MAGNETIC NANOPARTICLES FOR DRUG DELIVERY

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ABSTRACT

Nanoparticles can be defined as any particle that has at least one dimension in the nanometer scale – that is in the region of billionths of a meter. These materials differ from molecular or bulk species with their high surface areas and unique optical, magnetic, and electronic properties. The potential of magnetic Nanoparticles stems from the intrinsic properties of their magnetic cores combined with their drug loading capability and the biochemical properties that can be bestowed on them by means of a suitable coating. Here we review the problems and recent advances in the development of magnetic Nanoparticles for drug delivery, focusing particularly on the materials involved. Nanoparticles are submicron moieties (between 1 nm and 100 nm, although there are examples of Nanoparticles several hundreds of nanometers in size) made of inorganic or organic (e.g. polymeric) materials, which may or may not be biodegradable. Their importance relates to the fact that the characteristics of Nanoparticles are different from those of bulk materials of the same composition, which is mainly because of size effects, the magnetic and electronic properties, and the role played by surface phenomena as the size is reduced. Preparation methods for Nanoparticles generally fall into the category of so-called ‘bottom-up’ methods, where nonmaterial’s are fabricated from atoms or molecules in a controlled manner that is thermodynamically regulated by means such as self-assembly. Some biomedical applications require core-shell magnetic Nanoparticles.

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INTRODUCTION

The concept of magnetic drug targeting (MDT) has been around for over 30 years and simply entails retaining specially designed magnetic drug carrier particles (MDCPs) at a septic site in the body using an externally applied magnetic field which was first used in early 1940s as a new methodology in waste water treatment. Nanotechnology is at the leading edge of the rapidly developing new therapeutic and diagnostic concepts in all areas of medicine (Veronica et al., 2008). Magnetic nanoparticles (MNPs) are a class of engineered particulate materials of <100nm that can be manipulated under the influence of an external magnetic field. MNPs are commonly composed of magnetic elements, such as iron, nickel, cobalt and their oxides like magnetite ((Fe₃O₄), magnetite (γ-Fe₂O₃), cobalt ferrite (Fe₃CoO₄), chromium di-oxide (Cr₂O₃) (Yang et al., 2006), risk of particle aggregation. First, they have sizes that place them at dimensions comparable to those of a virus (20±500 nm), a protein (5±50 nm) or a gene (2 nm wide and 10±100 nm long). The magnetic nanoparticles, which used in bio-applications are usually made from biocompatible materials such as magnetite (Fe₃O₄) for which susceptibility is large. Targeting of drugs by nanoparticles is intended to reduce drug wastage, frequency of drug administration, side effects providing prolonged, sustained drug delivery to desired targeted organ. Iron oxide magnetic nanoparticles present a higher performance in terms of chemical stability and biocompatibility compared with metallic nanoparticles. Nanoparticles have a large surface that may be properly modified to attach biological agents.

CLASSIFICATION OF MAGNATIC NANOPARTICLES

Magnetic properties

The penetration of magnetic fields through human tissue and the ability to remotely detect or manipulate magnetic materials has been investigated for use in medicine for centuries. One of the more recent and significant applications of these properties has been in MRI as a non-invasive imaging modality capable of providing high resolution anatomical images. However, the potential of current clinical medical imaging can be greatly expanded through the use of MNPs to improve differentiation of malignant and healthy tissue. In addition, upon location of a malignancy or lesion, external magnetic fields can then be controlled to direct particle accumulations to deliver therapeutics. To better understand the advantages of MNPs as MRI contrast agents, we briefly review some of the fundamental concepts of magnetism and the properties of MNPs. More thorough and detailed discussion of this topic can be found in the literature. The classification of a material’s magnetic properties is based on its magnetic susceptibility (χ), which is defined by the ratio of the induced magnetization (M) to the applied magnetic field (H). In diamagnetic materials, the magnetic moment is antiparallel to H resulting in very small and negative susceptibilities (−10⁻⁶ to −10⁻³). They do not retain magnetic properties when the external field is removed. Materials with magnetic moments aligned parallel to H and susceptibilities on the order of 10⁻⁶ to 10⁻¹ are described as paramagnetic. While in ferri- and ferromagnetic materials, magnetic moments also align parallel to H, coupling interactions between the electrons of the material result in ordered magnetic states, i.e., magnetic domains, and large spontaneous magnetization. The susceptibilities of these materials depend on their atomic structures, temperature, and the external field. At small sizes (on the order of tens of nanometers), ferri- or ferro-magnetic materials, such as MNPs, become a single magnetic domain and therefore maintain one large magnetic moment. However, at sufficiently high temperatures (i.e., blocking temperature T_B) thermal energy is sufficient to induce free rotation of the particle resulting in a loss of net magnetization in the absence of an external field. This superparamagnetic property, marked by the lack of remnant magnetization after removal of external fields, enables the particles to maintain their colloidal stability and avoid aggregation making it feasible for their use in biomedical applications. Furthermore, the coupling interactions within these single magnetic domains result in much higher magnetic susceptibilities than paramagnetic materials. Although superparamagnetism is a favorable property of small particles, the reduction of particle size is not without some consequences. As particle sizes decrease, surface-to-volume ratios increase resulting in pronounced surface effects, such as no collinear spins, spin canting, and spin-glass-like behavior, which can significantly impact the magnetic properties of the material. Typically, the saturation magnetization (M_s) values of nanoparticles, corresponding to the complete alignment of all individual moments in a sample, are smaller than their corresponding bulk phases due to disordered crystal structure resulting from high surface curvature, which increases with particle size reduction. Furthermore, significant differences in magnetic properties are observed with MNPs obtained through different chemical processes. More detailed explanations of the physical properties of MNP and nanoscale magnetic phenomena can be found in recent reviews in this area.

