Synthesis of core/shell nanoparticles for dual contrast MRI

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**Abstract**

This master's thesis was performed at Genovis AB, Sweden, and with the purpose to synthesize a new contrast agent for MRI. MRI is a modern noninvasive tool for imaging biological tissue. To enhance the image quality two different contrast enhancing agents are used, in two different imaging modes, one is called $T_1$ and gives a positive enhancing of the MRI signal and the other is called $T_2$ and increases contrast by reducing signal strength. Commercial contrast agents is optimized for only one mode and both modes have drawbacks reducing image quality. This work details the synthesis of a dual core/shell contrast for imaging in both modes. A particle created by using a core of iron oxide and a outer shell of gadolinium and capable of achieving simultaneous $T_1$ and $T_2$ contrast. This was verified by in vitro MRI and the structure by TEM studies. The toxicity of the particles were evaluated by the use of cellcultures. The dual mode nanoparticles can by switching mode give complementary information that cannot be obtained by the use of only one type of contrast agent. This leads not only to more accurate and diagnostic useful images but also gives the particles a self-confirmation ability, allowing the MRI operator to easily identify the agent by shifting mode and making the interpretation of the image easier and faster.

**Keywords:** Dual mode contrast, MRI, core/shell nanoparticles
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1 Introduction

1.1 Background

Magnetic resonance imaging (MRI) is a powerful noninvasive diagnostic tool for looking at the fine structures in the soft tissue of a body. MRI works by surrounding a patient with a powerful magnetic field. The field causes the spin of the nuclei of hydrogen atoms to align in one of two possible orientations with a small excess in one of them. Radio wave pulses then systematically probe small sections of tissues, disturbing those atoms out of alignment. The MRI then measures the time it takes for the atoms to relax back into alignment. The contrast in a image is generated by the different relaxation times of different sorts of tissue. To increase the contrast and achieve clearer images for more accurate diagnosis, contrast agents are used. MRI contrast agents works by exerting an influence on the longitudinal ($T_1$) or transverse ($T_2$) relaxation times of the surrounding tissue. Paramagnetic complexes containing, for example gadolinium (Gd$^{3+}$) or manganese (Mn$^{2+}$) ions induce a local relaxation of the nearby water protons that shortens $T_1$. A shorter $T_1$ gives rise to positive contrast (bright signal) on $T_1$-weighted MRI images[1, 2]. For $T_2$ contrast, superparamagnetic magnetite nanoparticles have long been synthesized and utilized as ultrasensitive negative contrast for, among others, stem cell tracking and early detection of cancers due to their strong $T_2$ shortening effect[3, 4]. However, since magnetite nanoparticles (NP) give a negative signal represented by dark areas in MRI images, their negative contrast can often be confused with a weak MRI signal arising from adjacent tissues such as air filled pockets (lungs) and of course a reduction of the overall signal strength is never optimal. A dual mode strategy where both $T_1$ and $T_2$ imaging modes are utilized simultaneously can give the contrast a self-confirming ability, enabling the operator to always distinguish it from the background. Further more, depending on the tissue of interest, dual mode contrast can be selectively visualized by $T_1$- or $T_2$-weighted MRI in order to achieve complementary information that cannot be obtained by the use of only one type of contrast agent, thereby leading to more precise diagnosis.

1.2 Scope of this work

The purpose of this project is to synthesize a dual mode contrast agent with a core/shell structure, in which the $T_2$ contrast material consist of a superparamagnetic iron oxide located in the core and the $T_1$ contrast material is gadolinium based and placed as the shell. By inserting a thickness-tunable inert separation layer between the two, the magnetic properties of the agent can be modulated to find the optimum conditions for strong $T_1$ and $T_2$ effects. As a starting point silica was chosen as separation material because of its inert nature and well known synthesis reactions. The ultimate aim of this work is not to investigate the physics of nuclear magnetic resonance nor the chemistry in the synthesis process fully. Rather to combine both fields and use nano-engineering to build a new particle and show that designing on the nano scale can achieve new and superior results. The particles will be grown in steps, adding layers by utilize several well known methods described in literature and modifying them to fit together. The structure of the particles will then be determined using dynamic light scattering and a transmission electron microscope. The magnetic properties of the synthesized particles and their potential as a contrast agent will be evaluated using MRI.
2 The basic physics behind the magnetic resonance imaging.

MRI is a imaging technique mostly used in medical examinations as a noninvasive method for taking images of the inside of the human body. MRI is based on the nuclear magnetic resonance of the atomic nuclei. However, the word nuclear was early dropped from the name due to the false connection it made to the use of nuclear materials in the technique.

Every elementary particle has a property called spin. Spin is a fundamental property of nature like electrical charge or mass. Spin comes in multiples of \( \frac{1}{2} \) and can be either + or -. Spins tend to pair up and in a atom with a even number of elementary particles the spins will cancel each other out[5].

Some substances have several orbitals (a place in space where an electron has some probability of residing) at the same energy level. In this case the orbitals is filled with one electron at the time and all with parallel spins, creating paramagnetic materials. This leads to substances with unpaired electron spins and magnetic properties. A nuclei with uneven mass number, as in a proton, have an unpaired spin that can be used for MRI. Each spin have an intrinsic angular momentum, a vector often denoted \( \mathbf{I} \). This angular momentum also give a magnetic momentum given by equation 1.

\[
\mu = \gamma I
\]

Eq. 1

where \( \gamma \) is the gyromagnetic ratio. \( \gamma \) is constant, for hydrogen \( \gamma = 42.6 \text{MHz/T} \) [1].

Spin could be seen as the particle spinning around its axis, figure 1, but this is a misleading picture. Spin is derived from quantum mechanic theory and the magnetic moment is different from the magnetic moment a charged spinning sphere would give rise to. Spin is a intrinsic property and should not be confused with spinning spheres[6].

In a MRI the unpaired spin of a hydrogen proton (H) is used to generate images, mainly because its abundance in the body. Hydrogen protons can be found in for example water (H\(_2\)O) and in fat (-CH\(_2\)-).

When placed in a strong fixed external magnetic field, \( \mathbf{B} \), the magnetic moment of the protons will align in one of two possible orientations with a small excess in one of them. By convention is the direction of the static field always along the z-axis and the magnitude is \( B_0 \) as experienced by the proton.
The energy of a nuclear spin in the magnetic field then becomes
\[ E = -\mu_z B_0 = -\gamma I_z B_0 \quad \text{Eq. 2} \]

Eventually a small excess, about one in a million\[1\] of the spins, will occupy the orientation along the magnetic field, this being the energy state with the lowest energy. This will create a net magnetization, \( \mathbf{M} \), the sum of all spins. This net magnetization will build up under a few seconds until it reaches a maximum value. The time it takes is dependent on the strength of \( B_0 \) and the kind of tissue examined or more specifically on the mobility of the protons in that tissue\[1\].

When placed in a magnetic field the protons will experience a torque as a result of any imperfections according to equation 3. \[47\]
\[ \frac{dJ(t)}{dt} = M(t) \times B(t) \quad \text{Eq. 3} \]

where \( J(t) \) is the net spin angular momentum of all spins. Using equation 1 and multiplying each side with \( \gamma \) yields
\[ \frac{dM(t)}{dt} = M(t) \times \gamma \mathbf{B}(t) \quad \text{Eq.4} \]

This equation is the basis for understanding the physics behind MRI\[47\]. Felix Bloch formulated a set of equations emanating from equation 4 to describe what happens during a MRI, this is from a stationary frame of reference.

He observed that the nuclear spins relax back to their equilibrium values after being disturbed. The relaxation occur along the z-axis and the x-y plane at different rates called \( 1/T_1 \) and \( 1/T_2 \) respectively. The individual components of equation 4 with added terms for relaxation can be seen in equation 5.

\[ \frac{dM_x(t)}{dt} = \gamma (M(t) \times B(t))_x - \frac{M_x(t)}{T_2} \]
\[ \frac{dM_y(t)}{dt} = \gamma (M(t) \times B(t))_y - \frac{M_y(t)}{T_2} \]
\[ \frac{dM_z(t)}{dt} = \gamma (M(t) \times B(t))_z - \frac{M_z(t) - M_0}{T_1} \]
\[ \frac{dM_x(t)}{dt} = \gamma (M_y(t) B_z(t) - M_z(t) B_y(t)) - \frac{M_x(t)}{T_2} \]
\[ \frac{dM_y(t)}{dt} = \gamma (M_z(t) B_x(t) - M_x(t) B_z(t)) - \frac{M_y(t)}{T_2} \]
\[ \frac{dM_z(t)}{dt} = \gamma (M_x(t) B_y(t) - M_y(t) B_x(t)) - \frac{M_z(t) - M_0}{T_1} \]

If we for the moment ignore the relaxation term, that is \( T_1 \& T_2 \rightarrow \infty \).

By assuming \( M_{xy} = M_x + iM_y \)
\( B_{xy} = B_x + iB_y \)

and utilizing some algebraic tricks the equations can be simplified to
\[ \frac{dM_{xy}(t)}{dt} = -i\gamma (M_{xy}(t) B_z(t) - M_z(t) B_{xy}(t)) \]
\[ \frac{dM_{xz}(t)}{dt} = i\gamma (M_{xy}(t) B_{xy}(t) - M_{xy}(t) B_x(t)) \]

Eq. 7

Eq. 8
where \( \overline{M}_{xy}(t) = M_x - i M_y \)  \hspace{1cm} \text{Eq. 9}

If the magnetic field is constant \( \mathbf{B}(t) = (0,0,B_0) \) and assuming no RF pulse. Then the equations become

\[
\frac{dM_{xy}(t)}{dt} = -i \gamma M_{xy}(t) B_0 \\
\frac{dM_z(t)}{dt} = 0
\]

and have the solution

\[
M_{xy}(t) = M_{xy}(0) e^{-i B_0 t} = M_{xy} [\cos (\gamma B_0 t) - i \sin (\gamma B_0 t)] \\
M_z = M_0 = \text{constant}
\]

\hspace{1cm} \text{Eq. 11}

We see that the transverse magnetization vector, \( M_{xy} \), rotates around the z-axis with \( \omega_0 = \gamma B_0 \) \hspace{1cm} \text{Eq. 12}

the Larmor frequency when the spins are placed in a magnetic field. Where \( \omega_0 \) [Hz] is the angular precessional frequency or simply the resonance frequency of the protons in that specific field and \( B_0 \) [T] is the external magnetic field.

This is called that the protons precess. Remember that while the individual spins precess they are out of phase with each other and \( \mathbf{M} \) therefore still.

The system can easier be understood by changing to a rotating frame of reference according

\[
\dot{M}_z(t) = M_z(t) \\
\dot{M}_{xy}(t) = e^{i \Omega t} M_{xy}(t)
\]

\hspace{1cm} \text{Eq. 13}  \hspace{1cm} \text{Eq. 14}

where \( \Omega \) is the frequency.

Then we get

\[
\frac{d M_{xy}(t)}{dt} = \frac{d (M_{xy}(t) e^{i \Omega t})}{dt} = e^{i \Omega t} \frac{d M_{xy}(t)}{dt} + i \Omega e^{i \Omega t} M_{xy}(t) = e^{i \Omega t} \frac{d M_{xy}(t)}{dt} + i \Omega \dot{M}_{xy}
\]

\hspace{1cm} \text{Eq. 15}

and by substituting in equation 8, this time including the relaxation term

\[
\frac{d M_{xy}(t)}{dt} = e^{i \Omega t} [ -i \gamma (M_{xy}(t) B_z(t) - M_z(t) B_{xy}(t)) - \frac{M_{xy}}{T_2} ] + i \Omega \dot{M}_{xy}
\]

\[
= -i \gamma (M_{xy}(t) e^{i \Omega t} B_z(t) - M_z(t) B_{xy}(t) e^{i \Omega t}) - \frac{M_{xy} e^{i \Omega t}}{T_2} + i \Omega \dot{M}_{xy}
\]

\hspace{2cm} \text{Eq. 16}

To be able to account for inhomogeneities in the magnetic field we assume that \( B_z(t) = B_z(t) + \Delta B_z(t) \) \hspace{1cm} \text{Eq. 17}

i.e. that the magnetic field experienced by the protons consist of one constant part and one that can vary with time.

\[
\frac{d M_{xy}(t)}{dt} = -i \gamma (M_{xy}(t)(B_0 + \Delta B_z(t))) - M_z(t) \dot{B}_{xy} + i \Omega \dot{M}_{xy} - \frac{M_{xy} e^{i \Omega t}}{T_2}
\]

\[
= i(\Omega - \omega_0) \dot{M}_{xy}(t) - i \gamma \Delta B_z(t) \dot{M}_{xy}(t) + i \gamma B_{xy}(t) M_z(t) - \frac{M_{xy}(t)}{T_2}
\]

\hspace{1cm} \text{Eq. 18}
This equation is simpler than it looks.
The first term is the Larmor term for a frame of reference that is rotating with angular frequency $\Omega$.
This becomes zero, if $\Omega = \omega_0$, if the frame of reference rotates with resonance frequency.

If a electromagnetic wave with the Larmor frequency, $\omega_0$, is sent into the tissue the protons will start to resonate. Since this frequency is in the radio range this pulse is often referred to as the radio frequency pulse or RF pulse[1].
This is what is described by the last term, $i\gamma B_{xy}M_{xy}$. $B_{xy}$ is the new magnetic field caused by the RF pulse sent in perpendicular to the static field.
By analogy to equation 12 can we now see that the term $\gamma B_{xy}$ is the frequency of the magnetization vector precessing around the axis of the RF pulse. Therefore by applying the RF pulse during a certain time will allow different degrees of rotation around x-axis. The RF pulse is typically very short, shorter then both $T_1$ and $T_2$ to allow the vector to tip but not to rotate[47].
Since $B_{xy}$ is much weaker then $B_0$ the precession will also be much slower.
The result of this is a spiral motion flipping the sum magnetization vector of the protons into the x-y plane, figure 2.
When the magnetic moments precess around only the z-axis they will be randomly distributed around the central axis and out of phase with each other.
When the protons are affected by the new oscillating RF wave the magnetic moments will also be forced into one phase[1].

