites, while the other half occupy the tetrahedral sites. By adjusting the chemical identity of M^{2+} , the magnetic configurations of MFe_2O_4 can be molecularly engineered to provide a wide range of magnetic properties [22], making it a potential candidate for contrast agents in MRI.

MRI is a noninvasive technique that allows the characterization of pathological tissues and lesions. The image results from the spatial registration of hydrogen nuclei. As the signal of MRI depends on the concentration of protons and the nuclear relaxation time, including the spin lattice or longitudinal relaxation time (T_1) and the spin–spin or transverse relaxation time (T_2), the image contrast can be varied by modifying the instrumental or ‘extrinsic factors’ of the imaging process (i.e., the parameters of the pulse acquisition sequence), or by taking advantage of the ‘intrinsic’ parameters (e.g., the local concentration of the nuclei, their relaxation parameters, their relative bulk magnetic susceptibility) [23]. In addition to utilizing these extrinsic and intrinsic factors, it is possible to further improve contrast by exogenously administered contrast agents. In this article, some works on the synthesis and application of MFe_2O_4 as an MRI contrast agent will be described.

However, retrieving detailed biological information on a subcellular level is difficult, owing to the limited resolution and low sensitivity of the MRI technique [24]. Quantum dots (QDs) show superior optical properties and have potential applications in basic and applied biology. Compared to that of conventional fluorescence dyes, the sensitivity of QDs is several times higher as a result of a large Stoke’s shift (wavelength separation between the absorbance and emission maxima), where the vastly separated absorption and emission peaks increase sensitivity by reducing autofluorescence [25,26]. In

ultrasensitive detection experiments, a narrow-band pass filter centered on the emission peak can be employed to eliminate noise. Since the signal-to-noise ratio is already quite high, the sensitivity is not greatly affected. Thus, a single excitation light source coupled with a single filter set would greatly simplify the instrumentation. This article will also concentrate on the development of a MIONP/QD hybrid, as a dual-mode magnetic-fluorescent probe. The synthesis and physicochemical properties of this MIONP/QD hybrid will be described in detail, as well as its application as an MRI-fluorescent (Fluo) probe.

Strategies for the synthesis of MIONPs

In order to use MFe_2O_4 NPs in the future as a highly sensitive contrast agent for MRI, a practical route to monodisperse MFe_2O_4 NPs with diameters smaller than 20 nm and a tight size distribution (less than 10% standard deviation) are needed. From a chemist’s point of view, the preparation in their bulk form is a simple task; however, a somewhat more challenging aspect relates to phase purity, crystal structure and morphology, which are responsible for better performance in MRI applications [27]. This brings a new degree of complexity when synthesizing the crystal at a nanometer scale.

In order to prepare MFe_2O_4 NPs with uniform size and shape, it is necessary to control the kinetics of their nucleation, growth and coarsening. The best control is achieved when all three steps are separated in time, which means that the nucleation must be finished when the growth of nuclei begins, and this growth must be finished before the coarsening. It is easier to reach such levels of control in homogeneous solution systems, rather than in heterogeneous ones. Thus, the strategies of synthesis will be mainly separated into two classes: reactions in water and reactions in organic phase.

A commonly used water-phase procedure for making such particles has been the coprecipitation of M^{2+} and Fe^{3+} ions by a base, usually NaOH or NH_4OH , in an aqueous solution [28–30], or in a reverse micelle template [31,32]. The best example is the formation of spinel-structured ternary oxides via coprecipitation of M^{2+} and Fe^{3+} ions present in reaction solution with a 2:1 molar ratio. MnFe_2O_4 NPs of 5–25 nm have been similarly prepared from aqueous Mn^{2+} and Fe^{3+} at temperatures up to 100°C [33]. Variations in size and shape of the NPs have been observed under conditions of strictly controlled acidity and ionic strength in aqueous