Iron oxide nanoparticles

Colloidal iron oxide nanoparticles, such as SPIO and USPIO, have been the most extensively investigated MNPs for biomedical applications due to their excellent biocompatibility and ease of synthesis. Typically composed of nanocrystalline magnetite (Fe₃O₄) or magnetite (γFe₂O₃) protected with a polymeric coating, these ferrite nanoparticles possess a spinal crystal structure with oxygen ions forming a close-packed cubic lattice and iron ions located at interstices. In the case of Fe₃O₄, magnetization arises from electron hopping between the Fe²⁺ and Fe³⁺ ions that coexist at the octahedral sites. In addition to magnetic properties, the favorable biocompatibility and biodegradability of these MNPs have contributed greatly to their widespread use in biomedical applications.
Upon metabolism, iron ions are added to the body’s iron stores and eventually incorporated by erythrocytes as hemoglobin allowing for their safe use in vivo. Iron oxide nanoparticles have been produced by a variety of synthesis processes ranging from traditional wet chemistry solution-based methods to more exotic techniques such as laser paralysis or chemical vapor deposition. Currently, SPIO and USPIO utilized or under investigation for clinical application as MRI contrast agents are predominately synthesized by an aqueous co-precipitation process in the presence of the coating material. In these hydrolytic processes, the control of the solution pH value and the presence of the coating material serving as a surfactant are critical to particle formation and properties. Unfortunately, magnetization can vary vastly among synthesis methods even within particles of similar size due to incorporation of impurities disrupting the crystal structure, as well as the surface effects described previously. Typically, $M_r$ values of magnetite nanoparticles obtained by these methods are in the range of 30–50 emu/g, which is lower than the 90 emu/g reported for their bulk form. Recently, the use of high-temperature decomposition of organ metallic precursors has been examined to produce iron oxide nanoparticles with marked improvements in size control, size distributions, and crystalline. In this process, the size of the nanoparticle is controlled by varying the reaction temperature or changing the metal precursor. Sizes could be further tuned by a seed-mediated growth process to obtain larger particles. Utilizing this process, Sun et al. demonstrated the ability to synthesize highly uniform spherical $\text{Fe}_3\text{O}_4$ particles with size variation within 2 nm and mean diameters from 4 to 20 nm. One drawback of this approach is the use of hydrophobic oleic acid and oleylamine surfactants in the process which results in a hydrophobic coating on the particle surface necessitating additional modification to achieve nanoparticle solubility in aqueous media. Approaches such as the addition of an amphiphilic polymer or surface surfactant exchange have been utilized to overcome this problem. The need to improve magnetic properties for applications, such as molecular imaging, has generated interest in the development of metal doped iron oxides due to their enhanced magnetic properties. These spinal metal ferrites with a composition of $\text{MFe}_2\text{O}_4$, where M is $+2$ action of Mn, Fe, Co or Ni, have been fabricated by various methods to tune specific magnetic properties. Recently, Lee et al. reported the synthesis and characterization of $\text{MnFe}_2\text{O}_4$, $\text{FeFe}_2\text{O}_4$, $\text{CoFe}_2\text{O}_4$, and $\text{NiFe}_2\text{O}_4$ by high-temperature reaction between divalent metal chloride and iron tris-2,4-pentadioate. Through comparison of various metal-doped ferrite nanoparticles, this group has demonstrated that $\text{MnFe}_2\text{O}_4$ nanoparticles are nontoxic in vitro and possess higher magnetic susceptibility than magnetite nanoparticles, suggesting that they may be used as an ultrasensitive MR imaging probe. Cobalt and nickel ferrites have also been investigated recently for in vivo biomedical applications despite known toxicities of these elements. It has examined the synthesis and coating of $\text{CoFe}_2\text{O}_4$ MNPs for use as magnetic nanocarriers. Utilizing a polyl-based synthesis method this group produced 5.4 nm particles coated with mono- and dysfunctional phosphoric and hydroxamic acids. Cobalt leakage was monitored through inductively coupled plasma atomic emission spectroscopy (ICP-AES) and found to correspond with quality of surface coverage by the attached legend. Similarly, Rena et al. recently investigated the use of nanocrystalline $\text{NiFe}_2\text{O}_4$ as drug carriers.

**Metallic nanoparticles**

Metallic MNPs, made of iron, cobalt, or nickel, are often overlooked for biological applications due to their chemical instability. Readily forming oxides in the presence of water and oxygen, these metallic MNPs are typically protected by coatings, such as gold or silica, to form a core-shell structure. Despite complex synthesis processes, research continues on these metallic nanoparticles due to the unique advantages some of these MNPs can offer. For example, iron nanoparticles possess relatively high magnetization and are able to maintain superparamagnetism at larger particle sizes compared to their oxide counterparts. For iron nanoparticles, Pang et al. demonstrated that crystalline $\text{Fe}_3\text{O}_4$ shells were capable of providing a robust protective coating, while amorphous coatings could not protect the metallic core from deep oxidation. In this study, a thermal degradation process was used initially to create iron nanoparticles, while the oxide coating was formed and its thickness was tuned by controlled oxidation utilizing an oxygen transferring agent. $\text{FeFe}_2\text{O}_4$ nanoparticles were produced with a core radius of 4 nm and oxide thickness of 2.5 nm. Magnetic characterization of these MNPs confirmed that the particles were super paramagnetic and possessed a $M_r$ of 102.6 emu/g Fe. Alternatively, Qing et al. recently reported a method of producing stable $\text{FeFe}_2\text{O}_4$ nanoparticles using a nanocluster deposition system. The group demonstrated the ability to vary core sizes from 2 to 100 nm and shell thicknesses from 2.5 to 5 nm by controlling growth parameters. Nan particles generated by this process with a size less than 10 nm exhibited a $M_r$ of approximately 80 emu/g Fe.

