Physico Chemical Principles of Metal Based Paramagnetic/ Iron Contrast

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The main objectives of this lecture are to provide instructions/ updates on

(i) current status of MRI-based contrast and molecular imaging;
(ii) importance of nanotechnology in imaging science;
(iii) paramagnetic properties in the presence of external magnetic field;
(iv) physico-chemical properties of iron-oxide based contrast;
(v) physico-chemical properties of gadolinium-based contrast;
(vi) development of targeted agents by nanoparticle surface modification.

1. Contrast-Enhanced MRI: Diagnostic MRI routinely employs contrast agents to alter the relaxation rate of water protons as the signal intensity in MRI is dependent on the concentration of water in the area of interest. Thus, contrast agents are capable of increasing the contrast between a particular tissue of interest and the surrounding tissue. Effective contras agents must have a strong local effect on either the longitudinal (T1) or the transverse (T2) relaxation times, therefore shortening the relaxation time of the water protons (*Hasebroock and Serkova, 2009*). Arguably the most effective technique for altering the relaxivity of water protons is to introduce a contrast agent composed of a high spin paramagnetic metal, such as iron (Fe) or gadolinium (Gd). The water molecules bound to these high spin metals relax orders of magnitude faster than free water, resulting in the desired changes in signal intensities. However, not all contrast agents produce the same response in MRI; negative contrast agents produce darker signals due to reduction in T2 values, while positive contrast agents produce a bright image due to shortening of the T1 values. For example, iron oxide particles have a high transverse relaxivity (r_2) while Gd^{3+} chelates have a high longitudinal relaxivity (r_1). These results in Fe based contrast agents inducing a large shortening of T2 values and are thus negative contrast agents. Conversely, Gd-based contrast agents shorten the T1 values and are positive contrast agents.

2. Nanotechnology in Medicine: Over the past decade, the application of nanotechnology has become expansive area of research for the improvement of diagnostic imaging and the treatment of disease (*Ferrari, 2005; Nagahara et al., 2010).* One area where the application of nanotechnology has received considerable attention is in the development of targeted diagnostic imaging agents. Because of the versatility and control over the nanostructures that can be formed, nanoparticles have been used as imaging agents and molecular reporters for numerous imaging techniques including magnetic resonance imaging (MRI), computed x-ray tomography (CT), positron emission tomography (PET), and optical imaging (OI) (*Artemov et al., 2003; Sharma et al., 2006*). The effectiveness of nanoparticles for use in the various imaging technologies arises from their large surface area to volume and quantum confinement effects (*Schmidt, 2001*). As with other technologies based upon nanoscale systems, advances in nanotechnology currently provide the ability to specifically tailor critical features of nanoparticles, such as composition, size, and shape. In conjunction to these properties, the ability to functionalize the surface of nanoparticles with a variety of different moieties, including targeting ligands, therapeutics, and imaging agents, provides the ability to produce a wide range of novel multifunctional nanovectors. All of these advantages has led to the development of a wide range of nanoparticles systems for molecular imaging, some of the most interesting systems include superparamagnetic iron oxide (SPIO) and

gadolinium (Gd) nanoparticles for MRI (*Evanics et al., 2006; Hifumi et al., 2006; Sun et al., 2005; Sun et al., 2008*).

Over the past few years, there has been an increasing focus on the use of nanoparticle based contrast agents for MRI. This attention has been driven by limitations with conventional contrast agents based on chelates of metal ions, such as a low concentration of metal ion per molecule, short retention times *in vivo*, and difficulty in functionalization to enable use in nanovectors. Nanoparticle based diagnostic agents with targeting capabilities show advantages over conventional contrast agents such as increased retention time, stability, specificity, and enhanced imaging capabilities (*Sanvicens and Marco, 2008*). The addition of active targeting ligands, such as integrins, antibodies, and ribonucleic acid peptides, has increased the applicability of these novel imaging agents into biomedical imaging.