solutions containing no complexing agents, the changes of which will influence the electrostatic surface-charge density of the MnFe_2O_4 NPs, the interfacial tension and, consequently, their surface energy. Nanocrystalline NiFe_2O_4 , CuFe_2O_4 and ZnFe_2O_4 have been synthesized by rapid addition of aqueous solutions of metal salts into 1 M NaOH solution (preheated to 60–100°C) under vigorous stirring [34–36]. The obtained solids were used for the preparation of electric double-layer stabilized aqueous colloids. CoFe_2O_4 NPs with average particle size varying in the range of 2–14 nm have been obtained by controlling coprecipitation temperature [37]. As the precipitation temperature increased in the range of 20 to 80°C, the average particle size also increased from 2 to 15 nm, with a considerable change in x-ray diffraction crystallinity. The CoFe_2O_4 NPs prepared at temperatures below 40°C showed superparamagnetic relaxation at room temperature with blocking temperatures between 75 and 200 K, but the samples prepared at temperatures higher than 60°C consisted of both superparamagnetic and ferrimagnetic NPs that resulted in magnetic coercivity at room temperature. Mössbauer spectra of the samples also confirmed their magnetic properties and wide size distribution.

Although this coprecipitation method is suitable for mass production of MFe_2O_4 ferrofluids, it does require careful adjustment of the pH value of the solution for particle formation and stabilization [38], and makes it difficult to control sizes and size distributions, particularly

for particles smaller than 20 nm. An alternative approach to synthesize monodisperse iron oxide NPs (MIONPs) is via high-temperature organic-phase decomposition of a metal precursor, such as metal acetylacetonates or metal carbonyls, in high boiling point solvents. In combination with the use of surfactants, such as oleic acid and oleylamine, this procedure leads to the formation of monodisperse ferrite NPs with good crystallinity and uniform size, such as the decomposition of an intimately mixed binary metal–oleate complex, where metal included Fe and M (M = Co, Ni and Mn) [39–43]. Generally, there are two representative synthetic procedures. In the first one (as shown in FIGURE 1), the reagents (binary metal–organic complex) were rapidly injected into the preheated solution containing surfactants, which induced the simultaneous formation of many nuclei [44]. In the second procedure (as shown in FIGURE 2), all the reagents including the solvent were mixed at a low temperature, followed by slowly heating the resulting mixture in a controlled manner to generate nuclei. Subsequently, there were two ways leading to particle growth; namely, by the addition of reactive species and by aging at a high temperature through Oswald ripening, where smaller particles dissolve and deposit on the bigger particles. For the rapid-injection process, the most frequently applied size-selection process should be the addition of a poor solvent to precipitate the larger NPs, which is caused by the flocculation caused by Van der Waals, followed by centrifugation. During the process of

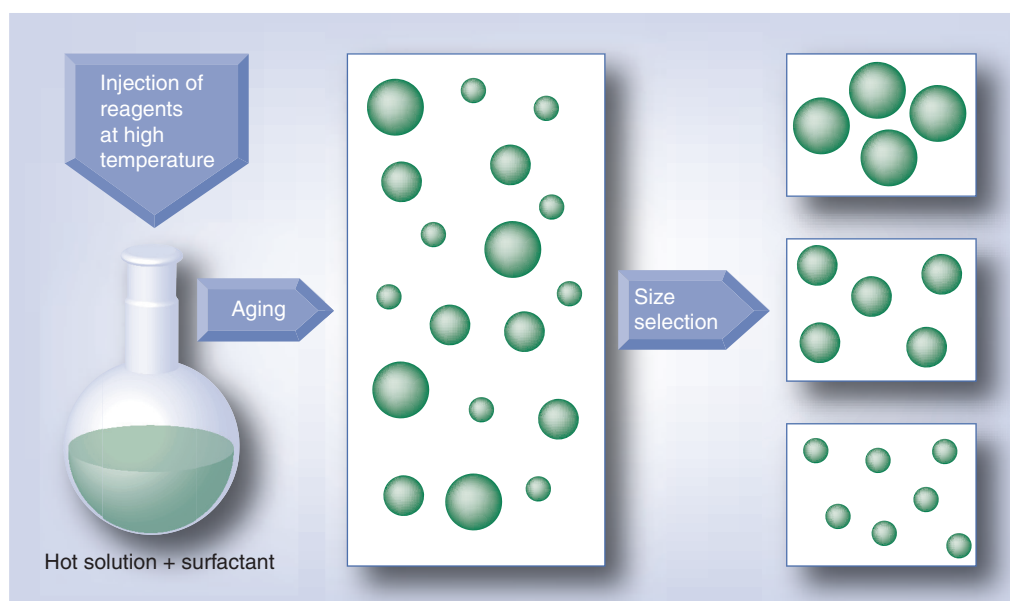


Figure 1. Synthesis of monodisperse MFe_2O_4 nanoparticles by injection of reagents into hot surfactant solution followed by size-selection process.

