Gadolinium-Loaded Nanoparticles: New Contrast Agents for Magnetic Resonance Imaging

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Abstract: We have developed a new class of metal-loaded nanoparticles that have potential as contrast agents for medical imaging. These polymeric materials have a core−shell morphology where the interior is a functionalized polymer, that provides high affinity for a specific metal, and the shell consists of a porous hydrophobic polymer that modulates access to the core. In the present case, the nanoparticles are loaded with Gd^{3+} to provide contrast in magnetic resonance (MR) imaging. The Gd^{3+}-loaded nanoparticles described in this paper have a diameter of 120 nm and are, therefore, small enough to pass easily through the vasculature. These particles have been shown to reduce relaxation times in vitro, and provide excellent contrast when used to image the heart and gastrointestinal tract in a rat animal model.

Introduction

The imaging of internal organs and structures is an important diagnostic procedure dating back to the first medical use of X-rays. It is now quite common to use some kind of contrast agent to improve the diagnostic value of images produced by virtually all imaging modalities[1] including X-ray, magnetic resonance (MR),[2−4] and most recently ultrasound (US).[5] Contrast agents aid diagnostic imaging by increasing the contrast between the specific tissue or organ of interest and surrounding tissues in the body. An example is angiography, where an X-ray dye is administered intravenously in order to delineate blood flow through the arteries of the heart for the purpose of diagnosing coronary artery disease.

Early in the development of MR it was felt that contrast agents would not be necessary, but it has become increasingly clear that in many clinical situations contrast agents can greatly improve the diagnostic value of MR,[2−4] just as they have for X-ray and CT. To be effective, MR contrast agents must have a strong local effect on the T_1, T_2 or T_2* relaxation times of water, have the proper pharmacokinetic properties and, obviously, be nontoxic to the patient. One of the most effective techniques for altering the relaxivity of water is to introduce a high spin paramagnetic metal such as Fe or Gd into solution. Water molecules bound to the high-spin metals relax orders of magnitude faster than free water, resulting in a dramatic change in T_1 that can be observed by MR.[2,3] Since it is impossible to dose patients with paramagnetic ions directly, given their inherent toxicity,[6] current MR contrast technology entails sequestering the metal using an organic chelate.[2−4] The chelating ligand acts as a carrier that provides safe transport in to and out of the body. With regard to MR, the first contrast agents to be used routinely are Gd^{3+}-chelates such as Gd^{3+}-DTPA and Gd^{3+}-DOTA.[2−4] These early MR contrast agents have shown great utility for imaging the brain, central nervous system, and other organs. The low molecular weight Gd chelates are essentially first pass agents, which may limit their use in other parts of the body.

There is a significant need for agents that target specific organs, regions of the body, or diseased tissue to gain the greatest diagnostic value from MR. One example is imaging of the gastrointestinal (GI) tract,[7−9] where one would prefer that the agent be localized in the gut. Another example is intravascular imaging[10,11] where the objective is to follow the flow of blood and identify areas of potential stenosis. This would include

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(1) Perkins, A. C. Polymer diagnostics: the next generation of image contrast agents. *J. Drug Targeting* **1998**, *6*, 79−84.
(2) Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. Gadolinium(III) Chelates as MRI Contrast Agents: Structure, Dynamics, and Applications. *Chem. Rev.* **1999**, *99*, 2293−2352.
(3) Lauffer, R. B. Paramagnetic metal complexes as water proton relaxation agents for NMR imaging: theory and design. *Chem. Rev.* **1987**, *87*, 901−927.
(4) Goldstein, H.; Lumma, W.; Rudzik, A. Contrast media for magnetic resonance imaging. *Annu. Rep. Med. Chem.* **1989**, *24*, 265−276.
(5) Goldberg, B. B. *Ultrasound Contrast Agents*; Goldberg, B. B., Ed.; Martin Dunitz, 1997.
(6) Cacheris, W. P.; Quay, S. C.; Rocklage, S. M. The relationship between thermodynamics and the toxicity of gadolinium complexes. *Magn. Reson. Imaging* **1990**, *8*, 467−481.
(7) Balkus, K. J., Jr.; Shi, J. Studies of Gadolinium(III)-Modified Hectorite Clays as Potential Oral MRI Contrast Agents. *J. Phys. Chem.* **1996**, *100*, 16429−16434.
(8) Balkus, K. J., Jr.; Shi, J. A Study of Suspending Agents for Gadolinium(III)-Exchanged Hectorite. An Oral Magnetic Resonance Imaging Contrast Agent. *Langmuir* **1996**, *12*, 6277−6281.
(9) Braybrook, J. H.; Hall, L. D. Synthesis and evaluation of paramagnetic particulates as contrast agents for magnetic resonance imaging (MRI). *Polym. Int.* **1991**, *26*, 251−259.