3. Paramagnetic Principles: To understand the magnetic properties of SPIO and Gd nanoparticles it is useful briefly outline the classification of magnetic materials. The magnetic properties of materials are classified based upon their volumetric magnetic susceptibilities (χ), which describes the magnetization induced in a material by an applied magnetic field. In ferromagnetic materials, ordered magnetic states are present and, as such, these materials are magnetic even without an applied external magnetic field. However, the majority of materials demonstrate little magnetism and, typically, this is displayed only in the presence of an applied magnetic field. In this case materials are defined based upon the magnitude of their χ, with diamagnetic materials having a value between $10^{-6} – 10^{-1}$ and paramagnetic materials having a value between $10^{-6} – 10^{-3}$. For these materials, once the applied magnetic field is removed, the magnetic property of the material is not retained. This is particularly important in the preparation of nanoparticles, as the lack of magnetization in the absence of an applied magnetic field allows for nanoparticles to preserve colloidal stability and suppresses aggregation, which is critical for the biomedical application of these nanostructures. Superparamagnetic materials typically consist of ferromagnetic materials on the nanoscale. At this size scale, the materials can maintain one large magnetic moment due to the presence of a single magnetic domain. However, the overall magnetization of the nanomaterials can be removed at temperatures high enough to induce sufficient thermal energy to provoke free rotation of the particles.

4. Chemical and Physical Properties of SPIO Nanoparticles: Most of the focus on nanoparticle contrast agents has been on the use of negative contrast SPIO nanoparticles, with various oral agents being already approved for clinical use (Ferumoxsil, Ferumoxide) and other being in clinical Phases II and III (Ferucarbotran, Ferumoxtran).

SPIO nanoparticles are a classic example of a superparamagnetic material and have significantly larger χ values when compared to paramagnetic materials due to their single crystal nature which results in the entire particle aligning with any externally applied magnetic field. SPIO nanoparticles are typically composed of either magnetite (Fe_3O_4) or maghemite (γFe_2O_3) and consist of single crystal containing regions of unpaired spins. The crystal structure of these ferrite nanoparticles is commonly a close-packed cubic lattice, with oxygen atoms present on the lattice and iron cations at the interstices. When an external magnetic field is applied to SPIO nanoparticles, the magnetic moments of the nanoparticles are aligned in the direction of the magnetic field which results in an improved magnetic flux. The magnetization is believed to result from electron hoping between Fe^{2+} and Fe^{3+} cations that coexist at the octahedral sites of the crystal lattice. The ability of the large magnetic moments of SPIO nanoparticles, present

within an applied magnetic field, to cause considerable fluctuations in the local magnetic field results in rapid dephasing of surrounding protons. This ability to influence the relaxation time, particularly the T2 relaxation times, of surrounding protons makes SPIO nanoparticles particularly suitable for application as contrast agents for MRI.

While there are numerous ways to synthesize SPIO nanoparticles, solution synthesis using a precipitation-based approach is most commonly used to produce nanoparticles for biomedical applications (*Sanvicens and Marco, 2008*). The precipitation-based synthesis procedures for SPIO nanoparticles can be further broken down in to either coprecipitation in the presence of a stabilizing agent, or microemulsion synthesis. The coprecipitation procedure normally involves the coprecipitation of ferrous and ferric salts in the presence of a stabilizing agent, such as polyethylene glycol (PEG), poly(vinyl alcohol), or dextran, and is probably the most used technique to produce SPIO nanoparticles. The use of a stabilizing agent promotes nanoparticle stability, prevents aggregation during synthesis and is also used to introduce biocompatibility to the system. While the coprecipitation procedure for preparing SPIO nanoparticles allows for variation of the size of the nanoparticles by changing the temperature or pH of the aqueous solution used, the technique has only demonstrated limited control over the size distribution of the nanoparticles. This has lead researchers to examine the use of the microemulsion systems for the synthesis of well-defined SPIO nanoparticles.