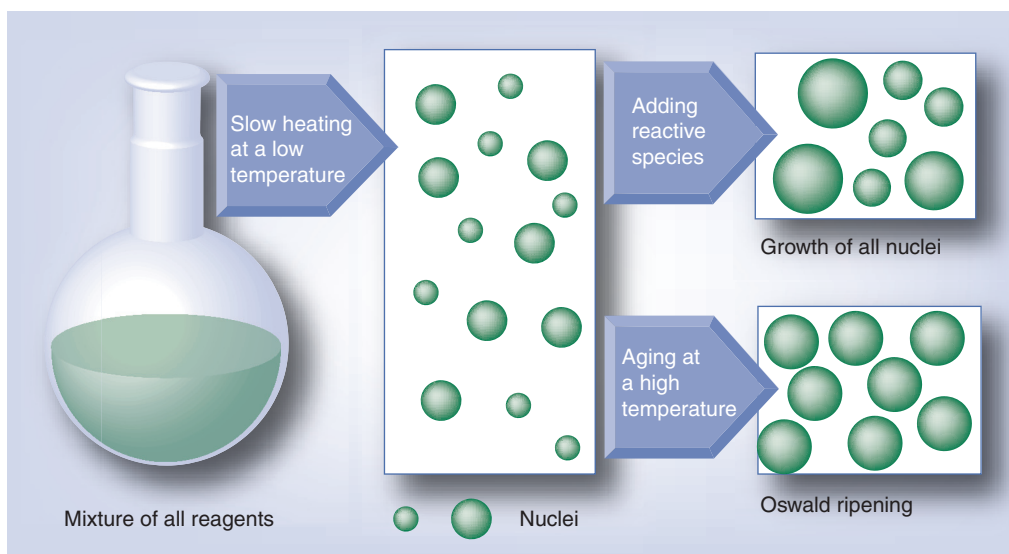


Figure 2. Synthesis of monodisperse MFe_2O_4 nanoparticles by programmed heating of the reagents.

'programmed heating', the growth of MFe_2O_4 NPs can be stopped by rapid decrease of the reaction temperature. The pre-mixing of all reagents followed by slow heating would perfect the size distribution of obtained NPs with standard deviation σ is approximately 5% [45].

The sizes of MIONPs can be controlled by systematically adjusting the reaction parameters, including time, temperature and the concentrations of reagents and stabilizing surfactants. In most cases, particle size increases with reaction time (as more monomeric species are generated) and reaction temperature (as the rate of reaction is increased).

Stabilizing the prepared MIONPs

Since metals and metal oxides are insoluble in the solvents used for their synthesis, it is necessary to adopt methods to stabilize the obtained MIONPs. Generally, there are two universal methods to achieve this purpose: using complexing agents and changing the surface potential.

As well as providing a link between the particle and the target site on a cell or molecule, a complexing agent has the advantage of increasing the stability of the magnetic colloid, whose functions are to coordinate both to metal ions or atoms that were just formed in solution as the reaction intermediates and to metal atoms on the surface of the growing crystal. Oleic acid and oleylamine were used in the high-temperature solution-phase reaction of iron(III) acetylacetonate, $\text{Fe}(\text{acac})_3$, with 1,2-hexadecanediol reported by Sun [22]. During the reaction, oleic acid and oleylamine are necessary

for the formation of MIONPs. The use of oleic acid resulted in a viscous red-brown product, which was difficult to purify and separate. On the other hand, the use of oleylamine alone produced MIONPs in a much lower yield than the reaction in the presence of both oleic acid and oleylamine, which suggested that the NH_2 group coordinated with $\text{Fe}(\text{III})$ on the surface of the particles.

These complexing agents, from the viewpoint of coordination chemistry, are classical ligands that act both as the spacer surrounding NPs to prevent the agglomeration and as surfactant to adjust the affinity of nanocrystals to the medium. Depending on the structure of the complexing agent, capping ligands, dendrimers or coordinating polymers can be distinguished. Capping ligands are discrete molecules containing a chain or branched-chain substituent; dendrimers are highly branched structures formed by condensation of a well-defined number of the monomer molecules [46,47], and coordinating polymers are macromolecules with the donor groups repeatedly attached to them along the chain.