The second term in equation 18, $-i\gamma \Delta B_z M_{xy}'$, describes the deviations in the magnetic field caused by local inhomogeneities.
It is this term that will be affected by the addition of contrast agents. The agents creates local inhomogeneities that promote a faster relaxation.

At the time right after the RF pulse ends and rotating with $\omega_0$. Assuming the $\Delta B_z$ is constant for small times will give:
$$\frac{d M_{xy}(t)}{dt} = (-i\gamma \Delta B_z - \frac{1}{T_2}) M_{xy}(t)$$
Eq. 19
with the solution
$$M_{xy}(t) = M_{xy}(0) e^{-i\gamma \Delta B_z t - \frac{t}{T_2}}$$
Eq. 20
The exponent in equation 20 is what actually measured and called $T_2^*$. $T_2^*$ is the theoretical time plus the time caused by any disturbances. The contrast agents induce changes in $\Delta B_z$ causing faster $T_2^*$ but from here on this is referred to as just shortening the relaxation time.

The same can be done for $T_1$ by adding additional terms for diffusion. $T_1$ relaxation are believed to work by the temporary binding of protons to the contrast agent[48]. By adding terms for diffusion and binding time the effect of $T_1$ contrast agents can be theorized and solved numerical.
2.1 $T_1$ and $T_2$ relaxation time

The spins absorb energy from the RF pulse to be able to get to the higher energy state that being misaligned from the strong $B_0$ represent. When the RF pulse ceases the spins will revert back to their equilibrium state aligned with $B_0$. This is called relaxation. $T_1$ is called longitudinal relaxation time and is characterized by the time it takes for the spins to realign along the $z$-axis, figure 2. $T_1$ is the time after the RF pulse ceases it takes for the net magnetization in $z$ direction, $M_z$, to recover to approximately 63% of its equilibrium value of $M_0$, given by equation 21 and figure 3.

Assuming $B'_z(t)=B_0$ i.e. the protons are exposed to a static eternal magnetic field, after the RF pulse and if $\Omega = \omega_0$, then $M'_{xy}$ will be experienced as stationary and only depend on relaxation, equation 21.

$$M_z(t) = M_0(1 - e^{-\frac{t}{T_1}})$$  \hspace{1cm} \text{Eq. 21}

For the $T_1$ process the excess energy is given of to the surrounding lattice of molecules in order to
get back to equilibrium. This means that $T_1$ also is specific for each type of tissue. A water molecule is mobile and have no fix lattice to lead away the energy, this gives a low probability per time unit to get rid of the energy. This leads to a long $T_1$ time, table 1 & 2, whereas fat tissue have a short $T_1$ time. [1]

$T_2$ characterize the time it takes for the $M_{xy}$ component to decay given by equation 22 and figure 4. Assuming $B'_z(t)=B_0$ i.e. the protons are exposed to a static eternal magnetic field, after the RF pulse and if $\Omega = \omega_0$, then $M'_{xy}$ will be experienced as stationary and only depend on relaxation.

\[
M_{xy}(t)=M_{xy}(0)(e^{-t/T_2})
\]  

Eq. 22

Due to precession and measured by a coil in the x-y plane will cause the decay curve to be superimposed on a sinus curve and look like figure 5.

The 5-10 times faster relaxation time of $T_2$ compared of $T_1$ is due to dephasing[1]. Dephasing is the randomization of the magnetization vectors of the excited spins with the same phase coherence after the RF pulse[8]. Dephasing happen because of the inhomogeneities, $\Delta B_z$, in the magnetic field. Inhomogeneities in the field are created by the magnet in the MR machine or are internal inhomogeneities caused by the spins themselves. Because the spins may point with $B_0$ or away from it, the spins affect their neighbors slightly different. Also the proton interactions with macromolecules, such as proteins, or contrast agents in the tissue can induce local changes in the magnetic field[8]. The disturbances in the magnetic field will cause the spins to have difference precess frequency following from equation 12 where $B_0$ instead will be $B_0 + \Delta B$. The difference in frequency will cause the spins to start to get out of phase directly after the RF-pulse. Since the net magnetization $M_{xy}$ is the summation of all vectors for all spins, when they start to get out of phase the vector sum decreases rapidly[1]. $T_2$ depends on how fast protons in that tissue loses their phase coherence. This is primarily depending on the density of protons, examples in table 2,[1]. Higher density means more spin-spin
interactions and faster dephasing, table 1.[1].
Fluids for example have relatively long distances between molecules and therefore long $T_2$
$T_1$ characteristics of a tissue has to do with how efficient the energy transfer away from the protons
are. Testing has shown that the most efficient energy transfer occurs when the natural
vibration/rotation frequencies of the molecules are about the same as the Larmor frequency. The
Larmor frequency depends on the magnetic field according to equation 12 while the vibrational
frequencies depend on the physical state of the tissue[1]. The vibrations of for example water causes
the magnetic field the proton creates to fluctuate at the same frequency and it is this disturbance in
the magnetic field that stimulates relaxation.
For fat the natural rotational frequency around the C—C bond is near the Larmor frequency and
therefore have a very efficient energy transfer[1]. The opposite is the small water molecule that
vibrates at much higher frequencies resulting in bad energy transfer[1].

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Approximate $T_1$ [ms]</th>
<th>Approximate $T_2$ [ms]</th>
<th>Main field [T]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipose tissues</td>
<td>240-250</td>
<td>60-80</td>
<td>1.5</td>
</tr>
<tr>
<td>Whole blood (oxygenated)</td>
<td>1350</td>
<td>200</td>
<td>1.5</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>2200-2400</td>
<td>500-1400</td>
<td>1.5</td>
</tr>
<tr>
<td>Grey matter</td>
<td>920</td>
<td>100</td>
<td>1.5</td>
</tr>
<tr>
<td>White matter</td>
<td>780</td>
<td>90</td>
<td>1.5</td>
</tr>
<tr>
<td>Liver</td>
<td>490</td>
<td>40</td>
<td>1.5</td>
</tr>
<tr>
<td>Kidneys</td>
<td>650</td>
<td>60-75</td>
<td>1.5</td>
</tr>
<tr>
<td>Muscle</td>
<td>860-900</td>
<td>50</td>
<td>1.5</td>
</tr>
</tbody>
</table>

*Table 2: Approximation values for the time constants in humans[9].*

2.1.2 $T_1$-weighted and repetition time, TR.
To be able to create a picture the same cut must be excited many times. TR is time between two
consecutive RF pulses. TR directly affects $T_1$-contrast because it regulates the amount of time
available to relaxation after the last pulse. A long TR allows a greater realignment along the z-axis
and a larger angle to flip with a RF. Choosing a short TR will highlight tissues with short $T_1$. Tissue
with short $T_1$, ex. fat, will be able to recover to a greater degree which gives a greater net
magnetization which induces a greater current which gives a brighter spot on the picture. Tissues
with a longer $T_1$ will not have time to relax as much and will consequently have a smaller magnetization. This kind of picture with short TR contains more information from $T_1$ and is therefore called $T_1$-weighted and is described mathematically by equation 24,[10].

2.1.3 $T_2$-weighted and time to echo, TE

TE is the time between the RF pulse and the measurement. TE influence $T_2$ contrast. Choosing a TE in the same order of magnitude as $T_2$ of the interesting tissue type will highlight differences in $T_2$ relaxation. Tissues with shorter $T_2$ will be more out of phase and lost most of their net magnetization, $M_{xy}$. Tissues with longer $T_2$ will still give a strong signal. Since a short $T_2$ will have a faster dephasing and a faster loss of signal, tissues with short $T_2$ will appear darker, figure 6. A bigger difference between the decay curves will give a sharper contrast. A contrast agent will seek to increase this difference.

A picture with long TR and long TE is called $T_2$-weighted and will give dark spots for tissues with short $T_2$ times, see table 2,[1].

![Figure 6: Different relaxation times give contrast. Adapted from [11]](image)

The intensity of the received signal, $SI$, can be described by equation 24, which also describes the relationship between TR and TE[1].

$$SI \propto N(H) e^{-\frac{TE}{T_2}} (1 - e^{-\frac{TR}{T_1}})$$  \hspace{1cm} \text{Eq. 24}$$

where $N(H)$ is the number of mobile protons. By varying TR and TE can the operator effectively chose to look at $T_1$ or $T_2$.

For example if $TR \to \infty$ then $1 - e^{-\frac{TR}{T_1}} \to 1$ and $SI \to N(H)(e^{-\frac{TE}{T_2}})$. If TR is 4 or 5 times $T_1$, then the $T_1$ effect becomes negligible.[1]

2.2 Magnetic susceptibility

All materials get magnetized to a certain degree when placed in a magnetic field. Magnetic susceptibility is a measure on how much a material can be magnetized and is defined as

$$M = \chi H$$  \hspace{1cm} \text{Eq. 25}$$

where $M$ is the magnetization and $H$[A/m] the field.

Ferromagnetic materials, ordinary magnets, are permanently magnetized due to small magnetic domains where all magnetic moments are aligned[12]. This causes each domain to have a strong magnetic field. However the domains themselves are randomly oriented with respect to one other causing the bulk material to often be unmagnetized. If placed in a external magnetic field the
domains will line up the bulk material becomes permanently magnetized. Paramagnetic substances becomes magnetized while in a external field but loses their magnetization when the field is turned off. Their induced magnetic field is in the same direction as the inducing field and consequently gives a small increase in field strength. Materials with a large number of unpaired spins, for example gadolinium with seven unpaired electrons, are strong paramagnetic substances[1]. The magnetic moments of these unpaired spins creates the net magnetization when they orient themselves after the external field.

Superparamagnetism occurs when the material is composed of very small single domain crystals, for example in a single nanoparticle with a hydrodynamic diameter, $D_{H} \leq 30$ nm. In this case even when the temperature is below the Curie or Neel temperature (the thermal energy needed to overcome the coupling forces between neighboring atoms and destroy any magnetic order, turning the material paramagnetic), the thermal energy is sufficient to change the direction of magnetization of the entire crystal.

The resulting rapid fluctuations in the direction of magnetization cause the total to average to zero. Thus the material behaves in a manner similar to paramagnetism, except that instead of each individual atom independently changing direction the whole crystal changes. When influenced by an external magnetic field, the magnetic moment of the entire crystal will align with the magnetic field[12]. Giving it a susceptibility 100 to 1000 times stronger then a paramagnetic material[1].

3 MRI contrast agents

To ensure as high quality picture as possible, contrast agents are used. A greater difference in relaxation time between two areas will give a sharper contrast in the picture, figure 6. Contrast simply refers to the signal difference between two regions, for example soft tissue and bone which gives a great contrast in a ordinary x-ray due to the large density difference. However a MRI is more complicated but can in return differentiate between tissues with the same density. Contrast agents are used to shorten the relaxation times of the selected tissue and thereby increase the contrast to surrounding tissue[8].

MRI contrasts today are sorted as $T_1$ or $T_2$ agents based on what relaxation process they enhance. All contrast agents promote both $T_1$ and $T_2$ but one mode usually dominates and the only way to say which mode is to test the substance.