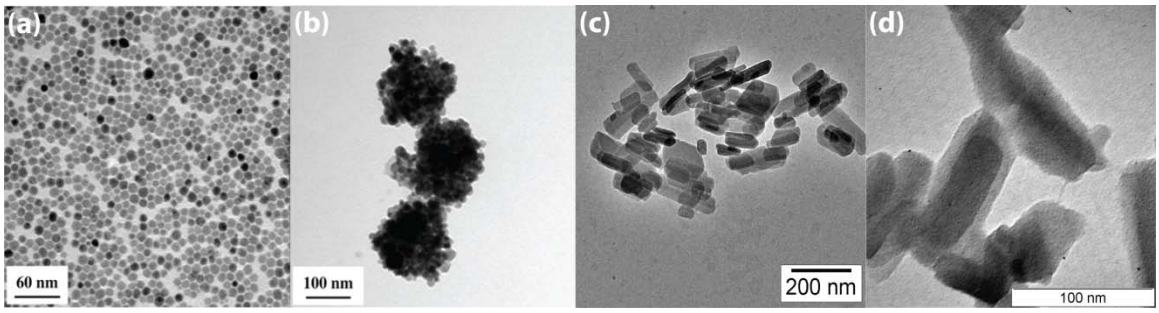

Figure 1. Transmission electron microscopy of (a) 12nm SPIO nanoparticles, (b) approximately 100nm SPIO nanoparticle aggregates following the controlled addition of acetic acid, (c) gadolinium metal-organic framework nanoparticles, and (d) RAFT polymer modified gadolinium metal-organic framework nanoparticles.

The synthesis of SPIO nanoparticles by the microemulsion technique can be achieved using conventional (oil in water) or reverse (water in oil) systems (*Pileni, 2003*). The use of a microemulsion system overcomes many of the problems associated with poor size distributions in SPIO nanoparticles prepared by the coprecipitation procedure. This is primarily due to the ability to produce well defined micelle sizes ranging from 2 to 12nm in microemulsions. The most common microemulsion system for producing SPIO nanoparticles is the reverse microemulsion system. This approach typically involves the use of aqueous ferrous salts that are selectively isolated in the water micelles and separated from the organic continuous phase by a suitable surfactant. The nanoparticles are formed within the micelles by the addition of an alkaline solution to the system and the size of the nanoparticles can be controlled by varying the temperature, surfactant concentration, ferrous salt concentration, or the base concentration. While this method produces SPIO nanoparticles of narrow size distribution, it is often difficult to prepare larger particles (> 20 nm) and the nanoparticles produced are typically soluble only in organic media which limits their use in medical applications (Figure 1).

5. Chemical and Physical Properties of Gd Nanoparticles: The currently employed clinical positive contrast agents for MRI typically consist of Gd^{3+} cations coordinated to various ligands to produce small molecule Gd chelates. Gd^{3+} as a metal ion is effective as a contrast agent for MRI for three basic reasons: (1) it has seven unpaired electrons, which gives it a large magnetic moment, (2) its electron states are symmetric providing much slower relaxation rates, which yields a more stable environment for electron spins, and (3) it has labile water coordinated producing enhanced proton relaxation. Additionally, Gd^{3+} cations are advantageous over Fe-based contrast agents because advances in MRI technology are yielding faster scans giving T1 weighted data, which saturates the images. T1 contrast agents, such as Gd^{3+} based systems, can effectively mitigate the saturation without imparting any distortion of the imaged tissue, due to changes in bulk magnetic susceptibility. Despite these advantages, the Gd^{3+} cation is highly toxic to cells and, as such, for application as a positive contrast agent for MRI the toxicity has been overcome by using chelating ligands (*Hasebroock and Serkova, 2009*). Examples of chelating ligands used include diethylenetriaminepenta acetate (DTPA), for the contrast agent Magnevist® ($[Gd(DTPA)(H_2O)]^{2-}$), and the benzoylmethyl analogue, BOPTA, which is used in the contrast agent Multihance® ($[Gd(BOPTA)(H_2O)]^{2-}$).

Recently researchers have reported procedures for the preparation of positively-enhancing nanoparticles containing Gd. The Gd-based nanoparticles reported include structures based on gadolinium fluoride (GdF_3), gadolinium phosphate ($GdPO_4$), gadolinium oxide (Gd_2O_3), gadolinium hexanedione (GdHex) and acetylacetenoate mixed with emulsifying wax, and gadolinium metal-organic frameworks (MOFs) (*Evanics et al., 2006; Bridot et al., 2007*). These Gd nanoparticles can exhibit relaxivities significantly higher than typical Gd chelates and also provide a positive contrast agent with higher molecular weights for improved retention times and a high concentration of Gd^{3+} ions per contrast agent particle.