As mentioned, the stability of MIONPs can also be achieved by changing the surface potential. In the 1980s, Massart proposed the chemical synthesis of aqueous ferrofluids with no surfactant [48,49], making H^+ (or OH^-) ions to be adsorbed onto the surface of the particles, which introduces an electric repulsive force between particles and forms a stable water-based ionic MIONPs colloid. However, with Massart's method for preparing acid ionic ferrofluids of cobalt ferrite (CoFe_2O_4), the obtained CoFe_2O_4

NPs must be heated in a boiling $\text{Fe}(\text{NO}_3)_3$ solution to make the particles stable as colloid, but this stability would be progressively destroyed in an acidic medium and the colloid would flocculate [50]. Therefore, a new method for preparing water-based ionic colloids by treating the MFe_2O_4 NPs in nitric acid was developed [38]. The acid treatment turned the surfaces of both the aggregated and the single particles into viscous layers different from the internal MFe_2O_4 in construction, which had voids. The surface layers prevented the decomposition of the internal MFe_2O_4 and made both NPs charged by adsorbing H^+ ions, which stabilized the obtained MFe_2O_4 colloid.

Synthesis of QD/ MFe_2O_4 bifunctional materials

Colloidal semiconductor nanocrystals or QDs are of great interest to scientists for their remarkable optical properties (sharp emission and broad absorption), which are governed by the quantum confinement effect. The application of QDs and MIONPs in biolabeling [51] and MRI, respectively, have recently undergone a major development in the fields of biological and biomedical imaging. A combination of optical and magnetic properties in a single material would enable simultaneous biolabeling/imaging and cell sorting/separation [52,53], integrating the advantages of high sensitivity from fluorescence imaging and high spatial and temporal resolution and deep tissue penetration from MRI.

Generally, two strategies have been utilized to prepare this hybrid material: one is to synthesize *in situ* the second component on the surface of the first one, and the other is to conjugate them with a linker or by packing them with a coating material.

Through the *in situ* growth method, core-shell structured MIONPs/QDs or QDs/MIONPs composites are always obtained. However, because of the lattice mismatch of the two components, it is difficult for them to coat each other directly. Alternately, a coating technology has been used to construct core-shell nanostructures, in which an amorphous layer (generally utilizing SiO_2) is deposited in advance on the surface of the core material, and then the shell substance can easily crystallize on the formed amorphous SiO_2 layer. However, the coverage area has been found not to be large enough to form a continuous layer when a single coating is performed. Halas and coworkers found that the coverage area ratio would not exceed 25% only by the electrostatic interaction

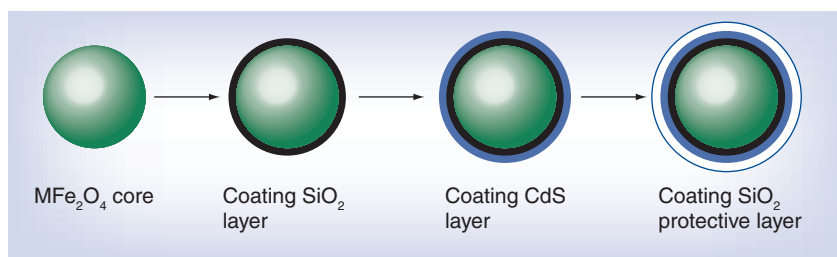


Figure 3. Synthesis of $\text{MFe}_2\text{O}_4/\text{CdS}$ nanoparticles, with SiO_2 as the spacer.

for the Au/SiO_2 system [54]. Therefore, layer-by-layer and seed-growth techniques were used to increase the coverage area and thickness of the shell. Several repetitions of the coating step are needed to achieve a desired thickness, as is the case for $\text{CoFe}_2\text{O}_4/\text{SiO}_2$ [55], $\text{MnFe}_2\text{O}_4/\text{SiO}_2$ and $\text{NiFe}_2\text{O}_4/\text{SiO}_2$ nanocomposites [56]. A schematic representation of this process is shown in Figure 3. The preparation of this magnetic luminescent hetero-nanosystem with core/shell structure involved the coating with SiO_2 and QDs on MFe_2O_4 NPs, respectively. In Chen's synthesis of cadmium sulfide-coated magnetite [57], denoted as $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{CdS}$, spherical Fe_3O_4 NPs were firstly synthesized via a modified solvothermal method, as reported by Li [58]. To overcome the difficulties of directly grafting active groups or bonding foreign molecules on to the surface of the prepared Fe_3O_4 NPs, a thin SiO_2 transition layer (amorphous) was coated on the magnetic core via a modified Stöber process, in isopropanol and water media, to change the surface characteristics and to increase the stability and oxidation resistance, as illustrated in Figure 3.