The strength of the contrast agents is usually measured in relaxivity, $R_1 = 1/T_1$ at 20°C and one molar [mM$^{-1}$s$^{-1}$].
<table>
<thead>
<tr>
<th>Name</th>
<th>Core size [nm]</th>
<th>Coating materials</th>
<th>Total size [nm]</th>
<th>$R_2^x$ [mM$^{-1}$s$^{-1}$]</th>
<th>$R_1^x$ [mM$^{-1}$s$^{-1}$]</th>
<th>$R_2/R_1$</th>
<th>B [T]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feridex</td>
<td>5-6</td>
<td>Dextran</td>
<td>80-150</td>
<td>1.00</td>
<td>10</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Resovist</td>
<td>~4.2</td>
<td>Carbodextran</td>
<td>62</td>
<td>151</td>
<td>10</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Combidex</td>
<td>4-6</td>
<td>Dextran</td>
<td>20-40</td>
<td>53</td>
<td>10</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>CLIO;MION</td>
<td>ca. 2.8</td>
<td>Dextran</td>
<td>10-30</td>
<td>69</td>
<td>1.5</td>
<td>1.5</td>
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<tr>
<td>Magnevist</td>
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<td></td>
<td>4.6</td>
<td>4.6</td>
<td>1</td>
<td>1.5</td>
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<tr>
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<tr>
<td>NIMT®FeODot</td>
<td>11</td>
<td>PEG-Amine</td>
<td>18</td>
<td>400</td>
<td>2.4</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Fe$_3$O$_4$</td>
<td>12</td>
<td>DMSA, PEG</td>
<td>15</td>
<td>218</td>
<td>4.6</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>MnFe$_3$O$_4$</td>
<td>12</td>
<td>DMSA</td>
<td>15</td>
<td>358</td>
<td>1.1</td>
<td>325.5</td>
<td>1.5</td>
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<tr>
<td>FeCo/GC</td>
<td>7</td>
<td>Carbon and phospholipid–PEG</td>
<td>30</td>
<td>644</td>
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<td>D-glucuronic acid</td>
<td>10.9</td>
<td>9.9</td>
<td>1.1</td>
<td>1.1</td>
<td></td>
</tr>
</tbody>
</table>

$x. The R_2 & R_1 values are taken from literature and can vary depending on the field strength and MR pulse sequences.$

CLIO;MION - Cross linked iron oxide; magnetic iron oxide nanoparticles
DMSA - Dimercaptosuccinic acid
PEG - Polyethylene glycol

Table 3: New and commercial contrast agents.[16][2][18][Genovis]

### 3.1 $T_2$ contrast agents

The commercial available $T_2$ agents are often iron oxide nanoparticles (NPs), Fe$_3$O$_4$, which exhibits superparamagnetic properties with a magnetic susceptibility many times greater than that of paramagnetic materials. $T_2$ agents based on paramagnetic materials exist but are less effective. Iron oxide is in its bulk form ferromagnetic and have a large magnetic susceptibility which persist without any external field. When in nanoscale, iron oxide particles lose their ability to hold magnetization without an external field and become superparamagnetic. When a magnetic field is applied they exhibit a strong magnetization which causes local field inhomogeneities described in equation 18. Disturbances in the magnetic field will cause the precession frequency of different spins to be slightly different. superparamagnetic substances cause strong disturbances leading to larger differences in precession frequency and hence a faster dephasing. Because $T_2$ agent give a signal-decreasing effect they are called negative agents and should have as large $R_2$ as possible to be effective. This signal-decreasing effect is the main drawback with $T_2$ agents, meaning that you lose some of the fine details. Also the dark areas can easily be confused with other pathogenic conditions, like bleeding or metal deposits, or with naturally dark organs for example the lungs which appear dark due to the relative low proton density of air. Other drawbacks are that the high susceptibility of $T_2$ contrast agents induces some distortion of the surrounding tissue. This distortion...
is caused by the steep gradient in magnetization and is called susceptibility artifacts or “blooming effect” [4]. On the positive side is the size and effectiveness of iron oxide nanoparticles. The size is the main factor that controls the characteristics of the particles, such as blood half-life and distribution. Smaller particles (D_{H}<20nm [14]) can easily penetrate blood vessel walls and into tissue and the lymphatics system. NPs opens up the possibility of intracellular imaging and stem cell tracking due to their small size[3].

3.2 $T_1$ contrast agents

Commercially available $T_1$ contrasts are usually paramagnetic metal complexes based on gadolinium (Gd), dysprosium or manganese which have a large number of unpaired electrons[1, 8]. Paramagnetic material become magnetized when placed in a external magnetic field and their induced fluctuating magnetic field amplifies the effective field strength locally[1]. The fluctuations arise from brownian motion. The strength of the magnetic moment of the ion, directly affecting relaxation, is roughly proportional to the number of unpaired spins. The size of the amplification is not the only factor affecting, remember that it is the effect of the substance that is measured not the concentration. Interactions with the environment is great part of it, also the frequency of the fluctuations should be in the same time span as the Larmor frequency, compare with efficient energy transfer in chapter 2.1,[1].

Due to the high toxicity of heavy metals such as gadolinium, all conventional agents are in the form of a large chelate complex (a ligand with multiple bonds to a single central ion). This lowers the toxicity but also diminish their effectiveness.

There is no biochemistry based on gadolinium in the human body and consequently no pathway to get rid of gadolinium if it becomes incorporated into the tissue. Therefore is there a need to bind the metal ion to a complex that will be excreted. Other ions such as manganese that do appear in the body are researched in spite of fewer unpaired electrons and lower magnetic moments[8]. The magnetization strength of the contrast is directly dependent on the number of metal ions[8] and the large complexes limit the number of ions per volume. Also the complexes tend to have short life spans in the body and work in an unspecific manner[8]. Most $T_1$ contrast agents do not enter any cells when they spread in the extra cellular matrix and usually interacts with the blood which limit their use for longer time tracking[8].

That why the design is crucial when developing a new contrast agent.

3.3 Why the need for dual mode contrast?

Contrast agents are administered to enhance the signal effects and produce a clear and sensitive image[17]. Presently, gadolinium ion complexes are used to decrease local $T_1$ and superparamagnetic iron oxide NPs are used for $T_2$-weighted images. In effect, $T_1$-weighted and $T_2$-weighted images show complementary information. Which means that both could be needed for a diagnosis and this means two separate injections of contrast agents.

With the creation a dual mode nanoparticle contrast this problem is solved. This dual mode agent not only maintains the superior sensitivity and small size of $T_2$ contrasts but have the ability to show positive contrast. Switching between $T_1$ and $T_2$ modes enables a form of self-confirmation in images and leads to a higher accuracy and easier interpretation of MRI images[2]. The ability to utilize both negative and positive contrast makes it possible to always find the particles regardless of the background. To illustrate the problem, figure 7 show a MRI image of a rat.
Figure 7 shows two separate MRI images of a cut through a rat. The large space in the top is the stomach. In the left picture fat has been suppressed. The purpose with the picture was to look at two lymph nodes, marked with arrows. One node is visible with fat as a background, the other in a second scan when fat is suppressed. In this case fat is used as a dual contrast agent. However this required two separate scans and only works when the background is fatty. For example is many tumors is surrounded with water.

With a dual contrast agent the same thing can be done in one scan and regardless of the background. Another example is the fact that it is hard to distinguish between collections of $T_2$ agents and certain tissue types like vasculature and bone. If a dual agent is used the mode can be switched and the $T_1$ component causes the particles appear brighter while blood vessels stay dark.

### 3.3.1 Core-shell structures

One of the difficulties in creating a dual mode contrast rises from the fact that in the case of direct contact, between the $T_1$ and $T_2$ materials, the magnetic field from the superparamagnetic $T_2$ agent perturbs the $T_1$ relaxation process. This phenomenon induces a undesirable quenching of the $T_1$ signal[2].

This can be solved by creating a particle with a core-shell structure in which the $T_2$ material is located in the core and the $T_1$ material in the shell.
By inserting an isolating layer between them the disrupting effects of the superparamagnetic material can be decreased and a thickness can be achieved where the conditions are optimal for simultaneous strong $T_1$ and $T_2$ contrast effects. The adjustable silica layer should give strong $T_2$ for small particles and $T_2$ should decrease as the core becomes more isolated. $T_1$ should follow the opposite, close proximity with the iron core should dampen it.

Optimal conditions is not entirely dependent on relaxation strength, size is also a important factor. Maximizing $T_1$ by increasing silica thickness may only be feasible to a limit where the particle becomes to large and unable to spread effective or have a too short blood half-life.

The $T_1$ contrast material is positioned on the shell of the particles so that it is in direct contact in order to facilitate the energy transfer away from the protons, figure 8,[2, 13]. The superparamagnetic $T_2$ is positioned in the core since it produces a long range magnetic field that promotes dephasing[2]. At least in theory this is what should happen, in reality very often unforeseen interactions take place when the distances are on nanoscale, resulting in surprising outcome.

Contradicting reports exist suggesting that some not fully explained interaction take place with different results for different studies. Ki Hyun Bae et.al. suggest in a recent article [4] a dual contrast nanoparticle with gadolinium ions anchored to iron cores with proteins. Their findings show that the gadolinium instead reduces the $T_2$ effect of iron oxide. This is however for a situation when the substances not are in direct contact making it less reliable in this case.

More research is needed before the interactions between iron and gadolinium can be explained.

Other ways to achieve simultaneous $T_1$ and $T_2$ contrast have been reported. FeCo particles coated in graphite shell show dual $T_1$ and $T_2$ properties, table 3,[18]. Also contrast in the form of a metastable colloidal solid solution of Mn$_x$Fe$_{1-x}$O$_x$ have been suggested[17].

### 3.4 Size effects on magnetic nanoparticles

As mentioned before is size one important parameter for contrast agents. The size determine the agents ability to spread and to enter the lymphatic systems[16]. The size also directly effects the magnetic properties of the particles. In the ideal case all of the magnetic spins in the particle will be aligned with the external field. However in NPs the surface spins tend to be slightly tilted due to the small size, forming a magnetic disordered surface layer, figure 9,[16, 19].

![Figure 9: Canted surface spins. Adapted from[19]](image)

Such canted surface spins have a effect on the contrast enhancing effects that becomes more pronounced as the particle get smaller. The relaxivity coefficient, $R_2$, gradually increases from approximately 78 to 106, 130, and to 218 mM$^{-1}$s$^{-1}$ for 4 nm, 6 nm, 9 nm, and 12 nm sized Fe$_3$O$_4$
While \( R_2 \) increase with particle size up to certain level, \( R_1 \) show nothing of this behavior. A study by Ja Young Park et. al. on the \( R_1 \) relaxivity of \( \text{Gd}_2\text{O}_3 \) shows that the optimal particle size is 1-2.5nm and decreases as the volume increases. This suggest that only the surface \( \text{Gd}^{+3} \) ions significantly contribute to the relaxation process, possible by binding to the protons[13, 2, 48]. One other possibility that can explain the higher values achieved by Gd oxides compared to chelates, for example Magnevist, is that the surface ions cooperate. On a nanoparticle several ions can cooperatively induce relaxation of one water proton and as a result accelerate it[13]. This is not possible in a chelate as the Gd ions are to separated.

3.5 Modes of targeting

Contrast agents can be either passive or active. Passive agent spreads by blood circulation and diffusion and the limiting factors are their size and charge. Larger nanoparticles (20<\( D_{\text{H}} \)<100nm) are rapidly cleared, in order of several minutes, by mainly liver and spleen[14]. This enables good imaging of those organs due to the accumulation of particles there. Smaller particles (\( D_{\text{H}} \)<20nm) have a much longer circulation time (hours to day) and easily spreads into the tissue and to the lymphatic system[14].

The surface of NPs can be functionalized with biomolecules, often antibodies, to actively select certain tissues to be highlighted. The antibodies bind to the tissue and help to retain the particles in that area for a prolonged time and in higher concentrations. This makes it possible to track migrating cells with high selectivity[3]. Also the particles needs to be coated with biocompatible materials to ensure that they are stable and do not start to wear down[14] or grow in size to much with attacking antibodies.

3.5.1 Coating

Synthesis of the nanoparticles are often carried out in non-polar organic solvents and this require hydrophobic surfactants e.g. oleic acid. The surfactants are used to stabilize the suspension, preventing the particles from aggregate. Before the particles can be used in a aquatic environment e.g. the human body, must the surface of the particles be treated with hydrophilic molecules that will give a high colloidal stability and to avoid aggregation in biofluids. In case of toxic cores the coating must also prevent any leaching out. The first coatings was done by introducing small bifunctional molecules as ligands[16]. The molecules typically consist of two parts, a region that can bind to the nanoparticle and a hydrophilic region facing the aqueous medium. Commercial contrast agents today utilizes dextran coating[8]. Other coatings are PEG[16], DMSA[16] and graphitic shells[18], see table 3. One interesting inorganic particle coat, considering its relative low cost and wide availability, is silica. Silica forms a protective barrier around the magnetic core, preventing contact with water. Also biomolecules can be covalently attached to the silica shell[20]. Biomolecules such as targeting probes; antibodies, oligonucleotides, aptamers, and other imaging probes[8]. How well this would work for a gadolinium based \( T_1 \) is unclear as literature[2, 13] suggest that gadolinium need direct contact with water to function effective.

4 Toxicity

Some issues regarding toxicity exist, mainly because of the use of gadolinium.

4.1 Iron oxide

Iron oxide is considered safe due to the large metabolic activity involving iron, iron oxide is
metabolized into elemental iron by either hydrolytic enzymes or the acidic conditions found inside lysosomes.[16] Records of the clinically approved Feridex reports an overall incidence of adverse events of 9.4% (114/1535 subjects) with back and leg pain the most common event reported (3.6%). Pain severe enough to cause interruption or discontinuation of the infusion was reported to have occurred in 55/2240 (2.5%) patients[2.6]. The largest pre-clinical study was done 1995 and included 208 patients in a phase III trial. The patients received 213 doses of 10 μmol Fe/kg given as a slow IV infusion. Eight percent experienced adverse reactions classified as possibly or probably related to drug administration. No serious adverse reactions were reported[21].

4.2 Gadolinium

Free Gd$^{3+}$ is acutely toxic due to its tendency to precipitate and be deposited in liver, lymph nodes, and bones. Since the body lack natural processes including gadolinium it also lack the ability to metabolize gadolinium. Gadolinium may also obstruct calcium-ion passage through muscle cells, and block the flow of calcium in nerve tissue cells, causing the arrest of neuromuscular transmission[21]. For these reasons is gadolinium always bound in a chelate when administered as a contrast. The primary factor contributing to the toxicity of gadolinium complexes is the extent to which gd$^{3+}$ can replace some metals, especially zinc. This phenomenon is called trans-metallation. This is depending upon the stability of the complex. A stable chelate is less likely to release gadolinium ions.

Headache, nausea, taste perversion, and hives are typically the most frequent adverse effects reported. Anaphylactoid reactions involving respiratory and cardiovascular shut down leading to death, have been reported in 1/500,000 cases[21].

New studies have also shown that giving gadolinium based contrast agents to patients suffering from renal insufficiency increases the risk for nephrogenic systemic fibrosis. This is considered so severe that FDA and Fass recommend avoiding the substances entirely unless no other alternative can be found[15].
5 Synthesis theory

The dual contrast particles are being synthesized in a multiple step reaction.

5.1 Synthesis of cores

The first step to synthesize the particles is the synthesis of the inner core. Several different cores was produced using the same reaction.