Due to the variety of Gd-based nanoparticles that have been prepared, a number of different synthetic techniques have been used (*Murphy et al., 2005*). GdF_3 nanoparticles were synthesized by heating an aqueous solution of $Gd(NO_3)_3{\cdot}6H_2O$ and NaF in the presence of a stabilizing agent, such as citrate or 2-aminoethyl phosphate groups, in order to increase their water stability. While the GdF_3 nanoparticles demonstrated higher relaxivity values when compared to Magnevist®, the particle size was relatively small (approximately 10 – 50 nm) and demonstrated a broad size distribution of particles. The $GdPO_4$ nanoparticles were prepared from a suspension of $Gd(NO_3)_3{\cdot}6H_2O$, $(NH_4)_2HPO_4$, dextran, and water by a hydrothermal method. The dextran acted as a stabilizing agent, which provided both water solubility and enhanced water retention at the surface of the nanoparticle. Nanoparticles formed from $GdPO_4$ once again showed relaxivity ratios similar to clinically employed contrast agents but also suffered from a high degree of size dispersity. Nanoparticles based on Gd_2O_3 were synthesized by a 'polyol' method and subsequently encapsulated within a polysiloxane coating. The Gd_2O_3 nanoparticles demonstrated good T1 relaxation times and also the ability to tune the T1 relaxation time based upon the average radius of the nanoparticle. However, as the size of the nanoparticles increased to greater than 8 nm, there was a lengthening of the T1 relaxation times which severely limited their clinical application.

One of the most interesting Gd-based nanoparticle structures synthesized are those based on MOFs (Figure 1). These nanoparticles are constructed from Gd^{3+} cations and organic bridging ligands such as 1,4-BDC, 1,2,4-benzenetricarboxylic acid (1,2,4-BTC) or benzenehexacarboxylic acid (BHC). Nanoscale MOFs are an up-and-coming field of materials research and are identified as porous polymeric materials consisting of organic bridging ligands linked together by metal ions, providing interesting characteristics such as structural stability, sorption and exchange of liquids and vapors, and nuclear

magnetic resonance and non-linear optical properties. Specifically, the Gd MOF nanoparticles are synthesized by a reverse microemulsion system, which can provide a range of sizes and shapes of nanoparticles by simply changing the type or concentration of bridging ligand or the water to surfactant ratio of the system. Gd MOF nanoparticles have shown exceptional MRI contrastability due to labile water interactions and have shown the ability to incorporate other imaging modalities, such as luminescence, to produce multimodal contrast agents.

The summary of SPIO and Gd based nanoparticle physico-chemical properties is presented in Table 1.

Table 1. Chemical and physical properties of diagnostic capable nanoparticles

Super paramagnetic iron oxide nanoparticles -	**Average Size (nm)**	**Stabilizing Agent**	**Relaxivity ($mM^{-1}sec^{-1}$)**
Ferumoxsil (GastroMARK)	400	Silicone	r_1=3.4, r_2=3.8, B_0=1.0T
Ferumoxide (Feridex)	80-150	Dextran	r_1=40, r_2=160, B_0=0.47T
Ferucarbotran (Resovist)	60	Carboxydextran	r_1=25.4, r_2=151, B_0=0.47T
Ferumoxtran (Sinerem, Combidex)	20-40	?	r_1=25, r_2=160, B_0=0.47T
Liposomes	.5-10	PEG-silane copolymer	Unknown
Gadolinium based nanoparticles -	**Average Size (nm)**	**Stabilizing Agent**	**Relaxivity ($mM^{-1}sec^{-1}$)**
Gadolinium fluoride	30-60	Citrate	?
Gadolinium phosphate	~25	Dextran	r_1=13.9, r_2=15.0, B_0=0.47T
Gadolinium oxide	~9	Polysiloxane	r_1=4.4, r_2=28.9, B_0=7T
Gadolinium hexanedione	~100	Emulsifying wax/PEG	Unknown
Gadolinium metal-organic frameworks	~100	PNVP/Silica	Unknown
Gadolinium metal-organic frameworks	~125	Multifunctional RAFT Copolymer	r_1=33.43, r_2=47.25, B_0=1.5T