**Bi-metallic nanoparticles**

Bimetallic or metal alloy nanoparticles can also exhibit super paramagnetic properties making them attractive candidates as MRI contrast agents or magnetic carriers for drug delivery. Recent advances in the synthesis and surface modification of FePt nanoparticles have made these MNPs a viable option for biomedical applications. Typically obtained from a variety of processes, such as vacuum-deposition or solution phase synthesis, FePt nanoparticles are known to possess a chemically disordered face-centered cubic (fcc) or chemically ordered face-centered tetragonal (fct) structure, both of which result in near-equal atomic percentages of Fe and Pt. Interactions between the two chemical species lead to greater chemical stability in comparison to other high moment metallic nanoparticles. Furthermore, the surface chemistry of these MNPs allows for binding of carboxyl ate- and amine-based surfactants.
which may be utilized to improve the water solubility of these nanoparticles. Hong et al. reported the modification of FePt nanoparticles with thiol terminated poly(ethylene glycol) (PEG) and dopamine legends to form a mixed-monolayer-functionalized MNPs. Utilizing FePt nanoparticles prepared by the method introduced by Sun et al., this group demonstrated that these coated MNPs were stable in biologically relevant media such as PBS and cell culture medium. Furthermore, the ability to bind DNA and protein to the surface of these MNPs was also demonstrated through the incorporation of charge functionality. Recently, Gao et al. developed a process to create FePt nanoparticles encapsulated with a shell composed of CoS₃ or CdO to serve as multifunctional nanostructures with cytotoxicity toward cancer cells or to provide fluorescence detectability. Although the toxicity of FePt nanoparticles themselves has not been thoroughly evaluated with only limited in vitro cytotoxicity assays reported throughout the literature, inert coatings such as gold shells are actively being investigated to improve the biocompatibility of these MNPs. Another form of binary metallic MNPs receiving increased attention are those composed of FeCo. With extremely high $M_r$ values, these nanoalloys require protective coatings to prevent them from oxidation and corrosion. Bai and Wang have reported a method of synthesizing high magnetic moment nanoparticles with 10–20 nm Fe₈₀Co₂₀ cores and 1–3 nm gold or silver shells through a physical deposition process. Cubic nanoparticles synthesized by this method were found to be super paramagnetic and have a $M_r$ three times as high as that of comparable iron oxide nanoparticles. Recently, Seo et al. reported the development of FeCo nanocrystals coated with a single-graphitic shell that were soluble and stable in aqueous solutions. Synthesized through chemical vapor deposition (CVD), 7 nm and 4 nm FeCo cores were produced with compositions of Fe₈₆Co₁₄ and Fe₅₄Co₄₆, respectively. The graphitic shell was then applied by heating in H₂ and subsequent methane CVD. The $M_r$ of the 7 nm and 4 nm nanocrystals were 215 emu/g and 162 emu/g, respectively. In addition to providing protection from oxidation and potential toxicity, the graphitic coating also provides near-infrared optical absorbance allowing for potential use of photo thermal ablation as a therapeutic application.

Surface coatings and functionalization

Polymeric coatings

Surface coatings are an integral component of all MNP platforms for biomedical applications. Although not attracted magnetically, due to their super paramagnetic properties, nanoparticles still have a significant tendency to agglomerate as a result of their high surface energy. Colloidal electrostatic stabilization arising from repulsion of surface charges on the nanoparticles is typically not adequate to prevent aggregation in biological solutions due to the presence of salts or other electrolytes that may neutralize this charge. Furthermore, upon intravenous injection the surfaces of MNPs are subjected to adsorption of plasma protein, or opsonization, as the first step in their clearance by the RES. Evading uptake by the RES and maintaining a long plasma half-life is a major challenge for many MNP applications in medicine. Polymeric coatings provide a steric barrier to prevent nanoparticle agglomeration and avoid opsonization. In addition, these coatings provide a means to tailor the surface properties of MNPs such as surface charge and chemical functionality. Some critical aspects with regard to polymeric coatings that may affect the performance of a MNP system include the nature of the chemical structure of the polymer (e.g. hydrophilicity/hydrophobicity, biodegradation characteristics, etc.), the length or molecular weight of the polymer, the manner in which the polymer is anchored or attached (e.g. electrostatic or covalent bonding), the conformation of the polymer, and the degree of particle surface coverage. Various monomer species, such as bisphosphonates, dimercaptosuccinic acid (DMSA) and alkoxysilanes have been evaluated as anchors to facilitate attachment of polymer coatings on MNPs. The molecular weight and geometric orientation of the polymer on the surface of the particles in the form of loops, tails or as end-grafted brushes) or as fully encapsulated polymer shells) not only affect the antifouling characteristics of the nanoparticle, but also contribute to their effective hydrodynamic size, which is another key factor in avoiding recognition by the RES,MNP structures and coating schemes. (A) End-grafted polymer coated MNP. (B) MNP fully encapsulated in polymer coating. (C) Liposome encapsulated MNP. (D) Core-shell MNP. (E) Heterodyne MNP. A variety of natural and synthetic polymers have been evaluated for use as coatings on MNPs. Readers are directed to several reviews on the topic for a comprehensive analysis of these materials. One of the most widely utilized and successful polymer coatings, in terms of in vivo applications, has been the polysaccharide dextrin. It has developed various formulations of dextrin-coated iron oxide nanoparticles also referred to as monocrytalline iron oxide nanoparticles (MION) and cross-linked iron oxide nanoparticles (CLIO), which have been evaluated extensively for a variety of MR imaging applications. Chemical functionality was established by treating CLIO with ammonia to provide primary amino groups for the attachment of biomolecules such as proteins or peptides. Poly(ethylene glycol) (PEG) is another widely used polymer for nanoparticle coating in biomedical applications. The antifouling nature of PEG has been shown to reduce nanoparticle uptake by macrophages and extend blood circulation time in vivo. Various methods have been utilized to attach PEG to MNPs including silane grafting to oxide surfaces, polymerization at the surface of MNPs, and modification through sol-gel approaches. To control polymer conformation and provide stable covalent linkages to the surface of iron oxide nanoparticles, Kohler et al. developed bifunctional PEG silanes capable of forming self-assembled monolayer’s (SAMs) and increasing the packing density of the polymer chains onto the nanoparticles surface. In addition, terminal amine or carboxyl groups extending out from the nanoparticle surface provide sites for conjugation of functional legends, as demonstrated by the attachment of folic acid in this study.