5.1.1 Fe$_3$O$_4$ cores

Synthesis of monodispersed metal oxide nanoparticles by thermal decomposition of a metal carboxylate salt[23]. Oleic acid was used both as stabilizing surfactant and as a reactant in forming the intermediate carboxylate salt, figure 10. At high temperatures FeO(OH) reacts with oleic acid to form iron(III) oleate which is the precursor for the pyrolysis reaction forming Fe$_3$O$_4$, as shown in figure 10,[23]. Amount of reactants are found in table 5.

\[ \text{FeO(OH)} + \text{Oleic acid} \rightarrow \text{Fe}_3\text{O}_4 \]

Figure 10: Synthesis of iron cores

5.1.2 Gd$_2$O$_3$ cores

Synthesis gadolinium oxide cores was carried out in the same way as iron oxide cores but with gadolinium acetate as precursor[22]. Gadolinium acetate reacted with oleic acid in a organic solvent, 1-octadecene, under high temperature. The formed gadolinium carboxylate decomposed under heat to gadolinium oxide particles. Results and reaction amounts are presented under results.
5.2 Silica shell

There are several methods currently used to synthesize silica-coated magnetic nanoparticles. The four most common are mentioned here. The first is the sol-gel method in which the silica is formed on colloidal magnetic particles in a simple alcohol/water mixture[24]. This is a popular method because of its relative non-toxic properties and that there is no need for a surfactant. The method is limited by problems with agglomeration when synthesizing certain sizes of particles. Approaches to reduce this problem such as ultrasound have been reported with somewhat success[25] but particles around 20-30nm are still hard to achieve with the sol-gel method[26]. The second method start with pre-synthesized silica particles and deposits metals in the pores of the silica to form the magnetic phase[24]. The third approach is aerosol pyrolysis and is done by letting a mixture of silicon alkoxides and a metal compound react in a flame environment[24]. More recently a fourth method was suggested, the water-in-oil microemulsion method. This is the method used in this work.

5.2.1 Water-in-oil TEOS reaction

SiO$_2$ coating of the Fe$_3$O$_4$ particles was done by the formation of a reverse water in hexane microemulsion to form a suspension of magnetic NPs. Microemulsions are defined as clear thermodynamically stable dispersions of two immiscible liquids containing a stabilizing surfactant[46]. A W/O (water-in-oil) consist of a oil phase, a water phase and a surfactant. The silica were then formed around the iron cores by the hydrolysis and condensation of tetaethyl orthosilicate (teos) in the water phase, figure 11[27, 2]. The iron cores were dispersed in hexane, still stabilized by oleic acid from their synthesis.

![Figure 11: Illustration of the process of reverse micelle and hydrolysis-condensation silica coating. Adapted from [43]](image)

Igepal CO-520 (polyoxyethylene nonylphenylether) was used as a nonionic surfactant to form micelles. When added to a suspension containing particles capped with oleic acid, the Igepal can interact with the oleic acid and partially or completely replace it[28]. This involves the hydrophilic carboxylate and polyoxyethylene groups of oleic acid and Igepal, respectively[28]. This will allow the normally hydrophobic iron oxide particles to be encapsulated within the water phase of the reverse microemulsion, figure 12,[29]. When teos is added it will diffuse in trough the surfactant layer and react in the water phase.
The hydrolysis/condensation reaction is catalyzed by ammonia according to reaction 1 and will deposit silica oxide on the surface of the iron oxide particles\[30\].

\[
\begin{align*}
\text{Si}(\text{OC}_2\text{H}_5)_4 + 4\text{H}_2\text{O} & \xrightarrow{\text{NH}_3} \text{Si}(\text{OH})_4 + 4\text{C}_2\text{H}_5\text{OH} \\
\text{Si}(\text{OH})_4 & \xrightarrow{\text{NH}_3} \text{SiO}_2 \downarrow + 2\text{H}_2\text{O}
\end{align*}
\]

Reaction 1

The required amount of tetraethyl orthosilicate (teos) needed for a specific shell thickness was determined theoretically, taking into account the number of core particles, using equation 26.

\[
V_{\text{teos}} = N_{\text{part}} \frac{\rho_{\text{SiO}_2} M_{\text{teos}}}{\rho_{\text{teos}} M_{\text{SiO}_2}} \left[ \frac{4}{3} \pi \left( (r + Si_{\text{shell}})^3 - r^3 \right) \right] 
\]

Eq. 26

Where \(Si_{\text{shell}}\) is the shell thickness, \(r\) the radii of the core particle and \(V, \rho, M\) is the volume, density and molecular weight. \(N_{\text{part}}\) is the number of \(\text{Fe}_3\text{O}_4\) particles.

5.3 Gadolinium layer

The adding of a gadolinium based \(T_1\) active shell was done by a homogeneous precipitation method. Homogeneous precipitation is based on the thermal instability of some organic bases, for example hexamethyltetraamine and urea\[31\]. Aqueous solutions of these substances are relatively stable at normal conditions. The increase of temperature above \(\sim 70^\circ\text{C}\) leads to their gradually decomposition and produce \(\text{NH}_3\) and carbonate ions, reaction 2, 3 and 4. The increase in pH leads to hydrolysis of metals ions present in the solution\[31\]. The gadolinium will react with carbonate ions, reaction 5. As mentioned above, the first step is the hydrolysis of urea\[32\]:

\[
\text{OC}(\text{NH}_2)_2 + \text{H}^+ + 2\text{H}_2\text{O} \rightarrow \text{HCO}_3^- + 2\text{NH}_4^+ 
\]

Reaction 2

which takes place in two steps. The first reversible

\[
\text{OC}(\text{NH}_2)_2 \xrightarrow{k_1} \text{OCN}^- + \text{NH}_4^+ 
\]

Reaction 3
and the second irreversible
\[ OCN^- + H^+ + 2H_2O \rightarrow HC0_3^- + NH_4^+ \] \hspace{1cm} \textit{Reaction. 4}

However the presence of metal salts alters the reaction. The reaction producing \( \text{Gd}_2\text{O} (\text{CO}_3)_2 \cdot \text{H}_2\text{O} \) could be:[33]
\[ 2[\text{Gd}_2 \text{O} (\text{H}_2\text{O})_n]^{3+} + 3\text{CO}_3^{2-} \rightarrow \text{Gd}_2 \text{O} (\text{CO}_3)_2 \cdot \text{H}_2\text{O} + \text{CO}_2 + (2n - 1) \text{H}_2\text{O} \] \hspace{1cm} \textit{Reaction. 5}

This reaction is not a continuous process but rather takes place in bursts. pH will rise gradually due to the decomposition of urea until it reaches the solubility limit of the metal ion. Precipitation will not occur at once because of the energy barrier caused by the large surface area of supposedly formed very fine nuclei[34]. However at some level of supersaturation the barrier is overcome and a burst of nuclei will form[34]. This will cause a drop in ion concentration and therefore in pH. After this the nuclei will grow and urea continue to decompose until another nucleation burst will occur. The last step is the precipitation of the finely dispersed metal carbonate which will heterocoagulate on the negative surface of the silica particles[35].

Heterocoagulation arise when two colloidal suspensions containing particles with opposite surface charges are mixed. Due to their charges the particles will attract each other with the one with a smaller size moving toward the bigger and creating a coating layer on the later, producing a layer of \( \text{Gd}_2\text{O} (\text{CO}_3)_2 \).[2, 33]

6 Analysis instruments

Description of instruments used to analyze the particles.

6.1 Zetaseizer Nano ZS. Malvern industries.

Measures hydrodynamic diameter, \( D_H \), with Dynamic light Scattering (DLS) using a 4.0 mW He-Ne laser at 633nm and using noninvasive 173° backscatter geometry. Particles in a suspension undergo brownian motion, if the particles are illuminated with a laser then the intensity of the scattered light fluctuates at a rate dependent on the size of the particles. Smaller particles moves more rapidly and analysis of the intensity fluctuations gives the velocity of the brownian motions or the diffusion coefficient, \( D \), and thereby the size of the particle using Stokes-Einstein relationship, equation 27,[39].

\[
D_H = \frac{kT}{6 \pi \eta D} \tag{27}
\]

where \( D \) is the diffusion coefficient, \( k \) is the Boltzmann constant, \( T \) the absolute temperature and \( \eta \) the viscosity. \( D_H \) is the hydrodynamic radii.

6.1.1 Hydrodynamic radii

The hydrodynamic radii is not the actual radii of the measured molecule rather it is the radii of a hard sphere that diffuses at the same rate as the molecule. This sphere include hydration and surface shape. Any irregularities on the surface such as remaining surfactant molecules will increase \( D_H \). Molecules that extend out from the surface will affect diffusion speed more then one laying flat the the surface and thus give a larger \( D_H \)[39].
Hydration is the process of attraction between the molecules of a solvent and the molecules of a substance dissolved and the attracted molecules will increase the radii. Also the ion concentration in the medium can affect the diffusion speed by changing the thickness of the electric double layer formed[39]. A low concentration of electrolytes will produce a thicker double layer and a larger radii[39].

6.1.2 Measurement

For iron oxide and manganese oxide cores; 100μl of the particles was diluted with 1 ml solvent and filtered through a 0.1 μm inorganic filter. 10μl of this suspension was transferred to a cuvette and diluted again with 390 μl solvent.

For gadolinium oxide cores the last dilution was considered unnecessary due to the more transparent color of this sample.

Teos and gadolinium layer samples was diluted 1:1.

For DLS measurements following constants was used.

<table>
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<th>Substance</th>
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<td>Diethyl ether</td>
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</tbody>
</table>

Table 4: DLS measurement constants.[Genovis][40][41]

6.2 µQuant Bio-Tek instruments Inc. Absorbance reader

Microplate reader for absorbance measurements. All absorbance measurements was carried out on 96 well plates.

Absorbance is defined as

\[ A_\lambda = \log_{10} \left( \frac{I_0}{I} \right) \]  

Eq. 28

where \( I_0 \) is the intensity of light at a specific wavelength \( \lambda \). \( I \) the intensity of the same light after it has passed through a sample. The measurements are done since the absorbance of the sample is proportional to the thickness of the sample and the concentration of absorbing particles in the sample[42].

6.3 MRI

MRI measurements was done on a Bruker Avance II coupled to a 2.4T magnet. \( T_2 \) is determined with multi spin echo (MSME) with starting echo times: 40, 80, 120, 160, 200 ms. \( T_1 \) with VTR-RARE (Variable Time of Recovery RARE) with starting TR: 100, 150, 200, 260, 400, 1000, 2000,
5000, 10000, 15000 ms
All measurements are in vitro and carried out in 1.2 ml glass bottles at 37°C to simulate biological environments.

6.4 TEM
A 80keV Jeol Jem-1230 transmission electron microscope (TEM) was used.

7 Experimental section
Description of the practical work done.

7.1 Chemicals used
All chemicals were bought from Sigma-Aldrich and used as received unless otherwise stated.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Source and Lot Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeO(OH)</td>
<td>Catalyst grade, 30–50 mesh; lot. # 05002DC</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>(CH₃(CH₂)₇CH=CH(CH₂)₇COOH), analytical standard, lot. # 03928TE-217</td>
</tr>
<tr>
<td>1-octadecene</td>
<td>(CH₃(CH₂)₁₅CH=CH₂) technical grade, 90%, lot. # 08525CB-105</td>
</tr>
<tr>
<td>MnCl₂</td>
<td>≥99%, lot. # 111K0151</td>
</tr>
<tr>
<td>Gadolinium(III) acetate</td>
<td>hydrate, 99.9% metal basis. lot. # 00805EJ</td>
</tr>
<tr>
<td>Urea</td>
<td>minimum 99.5%, lot. #51k0145</td>
</tr>
<tr>
<td>Dietylether</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td></td>
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<tr>
<td>Methanol</td>
<td></td>
</tr>
<tr>
<td>Hexane</td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td></td>
</tr>
<tr>
<td>Polyoxyethylene(5)nonylphenyl ether</td>
<td>(0.56 mmol, Igepal CO-520, containing 50 mol % hydrophilic group), lot.# MKBC5290</td>
</tr>
<tr>
<td>Ammonium hydroxide</td>
<td>25-35%, lot.# 221228</td>
</tr>
<tr>
<td>Tetraethyl orthosilicate (TEOS)</td>
<td>&gt;98%, Fluka lot.#1081428 51104105</td>
</tr>
<tr>
<td>TripLE express 12604 (Trypsin)</td>
<td></td>
</tr>
<tr>
<td>Celltiter 96 Aqueous one solution cell proliferation Assay (MTS), Promega</td>
<td></td>
</tr>
<tr>
<td>DMEM High Glucose 4.5g/l (Growth medium)</td>
<td></td>
</tr>
</tbody>
</table>

7.2 Synthesis
Synthesis procedures.

7.2.1 Fe₃O₄ cores
Magnetic iron oxide nanoparticles were synthesized in a three-neck flask equipped with condenser, magnetic stirrer, thermometer and heating mantle. A mixture of FeO(OH) fine powder, oleic acid and 1-octadecene was slowly, under approximately 35 minutes, heated while vigorously stirred to 320°C and then kept at this temperature for the desired time[23].

The suspension was then diluted and put trough a filter to remove all impurities larger then 0.1μm.

The size of the particles was then measured using a Zetaseizer Nano ZS.