6. Surface Modification for Targeted Nanoparticles: Critical to the advancement of the application of nanoparticles as contrast agents for molecular imaging has been the development of surface modification techniques for the nanoparticles. Surface modification of nanoparticles is an essential step in the biomedical application of these structures as it prevents aggregation of the particles, makes the particles biocompatible, renders the particles water soluble, and enables the introduction of almost endless functionality, including targeting moieties and therapeutics (*Schmidt, 2001; Sanvicens and Marco, 2008*). While there are many different techniques available for the surface modification of nanoparticles, including inorganic core-shell structures, liposomes, and small molecule ligands, arguably the most effective and versatile technique is the use of polymers (McCarthy and Weissleder, 2008). Polymeric coatings for nanoparticles provide all of the specific advantages of surface modifications mentioned above and also provide the ability to tune the desired properties by varying such things as the molecular weight of the polymer, the chemical structure of the polymer, the attachment mechanism of the polymer to the surface of the nanoparticle, the surface coverage of the polymer on the nanoparticles, and the conformation of the polymer on the surface of the nanoparticle.

One of the most significant advantages of the use of polymers for the surface modification of nanoparticles is the ability to introduce new functionalities, such as targeting moieties, to improve the *in vivo* performance and application of the nanoparticles (Figure 2). The introduction of targeting moieties to the surface of the nanoparticles provides the ability to selectively deliver the inherent image capabilities of some nanoparticle systems to specific sites of interest within the body. The development of these targeted nanoparticle imaging agents offers tremendous potential for the improvement in both the diagnosis and treatment of a wide range of diseases (*Serkova et al., 2010*). Traditionally the conjugation of targeting moieties to polymeric coating has been achieved via reaction between different reactive groups, such as amines and activated or non-activated carboxylic acids, on the polymers chain and the ligand of

interest. These conjugation reactions have been used to attach a wide range of targeting moieties, with most research focused on the attachment of either peptides or antibodies.

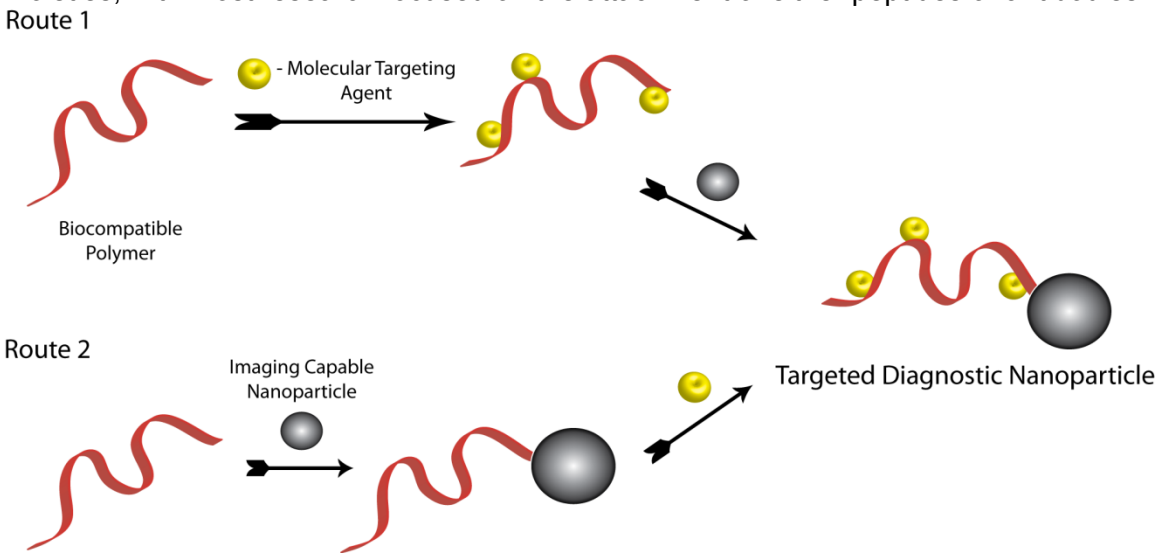

Figure 2: Two routes to incorporation of a molecular targeting agent with an imaging capable nanoparticle, such as a SPIO or Gd nanoparticle, to provide a targeted diagnostic nanoparticle.

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