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Similarly, Lee et al. reported the development of a protein resistant poly(TMSMA-r-PEGMA) copolymer comprised of saline anchoring groups and PEG branches. Utilizing this polymer to coat magnetite nanoparticles, this group demonstrated the accumulation of the MNPs in engraft tumors in mice as identified by MRI contrast enhancement. Recently, there has been an increased interest on the modification of polymers or development of copolymers to allow for in situ coating of MNPs during nanoparticle synthesis. These processes, often termed “one-pot” synthesis methods, have several advantages over stepwise surface modification, including reduced agglomeration due to immediate coating of the particles and less processing procedures. However, the presence of polymers during nanocrystal nucleation and growth can have a significant impact on the crystal structure and morphology of the MNPs obtained through these processes. For example, Lee et al found that crystalline decreased with increasing concentration of poly(vinyl alcohol) (PVA) present during the synthesis of iron oxide particles through a precipitation reaction. As noted, the imperfections in the crystal structure of these MNP can be detrimental to their magnetic properties. Another consideration to take into account while utilizing polymer coatings is their effects on the nanoparticle magnetic properties.

**Liposome’s and micelles**

The development of liposome’s as drug delivery vehicles can be considered one of the earliest forms of nanomedicine. These phospholipids bilayered membrane vesicles can range from 100 nm up to 5 μm in size and have been utilized for the delivery of small molecules, proteins and peptides, DNA, and MR imaging contrast agents. An advantage of liposome encapsulation is that their in vivo behavior has been well established with processes such as Pagination resulting in long circulation times. Another favorable feature of liposome’s is the ability to encapsulate a large number of MNP cores and deliver them together, avoiding dilution, to a target site. Combining a therapeutic agent in the payload further enhances the multifunctionality of these delivery vehicles. Similarly, multifunctional micelles formed with amphiphilic block copolymers have also been used to entrap MNPs for these applications. Martina et al. developed magnetic-fluid loaded liposome’s (MFLs) by encapsulating magnetite nanocrystals within unilamellar vesicles of egg phosphatidylcholine and DSPE-PEG2000. MFLs with hydrodynamic size of 195 ± 33 nm were formed by film hydration coupled with sequential extrusion and were capable of encapsulating up to 1.67 mol of iron per mol of lipid. In vivo evaluation in mice using MR angiography demonstrated that these MFLs were still present in the blood 24 hours after intravenous injection confirming their long-circulating behavior.

**Core-shell structures**

In addition to organic coatings, core-shell structures) utilizing biocompatible silica or gold to encapsulate the MNPs have become another attractive approach for developing MRI contrast agents or MTCs for drug delivery. As mentioned in the previous sections, these inert coatings, or shells, provide both protection against chemical degradation of magnetic cores and prevent the release of potentially toxic components. Furthermore, fictionalization chemistries are generally better established with these materials than those that comprise MNPs. Silica shells are attractive options to serve as protective coatings on MNPs due to their stability under aqueous conditions and ease of synthesis. Sol-gel processes using tetraethoxysilane (TEOS) are generally utilized throughout the literature to produce coatings of controlled thickness. The use of functional alkoxysilanes, such as 3-aminopropyltriethoxysilane (APS), allows for surface reactive groups to be easily added to these core-shell structures. In addition, the ability to encapsulate functional molecules, such as alternative imaging or therapeutic agents, within this protective matrix is a unique feature to these nanostructures. Recently, Ma et al. described one such multifunctional core-shell MNP composed of iron oxide cores of approximately 10 nm surrounded by a shell of SiO₂ 10–15 nm thick. In this study, an organic dye, Tris (2,2’-bipyridine) ruthenium, was doped inside a second silica shell to provide luminescence and prevent quenching by interaction with the magnetic core. With this core-shell structure exhibiting super paramagnetic and luminescent properties, the authors of this work proposed this nanostructure for use in biomedical imaging applications. Gold offers several advantages as a coat5603ing material for MNPs due to its low chemical reactivity and unique ability to form SAMs on their surface using alkanethiols. Unfortunately, this chemical inertness may also lead the difficulty in forming gold shells over MNPs. Recent advances in synthesizing gold-coated iron nanoparticles through a variety of methods ranging from reversed micro emulsion, combined wet chemical, to laser irradiation have been reviewed by Lu et al. Alternatively, heterodyne MNPs can be produced by similar processes as gold core-shell structures.