7.2.2 MnFe₂O₄ cores
FeO(OH) and manganese chloride were mixed in ratio 2:1 and added to a three-neck flask with oleic acid and 1-octadecene. [36] Previous heating protocol were thereafter followed. The size was measured using a Zetaseizer Nano ZS.
7.2.3 Gd$_2$O$_3$ cores

Gadolinium oxide nanoparticles were synthesized in the standard three-neck flask equipped with condenser, magnetic stirrer, thermometer and heating mantle, using a modification of known methods[13, 37]. Gadolinium acetate were added to a mixture of oleic acid and 1-octadecene. The mixture was heated to 100°C and stirred until all gadolinium was dissolved. The solution were then heated to 320°C under approximately 30 minutes and kept there under continuous stirring for the desired time.

The particles was precipitated by adding ethanol and washed twice in dietylether. The size was measured using a Zetaseizer Nano ZS after filtration.

7.2.4 Silica layer TEOS reaction

1.12mmol Igepal CO-520 and 30μl iron oxide particle suspension was dispersed in 8ml hexane by vortex. Next 70 μl ammonium hydroxide and varying amounts of teos were added and the brown transparent emulsion mixed again. Then the emulsion was aged for 20 hours under gentle stirring in room temperature.

The reaction was ended by adding 0.5 ml methanol, causing SiO$_2$/Fe$_3$O$_4$ NPs to go over into the alcohol phase. Aggregates were removed by centrifuging the sample at 2500rpm for 10min. The pellet was discarded. The resulting methanol suspension was either used directly or the particles was collected further by centrifuging at 10000rpm for 30 min and pellet dissolved in water. The size was measured with a Zetasizer Nano ZS.

7.2.5 Gd layer

The Gd$_2$O(CO$_3$)$_2$ shell was added with the homogeneous precipitation method[38, 2] using water-alcohol or water as a solvent and 0.038 mol · L$^{-1}$ Gd(CH$_3$CO$_2$)$_3$ as a metal precursor. The silica-coated iron particles acquired from the teos reaction was added along with 2 M (NH$_2$)$_2$CO to a sealed beaker. The suspension was heated in a water bath in order to keep the temperature constant. The temperature was kept at 81±1°C under a argon atmosphere and the liquid vigorously stirred for the desired time. Some occurrence of precipitation could be observed. The reaction was ended by quenching in cold water and the particles were collected by centrifugation. After adding acetone the particles was centrifuged at 2500rpm to remove formed byproducts then, after discarding the first pellet, 10000rpm to collect the particles. The result was redistributed in distilled water. The size and magnetic properties of the particles was determined by DLS, TEM and MRI measurements.

7.4 MRI measurements

A absorbance measurement was done to ensure that the samples had approximate the same concentration of particles.

The signal intensity was measured for different TR and TE. The results are given in the form of a image and the intensity calculated by measure the change in intensity of the pixels with a image software.

In figure 13 are the results from an $T_1$ measurement is shown. A picture of the seven samples are taken at 10 different TR, longer TR allows for greater relaxation och greater measured signal intensity.
By plotting intensity against TE and fitting the acquired curve to the exponential function seen in equation 22, $T_2$ can be determined.

$T_1$ can be found from plotting intensity and TR and fitting to equation 21, plotted curves can be found in attachments.

In the first measurement two coupled sample pairs was used to see the impact of gadolinium. The pairs consist of exactly the same particles, from the same production batch, where one in each pair have had a gadolinium layer added. Because the particles are intended for scanning live tissue all measurements were conducted at 37°C to give accurate values.

### 7.5 TEM measurements

A small amount of sample suspension was transferred to a copper grid and left to sediment for one minute. The excess liquid removed and the grid inserted in the TEM.

### 7.6 Cellcultures

To determine the toxicity of the synthesized particles cellcultures was used. Sample from Gd22 was dissolved in phosphate buffered saline (PBS), mixed with 0.01 M Phosphate and 0.14 - 0.15 M NaCl. This to minimize the osmotic stress on the cells.

The cells were of the type 786-O (adherent). The cells were detached from the growth plate using trypsin and incubating for short time. Growth medium was added. The concentration of cells were then determined using a Bürker type cell counting plate. If the concentration was deemed low the cells were concentrated by centrifuge at 300g for 7 minutes and dissolved in a smaller volume. The cells were transferred to a 96 wells plate and incubated for approx. 3 hours until the cells reattached themselves again after which the sample particles were added, with each concentration in triplicate.

After incubation over night, 18 hours, the particles toxicity was evaluated by use of the proliferation assay. The proliferation assay allows to determine the number of cells that are growing by measure the amount of certain proliferation agents they incorporate as the divide.
8 Discussion and Results

8.1 Fe cores

Fe$_3$O$_4$ cores have been synthesized and the results are showed in table 5. The nanosized magnetite particles was stored in 1-octadecene and at 4°C. There was no precipitation or aggregation over at least five months. This is most likely due to a stabilizing effect of the oleic acid. The acid functions as a surfactant both during the synthesis but also afterwards giving a stable suspension in most organic non-polar solvents. The NPs can however only be stored in a organic solvent since the, on the surface, ionically adsorbed oleic acid act as a hydrophobic shell making the particles insoluble in polar solvents. However to make the particles useful as contrast agents they need to be hydrophilic and and stable in aqueous solutions. This is done by coating the particles, in this work by SiO$_2$.

The average diameter of the particles increased with increased reaction time but when reaction times approaching 120 min the size distribution will start to broaden, possibly due to Ostwald ripening[23].

The size of the particles can also be tuned by changing the molar ratios of oleic acid to iron precursor. Higher ratios will give bigger particles. However ratios over 10 will not work, most likely is that the excess acid inhibits the formation of iron oxide nuclei[23].

Large scale production of iron oxide particles can be done by simply scaling up the reactant quantities.

8.2 MnFe$_2$O$_4$ cores

The experiment failed for unknown reasons. The reaction should be possible, it could be that this reaction demands another ratio. There is also indications from literature[49] that the reaction need a second a cationic surfactant to work with manganese chloride as a precursor. Result displayed in table 5.

8.3 Gd$_2$O$_3$ cores

The results gadolinium core synthesis is showed in table 5. The first attempt failed due to the high molar ratio, indicating that no particles form when the molar ratio oleic acid to metal precursor exceeds 10 to 1.[23].

The fact that gadolinium cores could be synthesized using almost the same protocol as iron cores suggest that the method could be seen as a general method for most metals. The synthesis done here showed that gadolinium acetate can be used as precursor.

The next step here, not done in this work, could be to cover the gadolinium cores with a coating. Perhaps SiO$_2$ to make them biocompatible and then test their ability as a $T_1$ contrast agent.
### Table 5: Synthesized core particles

*Measured by DLS*

#### 8.4 Silica shell, TEOS reaction

The coating with silica was done by the reverse W/O micelle microemulsion method and the results of the experiments are displayed in table 12, in attached files, and in graph 1 and 2. The theoretical amount of silica needed was determined from equation 26 with \( N_{\text{part}} \) calculated from the original amount of iron precursor and assumes no losses in any step. Also the equation is for a total reaction where all teos is condensed onto the particles and do not consider different reaction times. The theoretical equation was used as just a guideline to predict particle size. However, comparing the theoretical curve with the experimental result in this study as well as results from the experiments done by Dong Kee Yi et. al. [27] suggest that this estimate is fairly accurate if you make allowances for the larger hydrodynamic radii. Equation 26 is displayed as a curve in graph 1 & 2.

It is important to remember that the size in graph 1 & 2 is measured with Dynamic light scattering. DLS measures how the scattered light fluctuates with the brownian motion of the particles. It can not distinguish between the actual particle and water molecules coordinated around it or remnants of the micelle i.e. it will only give the hydrodynamic radius. The experimental results show a predictable increase in size with the increasing amount of precursor material and follows the theoretical curve very well. The gap between the experimental values and the curve is due to the

<table>
<thead>
<tr>
<th>Particle</th>
<th>Reactants</th>
<th>Molar ratio</th>
<th>Time [minutes]</th>
<th>Size* (diameter) [nm]</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe(_3)O(_4)#1</td>
<td>0.356 g FeO(OH) (4.00 mmol) 7.5g Oleic acid 10g 1-octadecene</td>
<td>1:6.64</td>
<td>60</td>
<td>13.2±1.8nm</td>
<td></td>
</tr>
<tr>
<td>Fe(_3)O(_4)#2</td>
<td>0.356 g FeO(OH) 4.52g Oleic acid 10g 1-octadecene</td>
<td>1:4</td>
<td>45</td>
<td>10.2±1.5nm</td>
<td></td>
</tr>
<tr>
<td>MnFe(_2)O(_4)</td>
<td>[MnCl(_2) 1:2 FeO(OH)] total of 0.356g 7.5g Oleic acid 10g 1-octadecene</td>
<td>1:6.64</td>
<td>60</td>
<td>Aggregation</td>
<td></td>
</tr>
<tr>
<td>Gd(_2)O(_3)#1</td>
<td>250.8mg Gd(C(_2)H(_3)O(_2))(_3) (\cdot)xH(_2)O 5.4ml Oleic acid 5.4ml 1-octadecene</td>
<td>1:30</td>
<td>60</td>
<td>No particles</td>
<td></td>
</tr>
<tr>
<td>Gd(_2)O(_3)#2</td>
<td>847.4mg Gd(C(_2)H(_3)O(_2))(_3) (\cdot)xH(_2)O 4ml Oleic acid 10.8ml 1-octadecene</td>
<td>1:4.2</td>
<td>60</td>
<td>9.15±1.37</td>
<td></td>
</tr>
</tbody>
</table>
hydrodynamic radii and is then around 8-12 nm depending on which point is chosen as reference. The first point should have a thin layer of silica, if iron oxide and silica coordinates water differently and give a gap of 7-8 nm. As a comparison can PEG-amine coated FeODot nanoparticles with a TEM determined diameter of 18 nm ± 2 nm and a hydrodynamic diameter of 30 nm i.e. an additional 6 nm added radii [Genovis] be used.

To produce an efficient dual contrast agent, there is a need to be able to adjust the thickness of the silica layer. A too thick layer would decrease the $T_2$ ability to much and with too thin layer the $T_1$ effect would be quenched. The thickness of the shell can be tuned by adjusting the microemulsion system. Shell thickness decreases with decreasing concentration of ammonium hydroxide and teos or increasing concentration core particles. Shorten the reaction time will also give a decreased shell thickness, see table 12, in attachments, for results. The ratio of Igepal and ammonium hydroxide can be used to control the size of the micelles so that only one magnetic particle can fit in each micelle [20].

Still, aggregation between iron oxide NPs prior or during the reaction can occur and will then lead to multiple cores in the silica shell. The occurrence of multiple cores can, besides keeping the micelles small, be minimized by decreasing the concentration of iron oxide particles and good mixing to ensure that the cores are well separated before the reaction starts. Later TEM images showed particles with multiple cores indicating that mixing inadequate. The mixing can be improved by the use of ultrasound, hopefully resulting in better separation. The TEM pictures confirmed the presence of multiple cores in approximately 20% of the particles.
Also the efficiency of the capping agents on the Fe₃O₄ cores, in this case oleic acid, is important to control the growth kinetics. The capping agents prevents Ostwald ripening and coalescence,[43] and in that way controls the size.

![Graph 2: Enlargement of graph 1](graph2.png)

Graph 2 shows a very rapid increase in size for small amounts of teos before it stabilizes. Tetraethyl orthosilicate is organophilic[44] and as such more readily dissolvable in the hexane phase. Teos need to diffuse into the micelles to react, a time consuming process. For small amounts of silica could the diffusion speed be the limiting factor. This could explain the rapid increase in silica thickness, the steeper gradient created by increasing concentrations of teos would increase the diffusion speed. At some point diffusion is sufficient and the limiting factor becomes the reaction speed.

The avoid the problem longer reaction times for small amounts of silica is recommended, this would ensure that more of the silica have time to diffuse into the micelles.

The largest and smallest particles was most difficult to synthesize. According to literature is it possible to synthesize shell thicknesses between $\sim 1.8\text{nm}$ to $\sim 30\text{nm}$[27]. For larger particles the upper limitations is due to when it is more energetically favorably to form new micelles instead of increasing the size. Evidence of core-free silica particles can be found in the TEM images. The particles synthesized here adhere to those limits as well, see graph 1 with silica thickness $\sim 2\text{nm}$ to $\sim 26\text{nm}$ assuming water adds $8\text{nm}$. 

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TEM pictures, figure 15, confirm that particles with a shell thickness of 30nm can be synthesized. However it is impossible to say if this particle contains more than one core.

When methanol was added the emulsion system was disturbed and the particles went over into the alcohol phase. A slow centrifugation, 2500rpm, of the methanol yielded a brown pellet of sticky consistency. The pellet was magnetic and likely a large portion of the synthesized particles was lost when the pellet was discarded. However, no other way to get rid of the impurities was developed. Not to centrifuge the sample gave a larger hydrodynamic radii, most likely igeapal and/or oleic acid adhered to the particles surface. This in turn would disturb the deposition of gadolinium in the next step. It was possible to disturb the emulsion system and precipitate the particles with other polar liquids such as ethanol and water but this produced unpredictable results and often the precipitates became unsolvable. The Teos reaction have taken place in hexane as a solvent which to my knowledge have not been done before. Taking into account that Tangi Aubert et.al.[44] synthesized particles in heptane makes it likely that a wide variety of organic solvents, not only cyclohexane which is predominately used in literature, will work. Later TEM pictures showed that the silica layer was very uneven. This could be because of the change of solvent or the sensitivity of the reaction to time, temperature or starting concentrations.