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imaging the arteries of the heart to diagnose coronary artery disease. Most current commercial agents exhibit poor performance for these applications because of their generally systemic biodistribution, but progress has been made in designing targeted MR contrast agents such as MS325[10,11] from Epix Medical for cardiovascular imaging and Gadolite[7,12] from Pharmacyclics for imaging the GI tract.

One of the major obstacles facing the design of targeted MR contrast agents is the necessity for producing detectable changes in the MR signal intensity of the target tissue or organ by altering its MR relaxation properties. These requirements dictate that there must be a large number of paramagnetic centers selectively bound to the target tissue and that these agents be of sufficiently high molecular weight to prolong vascular retention and thus slow tissue clearance.[13] Over the past several years a variety of different approaches have been used to address both of these issues. These approaches include the conjugation of paramagnetic chelates to macromolecules such as serum albumin[14] or linear polymers.[15] More recently, there have been several newer approaches reported in the literature which show a great deal of promise in fulfilling the two criteria discussed above. These include polymeric paramagnetic liposomes[16−20] and dendrimer-based metal chelates.[21−24]

The liposomes are made by incorporating diethylenetriaminepentaacetic acid (DTPA)-conjugated lipids into liposomes. These conjugated lipids can then form chelating sites for gadolinium ions. The chelates are so-called headgroups on the outer surface of the liposome. In the initial reports the liposomes were also conjugated with biotin for histochemical studies.[16] This indicates that the liposomes can be thought of as a two-stage delivery system, one stage is the targeting ligand (biotin) and the other is the detection system (Gd^{3+}-DTPA). Either system can be selectively altered. For example, Sipkins et al. have described a liposome-based MRI approach to detecting angiogenesis in vivo using paramagnetic Gd as the detection agent and targeting endothelial $\alpha_v\beta_3$ receptors, via the LM609 monoclonal antibody.[25] This approach is very attractive since in principle it can be extended to MR imaging of other receptors if suitable targeting ligands can be developed and conjugated to the lipids. Bulte et al. [26] have recently reported a slightly different liposomal approach in which superparamagnetic iron oxide particles were encapsulated into liposomes. These agents were employed as MR contrast agents for the imaging of bone marrow.

In addition to MR, other imaging/detection methods can be employed with liposomes by conjugating suitable moieties into the detection site. For example, a radionuclide may be employed for PET or SPECT studies. Alternatively a suitable fluorophore may be conjugated to the liposomes for use in optical studies. The possibility of employing complementary imaging strategies allow for the design of cross correlative studies aimed at determining the biodistribution of the liposomal agents. In this context it is important to note that there are radioactive analogues of Gd^{3+} (DTPA), namely Yb^{3+} (DTPA), which have been employed in radionuclide studies.[27−30] Weiner et al.[24] have employed the dendrimer approach to target tumor cells expressing the high-affinity folate receptor. The imaging method employed was MRI, and the dendrimer contained gadolinium.[23] As was discussed for the liposomes, the dendrimer approach can also be thought of having two parts: the targeting moiety and the detection system. This approach can potentially have the same opportunities as were discussed above. The choice of approach for the agent described in this paper will be based on a number of factors including ease of synthesis, toxicity, relative receptor affinity, and tissue clearance properties.