**Functional lagan’s**

It is representing another unique class of MNP. As discussed throughout this review, the ability to add components to MNPs in a modular fashion allows for specific features and functional moieties to be interchanged or combined. Ligands such as targeting agents, permeation enhancers, optical dyes, and therapeutic agents can all be conjugated on the surface or incorporated within these nanostructures. To perform such nanoscale engineering, bioconjugation chemistries and techniques utilized for protein coupling have been studied. Techniques such as avid in-biotin binding, use of heterobifunctional linkers to form amide, ester, or disulfide bonds, and more recently “click” chemistries, have all been shown to be useful in attaching functional ligands to MNPs. In addition to
understanding the mechanisms of these reactions, those utilizing these techniques on MNPs may also find it useful to review basic concepts of colloidal science to avoid unwanted flocculation or aggregation during these processes. MNPs possessing various legends to enable multifunctionality from a single nanoparticle platform. One example of adding functionality to MNPs has been the combination of organic dyes or fluorophores as optical imaging agents to allow for detection by multiple imaging modalities. Several groups have demonstrated the fluorescent imaging of cells in vitro after internalization of FITC or rhodamine labeled MNPs. Recently, the conjugation of near-infrared fluorescent (NIRF) dyes to MNPs has received significant attention due to the deep penetration of NIRF light through tissues. The integration of NIRF detectability may allow for these nanoparticles to be used for both presurgical planning by MRI and intraoperative resection of malignant tissues by optical imaging. Since both MRI and optical signals come from the same nanoparticles, the MR image can serve as a roadmap to the fluorescently labeled tumor cells. Josephson and co-workers have attached NIRF Cy5.5 dyes to CLIO MNPs and demonstrated in vivo accumulation of nanoparticles at tumor margins through macrophage uptake to improve brain tumor delineation. I constructed a multimodal agent composed of PEG-coated iron oxide nanoparticles conjugated to both Cy5.5 and a targeting agent, chlorotoxin, to improve specificity and internalization of nanoparticles into 9L glioma cells. Co focal fluorescent images of cells incubated with chlorotoxin-targeted iron oxide nanoparticles conjugated to Cy5.5. A: rat cardiomyocytes (rCM) representing normal cells. B: 9L glioma cells. C: MR phantom image of 9L (top) and rCM (bottom) cells cultured.

Pharmacokinetics and misdistribution

Blood half-life
The need to extend nanoparticles’ blood circulation time to allow for their accumulation in target tissues has long been recognized as one of the primary challenges in the development of MNPs. The ability to evade uptake by the RES are critical to achieving a long blood half-life. Like other colloidal carriers, the physicochemical properties of these MNPs platforms, such as size, morphology, charge, and surface chemistry, dictate their fate in vivo. The overall size of MNPs must be sufficiently small to evade rapid splenoid filtration but large enough to avoid renal clearance. Nanoparticles larger than 200 nm are sequestered by phagocytotic cells of the spleen, while particles smaller than 5.5 nm are rapidly removed through renal clearance. In addition to size, the shape and flexibility of MNPs have been suggested as physical characteristics that require more investigation to improve their performance in vivo. Particles that escape filtration are then subject to opsonization resulting in recognition and clearance by Kupffer cells and other tissue macrophages. As described in the previous sections, various coatings including hydrophilic polymers, such as PEG, have been utilized to create a non-fouling coating on the particle surface. In addition to the bio-fouling nature of MNPs, surface charge plays a critical role in blood half-lives of colloids and polymers. Positively charged polymers and particles tend to nonspecifically stick to cells. This nonspecific adsorption can have a significant impact on blood-half life as demonstrated in a study by Papoose et al., where the circulation time of cationic poly-L-lysine coated MION was found to be only 1–2 min in comparison to 2–3 hrs for their uncharged variant. Strong negative charges on the particle surface are also detrimental in that they result in increased liver uptake. Therefore, it is generally agreed that nanoparticles with a neutral surface experience extended blood circulation times.

Passive targeting
The development of long-circulating nanoparticles has allowed for many MNP platforms to exploit structural abnormalities in the vasculature of particular pathologies, such as tumors, inflammatory, and infectious sites. This phenomenon, known as the enhance permeability and retention (EPR) effect, is based on the mechanism that these tissues possess “leaky” vasculature which allows macromolecules and nanoparticles to extravagate and accumulate more readily. In the case of tumors, poorly organized vascular beds also result in impaired lymphatic drainage from these tissues. This non-specific accumulation, or passive targeting, has been demonstrated with nanoparticles ranging from 10–500 nm in diameter. Passive targeting can also occur through the inherent clearance by the RES. Comprised of bone marrow progenitors, blood monocots, and tissue macrophages, the uptake of MNPs by these phagocyte cells provides a means of delivering contrast agents and drug carriers to related organs. This RES-mediated targeting is the basis for the first clinical application of MNPs in the form of Ferrumoxides AMI- (Endorem and Feridiv IV) for liver imaging. The rapid uptake of these MNPs by Kupffer cells of healthy hepatic parenchyma allows for their differentiation from diseased tissue by the contrast enhancement observed under MRI.

Active targeting
One promising approach toward increasing the local accumulation of MNPs in diseased tissue, known as active targeting or specific targeting, is by the conjugation of targeting molecules that possess high affinity toward unique molecular signatures found on malignant cells. Often augmented by the EPR effect, these receptor-legend or antigen-antibody interactions provide an effective strategy to improve the residence time in malignant tissues, such as tumors. Targeting legends, such as proteins, peptides, aptamers and small molecules, have been investigated to increase the site specific accumulation of MNPs. In some cases, specific binding can
also facilitate internalization of the nanoparticle by receptor-mediated endocytosis. Illustration of tissue specific delivery of MNPs through active targeting facilitated by “leaky” vasculature. (A) Internalization of nanoparticles by (A) receptor-mediated endocytosis and formation of an endosome. (B) Endosomal acidification. Monoclonal antibodies (mAbs) were the first targeting agents to exploit molecular recognition to deliver MNPs and continue to be widely used due to their high specificity. Recently, the development of Perception™, an FDA-approved mAb to the HER2/neu (erbB2) receptor, has made it a popular targeting agent for nanoparticles. Huh et al. demonstrated specific delivery of Herceptin™ targeted DMSA-coated magnetite nanoparticles to NIH3T3.7 cells expressing the HER2/neu cancer marker in vivo. MR imaging of mice bearing engraft tumors showed a T2 decrease of ~20% as a result of accumulation of this nanoprobe. One drawback of mAbs is their large size and inherent immunogenicity which can cause conjugated nanoparticles to diffuse poorly through biological barriers. Another area of extensive investigation has been the targeting of MNPs to receptors over expressed on tumor neovasculature. The formation of new blood vessels, or angiogenesis, is an essential component of tumor growth and has been shown to be highly specific for neoplasia. A relatively large number of angiogenesis markers, which include the αvβ3 integrin, vascular endothelial growth factor (VEGF), cell surface nucleoli, and heparin sulfates, have been identified as potential targets for the delivery diagnostic and therapeutic agents. Targeting agents, such as the Arg-Gly-Asp (RGD) peptide demonstrating high affinity for the αvβ3 integrand, have been evaluated for the delivery of MNPs to a variety of eukaryotic tissues including breast tumors, malignant melanomas, and squamous cell carcinomas. In a recent study by Reddy et al., the F3 peptide, which binds to nucleoli expressed on tumor endothelium and cancer cells, was utilized to deliver a multifunctional MNP to brain tumors. Through combination with photodynamic therapy (PDT), this group was able to monitor the treatment efficacy of 9L gliomas in rats using the MNP component as a contrast agent for MRI. Chlorotoxin (CTX), a peptide originally purified from the venom of the Leirus quinquestriatus scorpion, has also been shown to be an effect targeting agent for tumors of neuroectodermal origin. Studies suggest the target of CTX is associated with the membrane-bound matrix metalloproteinase-2 (MMP-2) protein complex, which is up-regulated on gliomas, as well as a variety of other tumors. CTX has been shown to be an effective targeting agent to deliver MNPs to brain tumor cells. The use of short peptides and small molecules as targeting agents also offers the advantage of increased binding affinity through multivalent attachment. This targeting phenomenon has been examined with folic acid, a vitamin whose receptor is over expressed on the surface of many human tumor cells, including ovarian, lung, breast, endometrial, renal, and colon cancers. In our previous work, we demonstrated the highly selective binding of folic acid conjugated MNPs to a variety of tumor cells to improve their detestability by MRI. Another advantage of utilizing small molecules as targeting agents is that they are generally more robust than proteins or peptides thereby reducing possibility of loss of functionality through the synthesis of such MNPs.