8.5 Gd₂O₃(CO₃)₂ shell

There were no way to easily measure the thickness of gadolinium in this step of the synthesis available to me. The size as showed in table 13, in attachments, is highly approximately, since the exact starting size Fe₃O₄@SiO₂ particles are unknown because all measurements are made with DLS which only can give a hydrodynamic radii. Furthermore, is it possible that some of the original micelles still remains and contribute to the size. Remaining surfactants will also affect how much water is coordinated around the particles, which also increases the unreliability since it is unclear how well gadolinium coordinates water in comparison to silica. MRI measurements confirms that there is gadolinium deposited on the particles also proving that gadolinium acetate can be used as a precursor. Two population of particles was observed after the reaction. The expected gadolinium coated particles and some unexpected small particles. They show a Dₜ of 7-11nm which without hydrodynamic radii should be approx. 1-4 nm big, assuming same coordination of water as silica. This is most likely free gadolinium-based particles yet to be attached to the larger particles. The thickness of the formed gadolinium layer can be predicted from measuring these formed free gadolinium-based particles. The formed layer on the silica particles is likely only one or a few gadolinium particles thick, after that the surface will be covered and all charges saturated. It is unclear how well the gadolinium is bound to the silica. It is possible that there is some leakage. A way to prevent this would be to coat the particles with a second layer of silica or to anneal the gadolinium at 800°C changing it into gadolinium oxide. However the exact thickness of the gadolinium layer are somewhat unimportant. Jin-sil Choi et. al. [2] showed that the R₁ relaxivity value per concentration gadolinium was roughly the same regardless of the size of the particle. This would mean that only the surface molecules contribute to the relaxation process[2, 13].
There was some indication in the literature[45] that the basic solution in the gadolinium deposition process could corrode away some of the silica. Considering strong concentrated bases being one of the few thing capable of etching SiO\textsubscript{2} makes this sound likely and could then create an uneven surface on which less gadolinium would be able to bind[45].

This can be solved by using a water-alcohol mix instead of pure water[25, 45]. Most commonly a mix of propanol and water have been used[45]. Propanol dielectric constant is low compared to water so by adding of propanol the dissociation of ammonium hydroxide can be controlled[45] and as a result should the solution not be as basic as with pure water. This should minimize the corrosion. A high dielectric constant indicates that it will be a good solvent for ionic species and thus promoting the dissociating of urea[42].

For this reason both pure water and water-alcohol solvents have been tested, but in this work methanol was used instead of propanol. Methanol have similar dielectric constant to propanol, 33 to 20.1, compared to water 80,[42]. The use of methanol instead of propanol was mostly out of convenience since the particles already was suspended in methanol. I found some indication that a water-alcohol mix improved the results. Reactions not involving methanol were more likely to fail of reasons including agglomeration and large size distributions. Even though no conclusive answers could be found, the results indicates that using methanol in the reaction have no adverse effects and most likely improves the synthesis by increasing the binding of gadolinium.

It seems that the homogeneous precipitation of gadolinium is strongly affected by aging time, temperature and pH. It seemed however not to be affected by concentration of silica coated seeding particles.

To achieve perfectly monodispersed gadolinium particles is not the goal with this work, some polydispersity is fine. However if the gadolinium particles start to aggregate they will drag the silica-coated particles down with them.

Several different aging times between 0.75 to three hours where tested, table 13. Both long time (3h) and short time (45min) appear to have a higher probability to aggregate. A optimum time would be somewhere ~1.5 to ~2h. Longer times will give larger free gadolinium particles and a higher polydispersity since it hits the solubility limit more times. Exactly why this leads to aggregates is unclear but it could be that when a to large gadolinium particle adhere to the surface of a silica-coated particle, the gadolinium sticks out so far that the surface charges on it can start o attract other silica-coated particles. While the negative charges of the silica being to far away to repel each other.

It is also known that particles formed this way will spontaneously start to agglomerate when they become to large[34]. The aggregation at short times probability comes from the fact that the reaction takes time. The gadolinium based particles need time to adhere to the silica. Some aggregation is likely in the beginning where surface charges on gadolinium particles attached to silica attract silica particles not yet covered by gadolinium particles. This will reverse it self with time when the gadolinium coverage is more complete.

A fine tuned control of the temperature was necessary for a good result. When the temperature fluctuated during the synthesis the results were a broader curve, for example in the experiment gd 20 in table 13 (in attachments) during which there was large fluctuations in temperature. A high
temperature will lead to faster decomposition of urea and a greater polydispersity. On the other hand temperatures below the aim of 81°C can slow the reaction so particles of sufficient size won't have time to grow.

Best results would be achieved if the reaction could be monitored so well, both in temperature and pH, that the first burst of nucleation could be detected and the reaction ended before next burst. This would give monosized particles.

The fact that the concentration of starting particles didn't seem to affect the reaction was not surprising as the growth of the gadolinium based layer stops after a certain thickness, probably due to saturation of the surface charges, and gadolinium was present in excess. As long as there is enough gadolinium relative to silica it will work.

8.6 MRI measurements

Attempts to determine iron concentration failed because of the silica layer and atomic absorption spectrometry was unavailable.

So to ensure that the MRI samples had the same concentrations of particles absorbance measurements were done. Because of the limited thickness of the gadolinium layer, a few nm, and the fact that silica has low absorbance compared to iron oxide, the assumption was that they could be neglected. This allowed for making a comparison between particles with varying thickness of silica-coating and gadolinium layer. Since the exact concentration is unknown the values can only be used for comparison.

By fitting the experimental values to equation 24 assuming TR → ∞ for T<sub>2</sub> and vice verse, following results were found, tables 6-9 and tables 14-15 in attachments.

Where the constant a describes the proton density but due to the varying amounts of silica in the samples a can not be used to do comparisons.

There is likely to be additional particles in the samples besides the synthesized dual contrast agents such as silica particles without cores and free gadolinium particles. These particles will affect the measurements and contribute to the measured T<sub>1</sub> and T<sub>2</sub> values.

8.6.1 First measurement

First measurement was to determine the impact of the gadolinium layer. The samples are coupled, where Gd22 and T40 are the same particles with and without gadolinium. Same for T39 and Gd21. Absorbance measurements ensures that the concentrations are approximately the same within the couple. The proton density of the particles, a in table 6, increases due to the higher density of gadolinium compared to silica. This is the first indication that there in fact are a gadolinium layer.
1.1 $T_2$ measurement

<table>
<thead>
<tr>
<th></th>
<th>Pair 1</th>
<th>Pair 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR: 800ms, TE: 20, 40, 60, 80, 100 ms</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Particle $\text{D}_n$ [nm] and amount of teos [µl]</strong></td>
<td>T40 ~24nm or 32µl</td>
<td>Gd22 ~24nm or 32µl</td>
</tr>
<tr>
<td>$T_2$ [ms]</td>
<td>161±21</td>
<td>55±2.5</td>
</tr>
<tr>
<td>$a$</td>
<td>1.8±0.1</td>
<td>4.2±0.15</td>
</tr>
</tbody>
</table>

Table 6: The MRI $T_2$ results for the coupled pairs.

The measurement done in table 6 is not done with an optimal time span. If $T_2$ is longer then the measured time span will this increase the uncertainty. Likewise if the time span is much longer then the measured values, both cases will make less information available to fit the values to an expression. The values with their fitted graphs can be seen in attachments.

One unexpected observation is that $T_2$ is shorter for particles with gadolinium layer, table 6. To be able to lower the time this much require a $T_2$ effect much stronger then expected. This suggest gadolinium exhibit strong $T_2$ effects as well as $T_1$. This is also reported by Ja Young Park et.al. [13] who shows that ultrasmall gadolinium oxide, Gd$_2$O$_3$, nanoparticles exhibit nearly the same $T_2$ as $T_1$ properties. This indicates that Gd$_2$O(CO$_3$)$_2$ does as well. This is not a problem but instead simply increases the effectiveness of the agent.

This have not been reported from dual iron and gadolinium particles before. Ki Hyun Bae et.al. [4] actually reports the opposite, that $R_2$ decreased when coupled with gadolinium, when the the iron and gadolinium is separated by proteins. Clearly more research is needed before the interactions between $T_1$ and $T_2$ materials can be fully explained.

From table 6 we also see that T39 have a stronger $T_2$ effect giving rise to a shorter measured $T_2$ relaxation time, most likely due to the thinner silica shell in accordance with the theory.

1.2 $T_1$ measurement

<table>
<thead>
<tr>
<th></th>
<th>Pair 1</th>
<th>Pair 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR: 100, 129, 162, 200, 245, 300, 370, 467, 630, 1240 ms</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Particle $\text{D}_n$ [nm] and amount of teos [µl]</strong></td>
<td>T40 ~24nm or 32µl</td>
<td>Gd22 ~24nm or 32µl</td>
</tr>
<tr>
<td>$T_1$ [ms]</td>
<td>1251±190.5</td>
<td>212±2.2</td>
</tr>
<tr>
<td>$a$</td>
<td>2.1±0.2</td>
<td>2.8±0.01</td>
</tr>
</tbody>
</table>

Table 7: The MRI $T_1$ results.

The $T_1$ measurements in table 7 clearly shows that there is a gadolinium layer and the impact it has. The layer shortens the $T_1$ relaxation time considerably, by approximately a factor of 10 for the second pair.
$T_1$ is shorter in Gd21 compared to Gd22 which deviates from the theory but it could simply be from concentration differences either in total or in gadolinium.
That $T_40$ has shorter $T_1$ compared to T39 could be an effect from its weaker $T_2$ ability. The theory gives that a strong $T_2$ could disturb $T_1$. Or it could simply be differences in concentrations.

8.6.2 Second MRI measurement

Same absorbance measurement done in the first test is repeated to ensure approximate same concentrations.
This measurement is done to test the effect of different thickness of the silica layer and the effect of using methanol in the synthesis process. Five different thicknesses of the silica layer were tested.

<table>
<thead>
<tr>
<th>2.3 $T_2$ measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>TE: 20, 40, 60, 80, 100 ms</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>1 Gd14</td>
</tr>
<tr>
<td>Particle D_H [nm] and amount of teos [μl]</td>
</tr>
<tr>
<td>$T_2$[ms]</td>
</tr>
<tr>
<td>$a$</td>
</tr>
</tbody>
</table>

Table 8: The MRI T2 results for particles with different silica thickness with short time span.

<table>
<thead>
<tr>
<th>2.4 $T_2$ measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>TE: 40, 80, 120, 160, 200 ms</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>1 Gd14</td>
</tr>
<tr>
<td>Particle D_H [nm] and amount of teos [μl]</td>
</tr>
<tr>
<td>$T_2$[ms]</td>
</tr>
<tr>
<td>$a$</td>
</tr>
</tbody>
</table>

Table 9: Second MRI T2 results for particles with different silica thickness with long time span.
Two measurements with different times were performed on each sample, both results are shown in graph 3.

For the time being we ignore the samples synthesized without methanol.

First observation is that sample 7 seems to deviate in both $T_1$ and $T_2$ measurements. Can also notice a higher uncertainty for this measurement. The problem could lie somewhere in the synthesis process, where experience makes its clear that particles at the end of the size spectrum, large or small, are more likely to fail.

It is possible that sample 7 has reached the limit for what sizes are possible to synthesize with this method. According to S. Lee et.al. [27] is the maximum shell thickness $\sim 30\text{nm}$, Sample 7 have a $D_i$ of $\sim 40\text{nm}$ minus the core radii of $6\text{nm}$ and assuming that water adds $\sim 10$, then the limit is almost reached. The particle could be so large that it is no longer energetically favorably to continue increasing the size and the reaction instead favors the formation of core free silica particles.

Anyway seems that both $T_1$ and $T_2$ relaxation times are longer. This could be explained by if, for some reason, less gadolinium have been able to bind which would affect both $T_1$ and $T_2$. This could happen if the surface of the particle have somehow been altered.

The possibility that some other step in the synthesis other then the gadolinium deposit is responsible for the deviating times is unlikely. The same iron cores was used in all particles and is unlikely to have been altered in some way. The silica reaction is more proven and there has to be large deviations in the silica layer for in to be the responsible.

If we assume that sample 7 is non-representative and exclude it then $T_2$ in graph 3 is slightly decreasing with increasing amount of teos. This is surprising since the thicker silica layer should shield the iron core more efficient and $T_2$ time should be longer. This might be explained by the $T_2$ property exhibited by the gadolinium layer seen in the first MRI measurement.

Assuming a even layer of gadolinium the local concentration of gadolinium will increase as the particles becomes bigger. Because of the constant amount of iron precursor in all batches leading to approximately the same number of particles. It is possible that the $T_2$ effect of the gadolinium layer increases with concentration faster then the iron cores effect decreases with thicker silica. This could explain the findings but is unlikely since earlier studies suggest that gadolinium ions need
direct surface contact to facilitate relaxation[13, 2]. Larger particles would not mean more surface ions in contact with the same proton, rather the opposite. A logical next step would be to test the effect of the iron core and the gadolinium layer with different silica thickness separately. However the results here suggest that some sort of interactions take place when the iron core and gadolinium are combined, otherwise it is hard to explain why the $T_2$ times decreases when the iron core becomes more isolated. To confirm this more testing of core/shell particles of different size is needed possible after TEM examination for ensured correct structure and size.