As a proof of feasibility for depositing amorphous material on the surface of the obtained crystalline core, Xu synthesized heterodimer $\text{FePt}-\text{CdS}$, where amorphous CdS was deposited on the surface of FePt NPs to form a metastable core-shell structure, followed by the transformation of CdS into a crystalline state upon heating [59], as illustrated in Figure 4. Given the

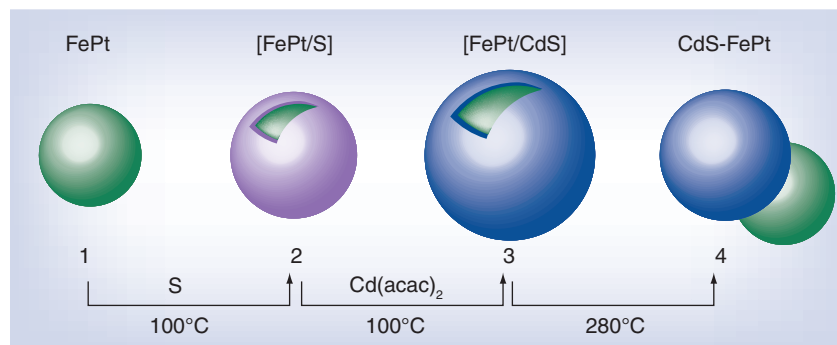


Figure 4. Synthesis of heterodimers of $\text{FePt}-\text{CdS}$ nanoparticles. For further details see [59].

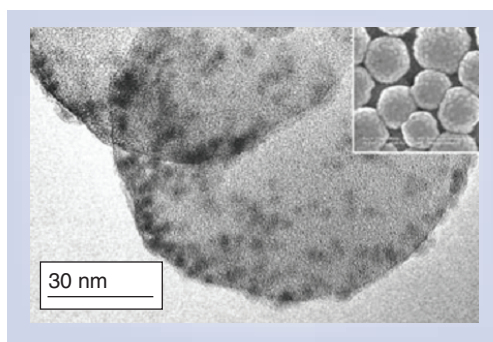


Figure 5. Transmission electron microscopy image of nanospheres embedded with both CdSe/ZnS quantum dots and Fe_2O_3 nanoparticles. The inset is a scanning electron microscope image of $\text{H}_2\text{N-St-AAm}$ nanospheres. Reproduced with permission from [60].

incompatibility of the FePt and CdS lattices and the surface tension when they are dispersed in solution, the FePt/CdS core-shell NPs evolve into heterodimers of CdS and FePt nanocrystals at 280°C , which have sizes of less than 10 nm and exhibit both superparamagnetism and fluorescence. This proves that only amorphous material can be equably deposited on the surface of a crystalline core and is the reason why an amorphous SiO_2 layer is necessary before the loading of QDs. The other strategy for integrating QDs and MIONPs is to synthesize the two materials separately and then conjugate them together by linking or packing, which is rarely reported. Xie and coworkers reported the synthesis of cell-targeting Fe_2O_3 -CdSe/ZnS multifunctional nanospheres, where hydrazinized styrene/acrylamide ($\text{H}_2\text{N-St-AAm}$) copolymer was used as the main material to build the framework [60]. In order to ensure that both

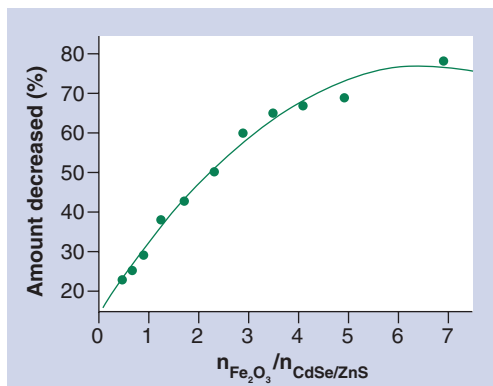


Figure 6. Influence of ratio between two components on the fluorescence intensity of CdSe/ZnS quantum dots (with the diameters of Fe_2O_3 nanoparticles and quantum dots being 8 and 5 nm, respectively). Reproduced with permission from [60].