Intracellular delivery and controlled release
An essential step in the use of MNPs for drug delivery is the internalization of the MNP and/or its therapeutic payload, as well as the subsequent release of these therapeutic agents to cell cytoplasm for desired actions to take place. Several mechanisms have been proposed to describe the uptake of nanoparticles into cells, including receptor-mediated endocytosis and internalization by caseload structures. Nan particle size and surface properties play a critical role in moving across the plasma membrane. Nan particles smaller than 50 nm or those coated with lipophilic polymers, such as PEG, have been shown to efficiently diffuse through cell membranes. In addition, permeation enhancers, such as the Tat peptide, can also be attached to MNPs to facilitate delivery to cytoplasm. Using Tat-labeled CLIO MNPs, Koch et al. demonstrated the effective internalization and slow excretion of the nanoparticles for cell tracking and drug delivery applications. Upon particle internalization by target cells, another significant challenge for MNPs to serve as drug carriers is the release of the therapeutic agent to targeted sub cellular organelles, such as the nucleus or mitochondria, prior to being trafficked to lissome where their biological activity may be destroyed. Although cleavage from MNP carriers under the hostile environment of the liposome’s may be suitable for some stable therapeutics, the effectiveness of other compounds, such as protein/peptides and oligonucleotides, may be severely compromised. Strategies to achieve endosomal release after cellular internalization include tailoring of cleavable linkers responsive to pH, osmolarity, or enzymatic activity. In addition, integration of cationic polymers to induce osmotic swelling, or “proton sponge” effect, has also been examined to facilitate escape from endosomes.

Misdistribution and clearance
The long-term fate of MNPs in vivo is a major concern in the development of these nanoparticle platforms. Although general guidelines, such as those discussed in regard to the physicochemical properties of MNPs, may provide some insight on their behavior in the body, no universal set of criteria has been elucidated to predict this critical aspect of nanomedicine. Mechanisms of clearance can vary significantly depending on the wide range of structures that are employed in the development of MNPs. One can make the obvious distinction that the metabolism, clearance, and toxicity profiles associated with a gold-coated FePt core-shell nanoparticle will be drastically different from that of an iron oxide filled liposome. These unique structures therefore necessitate their individual
evaluation. Recently, increased emphasis has been placed on standardizing preclinical characterization of biomedical nanoparticles to better elucidate structure-activity relationships (SARs).

Advantages of Magnetic Nanoparticles
There had been a growing interest on the properties and broad range of applications of colloids in recent years. Magnetic biomaterials provide the ability to be directed and concentrated within the target tissue by means of external magnetic field and to be removed once therapy was completed. Magnetic nanoparticles display the phenomenon of superparamagnetism, not keeping magnetized after the action of magnetic field, offering advantage of reducing risk of particle aggregation (Ruiz-Hernandez et al., 2008). First, they have sizes that place them at dimensions comparable to those of a virus (20±500 nm), a protein (5±50 nm) or a gene (2 nm wide and 10±100 nm long)

Advantages of the Magnetic Particle method of Non-Destructive Examination are:
- It is quick and relatively uncomplicated
- It gives immediate indications of defects
- It shows surface and near surface defects, and these are the most serious ones as they concentrate stresses
- The method can be adapted for site or workshop use
- It is inexpensive compared to radiography
- Large or small objects can be examined
- Elaborate pre-cleaning is not necessary

Disadvantages
- It is restricted to ferromagnetic materials - usually iron and steel, and cannot be used on austenitic stainless steel
- It is messy
- Most methods need a supply of electricity
- It is sometimes unclear whether the magnetic field is sufficiently strong to give good indications
- The method cannot be used if a thick paint coating is present
- Spurious, or non-relevant indications, are probable, and thus interpretation is a skilled task
- Some of the paints and particle suspension fluids can give a fume or fire problem, particularly in a confined space
- Books on the subject are:

Magnetic Nanoparticles: Magnetic property
The classification of a material's magnetic properties is based on its magnetic susceptibility (χ), which is defined by the ratio of the induced magnetization (M) to the applied magnetic field (H). In ferri- and ferromagnetic materials, magnetic moments align parallel to H, coupling interactions between the electrons of the material result in ordered magnetic states. The susceptibilities of these materials depend on their temperature, external field H and atomic structures. At small sizes (in the order of tens of nanometers), ferri- or ferromagnetic materials, such as MNPs, become a single magnetic domain and therefore maintain one large magnetic moment. However, at sufficiently high temperatures (i.e., blocking temperature, TB) thermal energy is sufficient to induce free rotation of the particle resulting in a loss of net magnetization in the absence of an external field. Lack of remnant magnetization after removal of external fields enables the particles to maintain their colloidal stability and avoids aggregation making it feasible for their use in biomedical applications. The coupling interactions in single magnetic domains result in much higher magnetic susceptibilities than paramagnetic materials (Lu et al. 2007).