<table>
<thead>
<tr>
<th>2.2 $T_1$ measurement</th>
</tr>
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<tr>
<td>TR: 100, 135.0, 174.6, 220.2, 274.1, 339.8, 424.0, 541.5, 737.4, 1500.0 ms</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Particle $D_H$ [nm] and amount of teos [μl]</th>
<th>1 Gd14</th>
<th>2 Gd16</th>
<th>3 Gd12B</th>
<th>4 Gd12A</th>
<th>5 Gd20</th>
<th>6 Gd10</th>
<th>7 Gd18</th>
</tr>
</thead>
<tbody>
<tr>
<td>~22.5nm or 8μl</td>
<td>415.5±31</td>
<td>149.4±1.2</td>
<td>181±1.1</td>
<td>287±40</td>
<td>308±7.2</td>
<td>133.5±1.1</td>
<td>346±36</td>
</tr>
</tbody>
</table>

| $a$ | 2.56±0.1 | 2.5±0.01 | 2.5±0.01 | 2.6±0.16 | 2.6±0.03 | 2.5±0.01 | 2.75±0.1 |

*Table 10: The MRI $T_1$ results for particles with different silica thickness.*

Graph 4 shows the results from the $T_1$ measurements, both tables 11 and 12, except sample 2 and 4, those without methanol. The graph shows a weak tendency that the curve decreases with larger particles. This is in accordance with the theory and is supported by the fact that sample 7 most likely have a too long time. But it is very inconclusive, more data is needed. You could argue that the small particles, 8μl,
have a long relaxation time because their silica shells are thin and the $T_2$ quenches $T_1$ in accordance with the theory. The other alternative is that shorter $T_1$ is a result of increasing concentration of gadolinium. However the larger particles means less surface area, something that should have a negative impact on $T_1$.

There exist some interactions between the layers and it is clear that more research is needed to fully explain them.

![Graph 5: Showing calculated $T_2$ times against amount of silica including reactions without methanol (romb dots).](image)

When it comes to using methanol or not in the synthesis is it hard to say definitely since I have results pointing in both directions in graph 5. 8μl sample indicates shorter relaxation time i.e. more inbound gadolinium with synthesis without methanol. 32μl gives the opposite.
Again a large difference between those made with methanol and those without for the first sample. In graph 6 we see that the 8µl sample without methanol seems to have a strong binding to gadolinium and a short $T_1$. It could actually be that the reaction without methanol is better for small particles but more data is needed to make a argument. However the experience of more failed attempts without methanol makes me more inclined to believe that the surface indeed becomes more rough without methanol, limiting the binding of gadolinium.

8.7 Transmission electron microscope images

The evaluate the structure and actual size of the particles in Gd22 a 80keV TEM was used. Gd22 have a radii of $D_{H} = \sim 24$ from graph 1. Figure 14 shows three particles of different size and different numbers of iron cores. To support the assumption that the dark spot is in fact are a iron core, comparisons was made to [2] and [Genovis] which gave that the contrast is the same. Further more was the black core measured to a diameter of $\sim 12$nm which is consistent with the synthesized iron cores.
The whole particle in the lower left corner was measured to a diameter of approximately 37-39nm. One particle, the small to the left, is a core-less free formed silica particle. The uppermost one is either one particle with 4 cores or a heap of several particles. A estimate of 20% of the particles had multiple cores. Multiple cores coupled with the very uneven form of the silica layer resulted in large variations in size on the particles.

Figure 15 show a common occurrence, that when the sample dries will the particles get stuck on each other. The lower particle here is probably two separate particles stuck together. On the upper particle inbound gadolinium can be seen as small black dots. The image support the theory that gadolinium forms as small free particles that are attracted and incorporated into the surface of the silica. The size of this particles was measured to 70-75nm. That gadolinium is not visible on the particles in figure 14 indicates that there is different amount of gadolinium bound the each particle. Figure 16 shows the opposite, a very strong binding of gadolinium as shown by the very dark surface of the particles. The TEM images show that there is formed particles besides the intended dual. There is silica particles without a core but with gadolinium particles on the surface in a wide variety of sizes. No free gadolinium based particles could be seen, the attraction between the gadolinium particles and silica appears to be strong enough to ensure binding. The small particles seen in the DLS must have been gadolinium bounded to small silica particles or it is possible that the reaction was not yet complete. Gd22 was stored for approximate 2 weeks before the TEM study, in which the binding could have taken place.
Figure 17 show a picture of an average particle with a diameter of ~48nm showing bounded black gadolinium against grey silica.
Most particles were in the range of 30-45nm, when compared to a hydrodynamic diameter of 48nm for Gd22 supports the assumption that water adds ~8-12nm made earlier.

8.8 Cellcultures
To get some feeling for the relative toxicity of the core/shell, Gd22 D_{ht}~24nm, particles they were compared to NIMT® FeOlabel nanoparticles from Genovis AB. NIMT® FeOlabel are considered to have a relative low toxicity and will have toxic effects on 786-O cells at approximately 200-250 pikogram iron/cell.[Genovis] The core radii of the NIMT® FeOlabel particle is around 5nm and with coating a total of around 9nm. A estimation give that a lethal concentration is 9.1e6-11.4e6 particles/cell.
All comparisons done are very approximate as NIMT® FeOlabel is smaller then the synthesized particles and the increased surface area should make them more reactive. Also all concentration given for the core/shell particles are the utmost maxima for what there can be. The somewhat high starting concentration of particles used in the first test was due to the belief that the losses was greater then what they actually appears to be.
8.7.1 First test

5,10,20,30,40,50,75,100 μl gd22 sample suspension was used.

Estimation based on numbers of particles:
Assuming no losses in any step and that there is only one core per particle. Starting from the measured amount of iron precursor will give a concentration of approximate 9.15e13 particles per ml pbs. With 8000 cells per well gives approx. 57.2e6 - 11.4e8 particles per cell, around a factor 100 greater then the comparable nanoparticles to compensate for losses.

Estimation based on mass of particles:
Assuming no losses in any step and that there is only one core per particle and each core give a complete particle. Starting with the amount of iron precursor to get the number of cores then adding the weight of the silica and gadolinium. The result is approximately 16mg particles per ml pbs, with 8000 cells per well gives 10e3 pg/cell to 2e5 pg/cell whole particles.
Because of the uncertainty in the thickness of the layers is the estimation based on mass more unreliable.

The optical observation of the cells showed what was later confirmed by the proliferation assay, almost all the cells were dead. It was also possible to see the large number of particles as a thin film covering everything. Further optical observations revealed cells that had absorbed particles and not so few had burst.

The slight increase in absorbance is explained by the higher concentration of particles capable of absorbing light, there is no proliferation.

8.7.2 Second test

The amount of gd22 was reduced to: 0.005, 0.01, 0.03, 0.05, 0.075, 0.1, 0.3, 0.5μl.

Still assuming no losses in any step and that there is only one core per particle. Starting from the measured amount of iron precursor will give a concentration of approximate 9.15e13 particles per ml. This time using 10000 cells per well will give approx. 45.8e3 - 45.8e5 particles per cell.
For mass estimate with 10000 cells per well and new volumes yields approx. 8 pg/cell to 800pg/cell.

This time the cells have multiplied. No indications that the proliferation is impaired can be seen, in fact the wells with sample have even higher concentrations of living cells then the control wells. This could be because the cells are stressed. They are affected by the particles and answer by increasing proliferation. Maybe this is why the higher proliferation is in the well with highest concentration or it could be the particles themselves that increase the absorption.

It is clear however that we now is below the lethal dose and somewhere between 45.8e5 and 57.2e6 particles/cell is lethal. However what exact concentrations that is equivalent to is unknown other then it has to be lower then the theoretical.

Comparing this to NIMT® FeOlabel (10e6 perticles/cell) shows that it is indeed more toxic but not so toxic that it is unusable, depending on how effective as a contrast it is.

Since gadolinium is the only toxic component on the particle it most likely it is gadolinium that kills. Either that it leaks or detaches from the particles due to changes in environment inside the cells or the small core free silica particle with gadolinium formed in the homogeneous precipitation reaction.

Both these problems can be minimized by different means. A second coating layer of silica can be added or the gadolinium can be annealed to possible increase stability.

8.7.3 Third test

To confirm the hypothesis that gadolinium is the dangerous part of the particle and not some other unforeseen thing. a batch of particles with the same size as Gd22 but without gadolinium was synthesized and tested.

The new batch was done by the same specifications as the previous tested but since the last reaction step was omitted will the losses be smaller. This batch will be more concentrated but how much more is impossible to say.
To have an overlap to both previous tests 0.5, 1, 5, 10, 30, 50, 75, 100 μl was used. With 10000 cells/well will the wells contain 4.6e6 – 9.2e8 particles per cell.

We can see that these particles have a lethal concentration which is approximately the same as NIMT® FeOlabel. NIMT® FeOlabel kills at 9.1e6 - 11.4e6 particles/cell compared to 9.1e6 – 45e6 particles/cell for the particles which is a high estimation, the concentration is probably lower.

The high proliferation in the well with 0.5 μl is possible due to the same stress factor mentioned before. Also there is an increase in absorption as the number of particles absorbing increases.

<table>
<thead>
<tr>
<th>Lethal dose</th>
<th>concentration</th>
<th>Particle</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.1e6 - 11.4e6 particles/cell</td>
<td>Norm concentration</td>
<td>NIMT® FeOlabel</td>
</tr>
<tr>
<td>9.1e6 - 45e6 particles/cell</td>
<td>Lower concentration</td>
<td>Particles without gadolinium</td>
</tr>
<tr>
<td>45.8e5 - 57.2e6 particles/cell</td>
<td>Lowest concentration</td>
<td>Core/shell particles</td>
</tr>
</tbody>
</table>

*Table 11: Toxicity of different particles.*

Exactly how toxic the core/shell particles are is unclear since no concentrations are known. However some educated guesses give that gadolinium indeed is more toxic, perhaps as much as tenfold compared to same particles without gadolinium and this only the acute toxicity. Nothing is said what effect it may have if it accumulates in tissue. However the acute toxicity is not that high so to make the particles unusable. With some methods to minimize gadolinium in free form, such as annealing and/or coating and given in low concentration, then this particle can probably be used for research purpose in the future however the long term toxicity of gadolinium limit it uses for humans.

**9 Conclusions**

The use of a dual mode method for MRI scanning, where $T_1$ and $T_2$ imaging modes are used simultaneously, can give easier interpretation and more accurate information for medical diagnostic. This work outlines a core/shell design for dual contrast $T_1$- and $T_2$-weighted MRI. The purpose of this study have successfully been achieved in the sense that the dual contrast core/shell particles have been synthesized and their function as contrast agents was verified. As proposed the particles consist of an iron core isolated with silica and a outer gadolinium based shell.
With the beginning of nano technology came a new concept, to build the particles from the bottom and up. Not to take a preexisting material and try to shrink it down but instead design the particle and by the means of that design control what properties the particle will have. This thought often changed the choice of material used, as many substances changes properties when the particles become smaller.

When different materials come very close together, as they do on the nanoscale, unforeseen interactions that can happen. The amplified $T_2$ effect in this work is one example. MRI studies confirms the presence of gadolinium in the samples and verified that the particles have the potential to be used as contrast agents. The MRI showed $T_1$ decreasing with thicker shell as expected but also that $T_2$ unexpectedly decreased with shell thickness indicating a interaction between iron and gadolinium. However their overall effectiveness is yet to be evaluated as I have not been able to determine relaxivity values or done any in vivo tests.

The understanding on how the the properties of the different layers affects changes in relaxivity is vital for designing the optimal magnetic contrast agents. This is important for medical applications, as higher contrast typically leads to a higher sensitivity and reduces the amount of contrast agent required for imaging.

By changing the materials and the thickness of the different layers can the magnetic properties be modulated to fit the specific need of a medical examination. In this work only the thickness of the silica was modulated but the same can be done with the other layers of the material. The $T_2$ effect might be increased by the use of a manganese-iron core or the $T_1$ effect might be deemed too strong and extinguish too much of the signal in which case the core can be made smaller.

Optimal parameters for the contrast agent is not entirely dependent on relaxation strength, size is also a important factor. Maximizing $T_1$ by increasing silica thickness may only be feasible to a limit where the particle becomes to large and unable to pass blood vessel walls or have a too short blood half-life time.

TEM pictures showed particles of varying size och uneven silica layers but with the right overall structure with three separate layers. The pictures also revealed a large distribution of core free silica particles with gadolinium layer but no free gadolinium particles. This is a problem as these particles affect the results, primarily the $T_1$ effect, and is likely responsible for much of the cell toxicity. The problem can be solved by better control of the teos reaction. This study proved that hexane will work as solvent in the teos reaction. In the next step a gadolinium based layer was successfully added using gadolinium acetate as a precursor. No conclusion could be made regarding the use of methanol in the reaction. The toxic effects of gadolinium is well known and a problem when designing contrast agents. Gadolinium could never be given by itself but by binding the gadolinium to a larger particle that is easily excreted, can the absorption into the tissue be greatly reduced. The proliferation tests reveal that the particles with gadolinium based outer layer indeed is toxic but not extremely so and can therefore be controlled. However these tests say nothing about long term effects.

To conclude, the dual mode nanoparticles have the possibility to become contrast agents. Especially as the unforeseen amplification of $T_2$ effect by gadolinium increases their effectivity. However the data is much to thin to say anything definitely, more testing is needed to understand the interactions between the layers.
9.1 Future works

This project is merely a beginning, lots of studies is needed before the particles can be used. The synthesis methods must be refined. During the silica deposition ultrasound should be used to ensure better separation of the iron cores to avoid multiple cores in the particles. The reaction itself must be better controlled to avoid formation of free silica particles. Perhaps by the use of a different solvent or a different method altogether.

The concentrations must be determined to see if the contrast effect is strong enough to merit further effort. With the concentration determined the cell toxicity must be tested again. It could be interesting to remove the particles and test the toxicity of the remaining fluid. To determine if further washing procedures are necessary.