the QDs and magnetic NPs are embedded in the polymer nanosphere, the particles must be small and well dispersed in identical solvent. As the hybrid nanospheres are synthesized in an aqueous solution, the hydrophilic groups of the polymer tend to be located toward the outer surface of the nanospheres, and the hydrophobic moieties are found at the interior, leading to the formation of hydrophobic hollow cavities. Thus, hydrophobic QDs (3–6 nm) and Fe_2O_3 NPs (5–20 nm) were embedded in a weakly polar organic solvent, as shown in FIGURE 5. Utilizing a human cervical carcinoma cell line (HeLa cells), a human breast cancer cell line (MCF-7 cells), and a diploid human lung fibroblast cell line (MRC-5 cells) as the targeted cells, fluorescent microscopic images of the obtained multifunctional nanospheres showed that the product has perfect representation. However, it was found that the existence of magnetic NPs had some influence on the fluorescence intensity of QDs, which changed with the particle diameter of the QDs and the relative dosages of both kinds of NPs (FIGURE 6). A similar result was also reported for Li and Xue's satellite-like MIONPs/ SiO_2 -QD/ SiO_2 nanosystems [61,62].

Applications of MIONPs in biomedical imaging

The main application of MIONPs has been reported without doubt as a T_2 contrast agent for MRI [47,63–65]. This kind of contrast enhancement, which is caused by dipolar coupling interactions between the synthesized MIONPs and protons in the bulk water, was found to be related to the saturation magnetization and initial susceptibility of the MIONPs, which in turn are related to their anisotropy energies [66]. The magnetic properties and relaxivities for different MFe_2O_4 ($\text{M} = \text{Mn, Fe or Co}$) are given in TABLE 1. To develop an efficient MRI contrast agent with strong signal, the magnetic properties of MIONPs need to be optimized. As shown in TABLE 1, the relaxivities of MnFe_2O_4 NPs were found to be mainly dependent on the initial susceptibility and anisotropy energy, as described previously. Normally, MnFe_2O_4 NPs showed ferromagnetic uncompensated magnetic moments between octahedral and tetrahedral sites. The divalent metal ions M^{2+} (including Mn^{2+} , Fe^{2+} , Ni^{2+} and Co^{2+}) occupied octahedral or partially tetrahedral sites, leading to different magnetic behaviors. For example, Mn^{2+} ions in MnFe_2O_4 NPs occupy both octahedral and tetrahedral sites, so the antiparallel magnetic moment for highest magnetization is reduced, leading to

Table 1. Magnetic properties of synthesized magnetic iron oxide nanoparticles, and their relaxivities measured using a 9.4 T MRI system at 37°C.

Magnetic iron oxide nanoparticle	Size (nm)	χ_i (emu/g ^{oe})	Ms (emu/g)	Ea (J/cm ³)	r_1 (s ⁻¹ mM ⁻¹)	r_2 (s ⁻¹ mM ⁻¹)	r_2/r_1	Ref.
MnFe ₂ O ₄	7.6 ± 1.4	0.042	53.1	0.056	18.6	227.6	12.2	[72]
Fe ₃ O ₄	6.0 ± 0.7	0.054	50.3	0.078	11.1	255.9	23.1	[73]
CoFe ₂ O ₄	8.0 ± 1.0	0.081	65.3	0.22	6.3	392.5	62.3	[74]

χ_i , Ms, Ea and $r_{1(2)}$ represent initial susceptibility, saturation magnetization, anisotropy energy and $T_{1(2)}$ relaxivity, respectively.

an r_2 relaxivity of 227.6 s⁻¹mM⁻¹, smaller than others in TABLE 1. However, CoFe₂O₄ NPs showed the highest saturation and r_2 relaxivity compared with other MFe₂O₄ NPs in the size range of 6 to 8 nm. Reports on different biomedical applications of MFe₂O₄ are listed in TABLE 2.

Magnetic iron oxide nanoparticles/QDs can be utilized as a dual-mode probe in medical imaging, as an integrated contrast agent for fluorescence imaging and MRI. There are limited reports on this work because of the lattice mismatch and quenching effect mentioned previously. The most widely studied Fluo-MRI probe has been the hybrid of MIONPs and organic dye [64–68], although organic dyes suffer the major drawback of photobleaching. Therefore, the development of new MIONPs/QDs dual-mode probes remains a challenge in the medical imaging field.