Synthesis of magnetite nanoparticles
The synthesis of magnetic nanoparticles of controlled size has long been of scientific and technological interest. Stable suspensions of magnetic fluids were first synthesized in 34 1964 by Papal. There are many synthetic procedures reported in the literature for preparing man-sized crystalline magnetite. Reported procedures for the synthesis of magnetite and magnetite are different. However, in practice mixtures of the two are often obtained even when a high level of care is taken experimentally. The sensitivity to reagent stoichiometry and a large number of other reaction parameters often complicate the ability to obtain pure magnetite crystalline structures. The review given here is mainly focused on the literature on magnetite. Due to the similarity of their magnetic properties, and of the synthetic procedures some references on magnetite are included. The synthetic procedures for magnetite can be broadly classified into three groups: aqueous precipitation, thermal decomposition of carbonyl complexes/cheats and high temperature alcohol reduction of Fe3+ chemical precursor, usually in non polar solvents.
The synthesis of magnetic nanoparticles by alkaline co precipitation

Magnetite synthesis by the aqueous precipitation of mixed Fe3+ and Fe2+ salts has known since early 1900s. The molar ratio of Fe3+ and Fe2+ salts used in the synthesis is 2:1 as the magnetite can be described as Fe0 .Fe2O3. As the Fe2+ ion is prone to oxidation, researchers emphasized the use of closed reactors for magnetite synthesis with nitrogen or argon gas purging through the reaction mixture. Some authors used the iron salts with Fe3+/ Fe2+ ratio greater than 2 due to the oxidative instability of Fe2+ ion. The complex mechanism of inverse spinal magnetite formation is not well understood. A very general mechanistic agreement on the formation of magnetite through aqueous precipitation is that the Fe2+ precursor hydrolyses to Fe(OH)2 and consequently reacts with other hydrous oxides to form magnetite . Farley et al. proposed that the formation of oxides occurs via surface adsorption of captions especially when the bulk solution concentration is below saturation with the solid phase There is general agreement that the process starts with the nucleation of magnetite followed by crystal growth to obtain monodisperse particles.

Magnetite nucleates when the concentration of ions becomes supersaturated, from that point the experimental conditions control the crystal growth process. It is known that acidity and ionic strength, which are responsible for the protonation-deprotonation equilibrium of surface hydroxylated groups, determine the electrostatic surface charge. At higher pH and ionic strength changes in the chemical composition of the interface, result in a decrease of the interfacial tension, y. As stated by Gibbs’s law, dy = -Fj dG, where Tj is the density of adsorbed species ‘i’, which increases with pH, of chemical potential (i). As the free enthalpy for the formation of particles is defined by $dG = ydA$, where dA is the change in surface area, reduced interfacial tension is consistent with a spontaneous increase in the system surface area. describe an interesting study of the effect of pH on the Fe-Cl-H2O system. They detail the operating conditions for co-precipitation of the precursor where it can be protected from undesired

The synthesis of magnetic nanoparticles by other methods

Other aqueous precipitation methods include: oxidation of Fe2+, reduction of Fe3+ salt followed by precipitation, precipitation through water-in-oil micro emulsions, vesicles and liposomal preparations. The experimental parameters control the size of these particles and the resulting physical and chemical properties. A wide range of techniques have been exploited in order to improve the control of the size distribution of magnetic nanoparticles.

The synthesis of magnetic nanoparticles by oxidation

It demonstrated that the mechanism of formation of magnetite from an aqueous alkaline slurry of Fe(OH)2 involves the sequence of dissolution, oxidation, nucleation on Fe(OH)2, and finally growth of Fe3 0 4. The dependence of the size and shape of the particles on experimental conditions is also discussed. It described the formation and stabilization of ferromagnetic iron oxide nanoparticles. Oxidation of Fe(II) at slightly elevated pH and temperature resulted in the formation of highly soluble nanocomposites of iron oxides which are stable under a wide range of temperatures and pHs. It described the synthesis of magnetite by the hydrolysis and precipitation of FeSO4 solution with NaOH. Hydrogen peroxide oxidation produces nanoparticles of size ~20 nm at pH 13 in presence of surfactant. While in another method described the synthesis of ultrafine (8-10 nm) magnetite particles by the precipitation of ferrous hydroxide from Fe(NH4)2(SO4)2.6H2O with excess NaOH. Hydrogen peroxide was used to oxidise the resulting green precipitate, in the presence of oleic acid and a commercial NNO surfactant at 60-70°C.

The synthesis of magnetic nanoparticles by reduction

It is successfully synthesized 3 to 10 nm magnetite particles by reduction of ferric chloride by Na2S03 before precipitating with ammonia. The authors emphasized 39 the importance of Fe /SO3 ’ ratio and the initial concentration of Fe ion in determining the particle size and surface properties of the magnetite. The researchers report that the most appropriate ratio is 3:1, and that the magnetite particle diameter decreased from ca. 11 to ca. 3 nm with a decrease of the concentration of aqueous ferric chloride from 0.45 to 0.075 molL’1. The advantage of this method lies in the fact that precipitation is done at the end of reduction process, and so precautions to prevent oxidation, e.g. by purging nitrogen or argon, are required.