Then an outer biocompatible coating must be added, possible another silica layer. The coating demands a lot as it has to be able to minimize leakage of gadolinium out but at the same time allow water contact with the gadolinium ions. In future work this coating could be further modified with different molecules. Antibodies can be used to guide the particles to desired locations, florescent molecules can be added for optical observations or radioactive isotopes. Opening up for the same particle to be used in several different types of examinations.

After the particles have been coated the results must be tested in vivo to see if the basic idea still works and that you in fact can get additional information from a MRI image.

Further studies is also needed to be able to understand the interactions between the different layers, MRI tests must be done with just iron core or gadolinium layer and silica of different thicknesses. More testing of dual particles is also needed to confirm the findings in this work.

It is possible that the $T_1$ effect can be further boosted by improving the gadolinium layer.

As it is now the gadolinium layer probably consist of $\text{Gd}_2\text{O(CO}_3)_2$ and have a proven $T_1$ effect. One possible way to improve the contrast could be to convert the $\text{Gd}_2\text{O(CO}_3)_2$ to $\text{Gd}_2\text{O}_3$ by calcination at $800^\circ\text{C}$. It is possible that $\text{Gd}_2\text{O}_3$ is more effective since the gadolinium is more tightly packed and more of them can cooperate on the same proton.
10 Literature cited


[13] Ja Young Park, Myung Ju Baek, Eun Sook Choi, Seungtae Woo, Joo Hyun Kim, Tae Jeong


### 11.1 Tables

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>TEOS #1 15µl Fe\textsubscript{3}O\textsubscript{4} suspension (21.1 mg/ml) 20µl TEOS</td>
<td>23</td>
<td>13.2±1.8</td>
<td>~</td>
<td>155±25 aggregates</td>
<td></td>
</tr>
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<td>–</td>
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<td>39±5.5 in ethanol</td>
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<td>–</td>
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<td></td>
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<td>13.2±1.8</td>
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<td>73±8 aggregates</td>
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<tr>
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<td>TEOS #7 3-4 days</td>
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<td>59 in methanol phase</td>
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<td>13.2±1.8</td>
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<td>126±23 aggregates</td>
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<td>44±5.5 in methanol phase</td>
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<td>62 in methanol phase</td>
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<td>13.2±1.8</td>
<td>51±8 in methanol phase</td>
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</tr>
<tr>
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<td></td>
<td>40</td>
<td></td>
<td>56 in methanol phase</td>
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<td>30μl Fe₃O₄ suspension (21.1 mg/ml) 15μl TEOS</td>
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<td>13.2±1.8</td>
<td>47±9 in methanol phase</td>
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</tr>
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<td>30μl Fe₃O₄ suspension (21.1 mg/ml) 19μl TEOS</td>
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<td>50.5±8 in methanol phase</td>
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<td>13.2±1.8</td>
<td>44±5 in methanol phase</td>
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<td>13.2±1.8</td>
<td>51±8 in H$_2$O</td>
<td></td>
</tr>
<tr>
<td>TEOS #28</td>
<td>30μl Fe$_3$O$_4$ suspension (21.1 mg/ml) 50μl TEOS</td>
<td>20</td>
<td>13.2±1.8</td>
<td>55±10 in H$_2$O</td>
<td></td>
</tr>
<tr>
<td>TEOS #29</td>
<td>30μl Fe$_3$O$_4$ suspension (21.1 mg/ml) 100μl TEOS</td>
<td>20</td>
<td>13.2±1.8</td>
<td>80±12 in H$_2$O</td>
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<tr>
<td>TEOS #31</td>
<td>30μl Fe$_3$O$_4$ suspension (21.1 mg/ml) 125μl TEOS</td>
<td>72</td>
<td>13.2±1.8</td>
<td>sedimenting</td>
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</tr>
<tr>
<td>TEOS #32</td>
<td>30μl Fe$_3$O$_4$ suspension (21.1 mg/ml) 32μl TEOS</td>
<td>20</td>
<td>13.2±1.8</td>
<td>45.6±7 in methanol phase</td>
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<tr>
<td>TEOS #33</td>
<td>30μl Fe$_3$O$_4$ suspension</td>
<td>20</td>
<td>13.2±1.8</td>
<td>52±8 in methanol</td>
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<tr>
<td>TEOS #34</td>
<td>30μl Fe₃O₄ suspension (21.1 mg/ml) 8μl TEOS</td>
<td>20</td>
<td>13.2±1.8</td>
<td>48±7 in methanol phase</td>
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<tr>
<td>TEOS #35</td>
<td>30μl Fe₃O₄ suspension (21.1 mg/ml) 125μl TEOS</td>
<td>20</td>
<td>13.2±1.8</td>
<td>80±11 in methanol phase</td>
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<tr>
<td>TEOS #36</td>
<td>30μl Fe₃O₄ suspension (21.1 mg/ml) 8μl TEOS</td>
<td>20</td>
<td>13.2±1.8</td>
<td>36±8 in methanol phase 39.5±7 in H₂O</td>
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</tr>
<tr>
<td>TEOS #37</td>
<td>30μl Fe₃O₄ suspension (21.1 mg/ml) 45μl TEOS</td>
<td>20</td>
<td>13.2±1.8</td>
<td>46±6 in methanol phase 57±14 in H₂O</td>
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<td>TEOS #38</td>
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<td>20</td>
<td>13.2±1.8</td>
<td>50±7 in methanol phase 47±15 in H₂O</td>
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<tr>
<td>TEOS #39</td>
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<td>20</td>
<td>13.2±1.8</td>
<td>47±10 in methanol phase 32±3 in H₂O</td>
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<td>13.2±1.8</td>
<td>45±6 in methanol phase 37±5 in H₂O</td>
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<tr>
<td>TEOS control</td>
<td>30μl Fe₃O₄ suspension (21.1 mg/ml) 0μl TEOS</td>
<td>20</td>
<td>13.2±1.8</td>
<td>28±3 in methanol</td>
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*Table 12: Synthesized silica shells around a iron core*
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<tbody>
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<td>Fe3O4@SiO2@Gd #1</td>
<td>120mg gd(acetate) 841mg urea 6ml H2O</td>
<td>3</td>
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<td>aggregates</td>
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<td>Gd #2</td>
<td>120mg gd(acetate) 841mg urea 6ml H2O</td>
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<td></td>
<td>aggregates</td>
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<td>Gd #3</td>
<td>60mg gd(acetate) 841mg urea 6ml H2O</td>
<td>2</td>
<td>49±5 (16μl)</td>
<td>84±10</td>
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<td>aggregate</td>
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<td>Gd #4</td>
<td>50mg gd(acetate) 841mg urea 6ml H2O</td>
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<td>46±6 (10μl) and 52±7 (40μl)</td>
<td>44±5 and 129±25</td>
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<td>Gd #5</td>
<td>50mg gd(acetate) 841mg urea 6ml H2O</td>
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<td>Bad measurement (50μl)</td>
<td>49±15</td>
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<td>Bad measurement</td>
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<td>120mg gd(acetate) 841mg urea 6ml H2O</td>
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<td>68.5±9</td>
<td>Centr. 2000rpm 95±20</td>
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<td></td>
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<td></td>
<td></td>
<td>Centr. 10000rpm 85±15</td>
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<tr>
<td>Gd #6</td>
<td>120mg gd(acetate) 841mg urea 6ml H2O</td>
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<td>68.5±9</td>
<td>Centr. 2000rpm ~91 bad</td>
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<td>Centr. 3000rpm ~69 bad</td>
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<td></td>
<td>Centr. 10000rpm 100 bad</td>
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<td>pellet 36 and 105</td>
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<tr>
<td>Gd #7</td>
<td>100mg gd(acetate) 841mg urea 6ml H2O</td>
<td>1</td>
<td>47±17</td>
<td>Centr. 2000rpm 96±25</td>
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<td></td>
<td></td>
<td>bad pellet 160</td>
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<tr>
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<td>47±17</td>
<td>Centr.</td>
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<td>120mg gd(acetate) 841mg urea 6ml H$_2$O</td>
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<td>51±8</td>
<td>Centr. 2500rpm 104±30 pellet 41±4 and 102±15</td>
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<td>Gd #9</td>
<td>120mg gd(acetate) 841mg urea 6ml H$_2$O</td>
<td>45min</td>
<td>55±10 (50μl)</td>
<td>Centr. 2500rpm 104±25 Pellet 88±25</td>
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<tr>
<td>Gd #10</td>
<td>120mg gd(acetate) 841mg urea 6ml H$_2$O</td>
<td>45min</td>
<td>80±12 (100μl)</td>
<td>Centr. 2500rpm 107±27 pellet 68±13</td>
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<td>Gd #10</td>
<td>120mg gd(acetate) 841mg urea 6ml H$_2$O</td>
<td>1.5</td>
<td>80±12 (100μl)</td>
<td>TEM, MRI</td>
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<tr>
<td>Gd #11</td>
<td>120mg gd(acetate) 841mg urea 6ml H$_2$O</td>
<td>1.5</td>
<td>59±11</td>
<td>Pellet 96±23</td>
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<td>GD #12A</td>
<td>120mg gd(acetate) 841mg urea 6ml H$_2$O</td>
<td>1.5</td>
<td>45.6±7 (32μl)</td>
<td>Centr. 2500rpm 63±7 Centr. 10000rpm 63±18 pellet 53.5±17 and 119±40 (2 particles) Reaction without methanol TEM, MRI</td>
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<td>Gd #12B</td>
<td>120mg gd(acetate) 841mg urea 6ml H$_2$O</td>
<td>1.5</td>
<td>45.6±7 (32μl)</td>
<td>Pellet 45.7±9 TEM, MRI</td>
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<tr>
<td>Gd #13</td>
<td>120mg gd(acetate) 841mg urea 6ml H$_2$O</td>
<td>1.5</td>
<td>52±8(44μl)</td>
<td>Pellet 34±4</td>
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<tr>
<td>Gd #14</td>
<td>120mg gd(acetate) 841mg urea 6ml H₂O</td>
<td>1.5</td>
<td>46±7 (8μl)</td>
<td>Centr. 2500rpm 36±6 Pellet 36±3</td>
<td>MRI</td>
<td></td>
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<tr>
<td>Gd #15</td>
<td>120mg gd(acetate) 841mg urea 6ml H₂O</td>
<td>1.5</td>
<td>80±11 (125μl)</td>
<td>Pellet 100±10 (1 measurement 84±6)</td>
<td>Reaction without methanol TEM</td>
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<tr>
<td>Gd #16</td>
<td>120mg gd(acetate) 841mg urea 6ml H₂O</td>
<td>1.5</td>
<td>39.5±8 (8μl)</td>
<td>Centr. 13000rpm 53±11 (40±8 1 measurement)</td>
<td>Reaction without methanol TEM, MRI</td>
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<td>Gd #17</td>
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<td>120mg gd(acetate) 841mg urea 6ml H₂O</td>
<td>1.5</td>
<td>84±13 (125μl)</td>
<td>Pellet 83±7</td>
<td>TEM, MRI</td>
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<td>Gd #19</td>
<td>120mg gd(acetate) 841mg urea 6ml H₂O</td>
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<td>47±15 (45μl)</td>
<td>Bad measurement</td>
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<td>120mg gd(acetate) 841mg urea 6ml H₂O</td>
<td>1.5</td>
<td>85±7(45μl)</td>
<td>Pellet 89±35</td>
<td>TEM sample, MRI</td>
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<td>Gd #21</td>
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<td>1.5</td>
<td>32±3 (10μl)</td>
<td>Pellet 33±3</td>
<td>MRI</td>
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<td>120mg gd(acetate) 841mg urea 6ml H₂O</td>
<td>1.5</td>
<td>37±5 (32μl)</td>
<td>Pellet 44±5</td>
<td>MRI</td>
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Table 13: Synthesized gadolinium layer around different silica-coated particles
11.2 Curve fit

The expression and values can be found in tables and text.

Figure 18:1.1 $T_2\ $Gd22 and Gd 21 measurement, table 6

Figure 19:1.1 $T_2\ $T40 and T39 measurement, table 6
Figure 21: $T_1$ Gd22 and Gd21 measurement, table 7.
11.4 MRI measurements with wrong time spans

Figure 22: 2.4 T2 for seven different silica thicknesses in falling order Gd12A, Gd18, Gd14, Gd20, Gd16, Gd12B and Gd10 seen in table 9.
1.1 $T_1$ measurement

<table>
<thead>
<tr>
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<tr>
<td></td>
<td>Pair 1</td>
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<tr>
<td>T40</td>
<td>Gd22</td>
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<tr>
<td>Gd21</td>
<td></td>
</tr>
<tr>
<td>Particle $D_n$ [nm] and amount of teos [μl]</td>
<td>~24nm or 32μl</td>
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<tr>
<td>$T_1$ [ms]</td>
<td>1905</td>
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<tr>
<td>$a$</td>
<td>2.8</td>
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Table 14: The MRI $T_1$ results for the coupled pairs.

2.1 $T_1$ measurement

<table>
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<tr>
<td></td>
<td>1 Gd14</td>
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<tr>
<td>$D_n$ [nm] and amount of teos [μl]</td>
<td>~22.5nm or 8μl</td>
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<td>$T_1$ [ms]</td>
<td>392.1</td>
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<tr>
<td>$a$</td>
<td>2.52</td>
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</table>

Table 15: MRI $T_1$ results for particles with different silica thickness with to long time span.

11.3 Links

Graphical illustrations
$T_1$ relaxation [http://www.youtube.com/watch?v=1Kp67IqQiH4](http://www.youtube.com/watch?v=1Kp67IqQiH4)
$T_2$ relaxation [http://www.youtube.com/watch?v=is8TscwFOvM&feature=related](http://www.youtube.com/watch?v=is8TscwFOvM&feature=related)