Future perspective

The synthesized MIONPs show an excellent T_2 contrast effect that potentially would be useful for MRI diagnosis and prognosis of cancer and other diseases. As the Fe²⁺ ions in Fe₃O₄ are apt to be oxidized to Fe³⁺ ions in air atmosphere, which causes a subsequent loss of magnetic properties [17], only by maintaining Fe₃O₄ NPs in an inert atmosphere or a protective layer can the product possess an extended lifetime. However, MFe₂O₄ (M = Mn, Ni and Co) NPs represent a better resistance to oxidation, because the standard electrode potential values of M³⁺/M²⁺ (M=Mn, Ni, Co) are obviously larger than that of Fe³⁺/Fe²⁺, for example E° of Co³⁺ + e⁻ ⇌ Co²⁺ is +1.82 v while that of Fe³⁺ + e⁻ ⇌ Fe²⁺ is +0.77 v. So the good

resistance to oxidation of MFe₂O₄ (M = Mn, Ni or Co) NPs allows a longer storage life of these contrast agents.

As a noninvasive technique, MRI allows the characterization of pathological tissues and lesions. However, it must be remembered that MRI of MFe₂O₄ NPs lack detection sensitivity, which is approximately 1 mM, at best, under practical conditions [68–71], while most pathological biomarkers, such as cell receptors, are available at 1–100 nM concentrations. By integration of QDs, the obtained dual-mode probe would have a higher sensitivity. For a specific targeting of pathological biomarkers, some molecular vectors (recognizing molecules) should be grafted on the surface of the MIONP–QD nanospheres with which to obtain a multimode probe. The molecular vectors should be specific ligands for targeted receptors, including antibodies, peptides, polysaccharides, aptamers and synthetic mimetics. With the fast development of gene technology, several laboratories are starting to use DNA-based peptides as molecular vectors, which includes the insertion of a foreign DNA segment in the structural gene of a bacteriophage. To date, it has yet to be achieved to use MRI contrast agents to routinely target *in vivo* cell receptors at physiologically relevant concentrations (i.e., not overstimulated with growth factors or hormones). For comparison, targeting *in vitro* matrix materials at higher concentration has been achieved, although extracellular matrix materials, such as fibrin or fibrinogen, do not always have the specificity for pathological assessments.

However, the released M²⁺ ions are toxic to surrounding cells and tissues, and the SiO₂-coating layer cannot stop this release because of

Table 2. Different applications of MFe₂O₄ nanoparticles in biomedicine.

Applications	Samples
Hyperthermic agent	CoFe ₂ O ₄ [75–79], MnFe ₂ O ₄ [80–82] and NiFe ₂ O ₄ [76,83,84]
MRI	CoFe ₂ O ₄ [47,63], MnFe ₂ O ₄ [64] and (Zn _{0.4} Mn _{0.6}) Fe ₂ O ₄ [65]
Drug (or gene) delivery	MnFe ₂ O ₄ [82,85,86], CoFe ₂ O ₄ [86,87] and NiFe ₂ O ₄ [88]
Bioseparations	CoFe ₂ O ₄ [89,90], MnFe ₂ O ₄ [91] and NiFe ₂ O ₄ [92–94]

its amorphous structure. Therefore, special attention should be paid to the investigation of the toxicology of such hybrid materials.

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Executive summary

- Several methods for synthesizing MFe_2O_4 have been described; these can be mainly divided into two kinds: coprecipitation of M^{2+} and Fe^{3+} ions by a base coprecipitation method, which is suitable for mass production of MFe_2O_4 , but entails the difficulty of controlling sizes and size distributions; and high-temperature organic-phase decomposition of a metal precursor, with which particle sizes can be controlled.
- Two universal methods, use of complexing agents and changing the surface potential, can stabilize the obtained MFe_2O_4 nanoparticles.
- To prepare magnetic iron oxide nanoparticle (MIONP)–quantum dots there are, generally, two strategies: *in situ* synthesis and conjugating by linking or packing.
- The application of MIONPs as a T_2 contrast agent for MRI depends on the magnetic properties of the MIONPs.

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