The synthesis of magnetic nanoparticles by water-in-oil methods

A range of techniques where particle growth is limited by precipitating Fe3+ and Fe2+ ions in micro emulsions, vesicles, polymer solutions, or gels have been reported Water-in-oil (w/o) micro emulsions are created by amphoteric surfactants. Water forms a micro droplet surrounded by a monolayer of surfactant molecules organized with their polar heads toward the aqueous core, known as the water-pool, and the hydrophobic tails in contact with the bulk no polar solvent. With appropriate surfactant, chemical composition and concentration, such micellar cores can serve as nonreactors for the co precipitation of aqueous iron salts. Ionic surfactants successfully used include sodium is(2-ethyl hexyl sulphonesuccinate) (AOT) and cetyltrimethyl ammonium bromide (CTAB) [98,
Limitations of magnetic drug delivery

Since the magnetic gradient decreases with the distance to the target, the main limitation of agnatic drug delivery relates to the strength of the external field that can be applied to obtain the necessary magnetic gradient to control the residence time of NPs in the desired area or which triggers the agglomeration. Permanent Nd-Fe-B magnets in combination with SPION, which have excellent magnetic properties, can reach effective magnetic field depths up to 10-15 cm in the body. However, it must be noted that the magnetic carriers accumulate not only at the desired site but also throughout the crosssection from the external source to the depth marking the effective field limit. Obviously, the geometry of the magnetic field is extremely important and must be taken into account when designing a magnetic targeting process. As a means to elude the limitations of using external magnetic fields, internal magnets can be located in the vicinity of the target by using minimally invasive surgery. Several studies have simulated the interaction between a magnetic implant and magnetic NPs, enabling drug delivery. In addition, work in several laboratories is addressing targeted drug delivery with magnetic implants. Another limitation relates to the small size of NPs, a requisite for superparamagnetism, which is in turn needed to avoid magnetic agglomeration once the magnetic field is removed (see below). A small size implies a magnetic response of reduced strength, making it difficult to direct particles and keep them in the proximity of the target while withstanding the drag of blood flow. Targeting is likely to be more effective in regions of slower blood velocity, and particularly when the magnetic field source is close to the target site. As for all biomedical applications, limitations also arise in extrapolating from animal models to humans. There are many physiological parameters to consider, ranging from differences in weight, blood volume, cardiac output, and circulation time to tumor volume/location/blood flow, complicating the extrapolation of data obtained in animal models. Related to this point is the fact that studies on toxicity (not only direct toxicity, but also toxicity of the degradation products and induced responses) and the fate of magnetic carriers are insufficient and, in many cases, there is insufficient characterization. Finally, state-of-the-art magnetic drug delivery seems mainly applicable to well-defined tumors, as treatment of metastatic neoplasm’s and small tumors in the early stages of their growth still remains a challenge. Treating emerging tumors will involve the development of a new generation of seek-and-destroy NPs, which specifically recognize small clusters of cancer cells and carry, the necessary elements (drugs or hyperthermia agents) for their destruction. A strong interest continues in this field given the capability of NPs to access tumors in regions where conventional surgery cannot be applied.

Tailoring magnetic NPs Essential requisites

Magnetic NPs for biomedical applications must be endowed with the specific characteristics required. As mentioned above, the first requirement is often superparamagnetism. Super paramagnetism occurs in magnetic materials composed of very small crystallites (threshold size depends on the nature of the material, for instance, Fe-based NPs become super paramagnetic at sizes <25 nm). In a paramagnetic material, the thermal energy overcomes the coupling forces between neighboring atoms above the Curie temperature, causing random fluctuations in the magnetization direction that result in a null overall magnetic moment. However, in super paramagnetic materials, the fluctuations affect the direction of magnetization of entire crystallites. The magnetic moments of individual crystallites compensate for each other and the overall magnetic moment becomes null. When an external magnetic field is applied, the behavior is similar to paramagnetic except that, instead of each individual atom being independently influenced by an external magnetic field, the magnetic moment of entire crystallites aligns with the magnetic field. In large NPs, energetic considerations favor the formation of domain walls. However, when the particle size decreases below a certain value, the formation of domain walls becomes unfavorable and each particle comprises a single domain. This is the case for super paramagnetic NPs. Superparamagnetism in drug delivery is necessary because once the external magnetic field is removed, magnetization disappears (negligible eminence and coercively, see ), and thus agglomeration (and the possible remobilization of capillary vessels) is avoided. Another key requirement is the biodegradability or intact excretion of the magnetic core. Thus, SPION are considered to be biodegradable with Fe being reused/recycled by cells using normal biochemical pathways for Fe metabolism. For no biodegradable cores, a specific coating is needed to avoid exposure (and possible leaching) of the magnetic core and to facilitate intact excretion through the kidneys, so that the half-life of the agent in the blood is determined by the glomerular filtration rate (e.g. contrast agents based on gadolinium).

Coatings on magnetic NPs

The coatings on magnetic NPs often serve multiple purposes. Their role in reducing leaching of the cores has already been mentioned. The coating also often facilitates the stabilization of NPs in an environment with a slightly alkaline pH or a significant salt concentration. For instance, the is electrical point of SiO2 is reached at pH 2.3, meaning that silica-coated NPs are negatively charged at the pH of blood, inducing electrostatic repulsion that helps avoid aggregate formation. Silica coatings also have additional...
advantages. On the one hand, the external surface of silica coatings can be functionalized to allow the binding of bimolecular. This is mainly related to the presence of hydroxyl surface groups in significant concentrations that provide intrinsic hydrophilicity and allow surface attachment by coval...
approach was made herein to review the history of magnetic guidance, concept of magnetic nanoparticles, their advantages, methods of preparation, characterization, applications explored in various fields of drug delivery. This review also deals up with the disadvantages of magnetic nanoparticles, the alternative way to overcome. It also focuses on exemplifying different drug formulations formulated into magnetic nanoparticles.

REFERENCES