We have discovered a new class of metalated 120 nm core−shell particles that exhibit good selectivity, MR contrast, and low toxicity. The size and composition of these nanoparticles provide favorable pharmacokinetic properties, including limited biodistribution and bioavailability. The materials discussed here contain $Gd^{3+}$to provide contrast in MR, but in principle, one could encapsulate other chemical entities within the nanoparticles, leading to enhanced contrast in other imaging modalities such as X-ray, CT, or nuclear imaging.

Metal-Loaded Nanoparticles. We have synthesized novel spherical metal-loaded core−shell nanoparticles that have an average diameter of 120 nm (Figure 1). The polymeric core is designed to have a high affinity for metals, such as gadolinium. The core consists of a large fraction of acidic methacrylic acid

(10) McMurry, T. J.; Sajiki, H.; Scott, D. M.; Lauffer, R. B. Diagnostic imaging contrast agents with extended blood retention. WO 9623526 A2, 1996.

(11) Parmelee, D. J.; Walovitch, R. C.; Ouellet, H. S.; Lauffer, R. B. Preclinical evaluation of the pharmacokinetics, biodistribution, and elimination of MS-325, a blood pool agent for magnetic resonance imaging. *Invest. Radiol.* **1997**, *32*, 741−747.

(12) Balkus, K. J., Jr.; Sherry, A. D.; Young, S. W., Zeolite-enclosed transition and rare earth metal ions as contrast agents for the gastrointestinal tract. U.S. Patent 5,122,363 A, 1992.

(13) Nunn AD.; Linder KE.; MF, T. Can receptors be imaged with MRI agents? *Q. J. Nucl. Med.* **1997**, *41*, 155−162.

(14) Brasch, R. C. Rationale and applications for macromolecular Gd-based contrast agents. *Magn. Reson. Med.* **1991**, *22*, 282−287; discussion 300−303.

(15) Shreve, P.; Aisen, A. M. Monoclonal antibodies labeled with polymeric paramagnetic ion chelates. *Magn. Reson. Med.* **1986**, *3*, 336−40.

(16) Sipkins, D. A.; Gijbels, K.; Tropper, F. D.; Steinman, L.; Bednarski, M. D. Antibody-Conjugated Paramagnetic Liposomes - Tissue-Specific Contrast Agents For Disease Processes. *Radiology* **1995**, *197* (Suppl.), 276.

(17) Li, K. C.; Bednarski, M. D.; Storrs, R. W.; Li, H. Y.; Trooper, F. D.; Song, C. K. H.; Sipkins, D. A.; Kuniyoshi, J. K. Targeted polymerized liposome contrast agents. U.S. Patent 5,512,294 A, 1996.

(18) Storrs, R. W.; Tropper, F. D.; Li, H. Y.; Song, C. K.; Kuniyoshi, J. K.; Sipkins, D. A.; Li, K. C. P.; Bednarski, M. D. Paramagnetic Polymerized Liposomes: Synthesis, Characterization, and Applications for Magnetic Resonance Imaging. *J. Am. Chem. Soc.* **1995**, *117*, 7301−7306.

(19) Tilcock, C. Liposomal blood pool agents for nuclear medicine and magnetic resonance imaging. *J. Liposome Res.* **1994**, *4*, 909−936.

(20) Torchilin, V. P.; Trubetskoy, V. S. In vivo visualizing of organs and tissues with liposomes. *J. Liposome Res.* **1995**, *5*, 795−812.

(21) Kim, Y.; Zimmerman, S. C. Applications of dendrimers in bio-organic chemistry. *Curr. Opin. Chem. Biol.* **1998**, *2*, 733−742.

(22) Schmitt-Willich, H.; Platzek, J.; Raduechel, B.; Muehler, A.; Frenzel, T. Cascade polymer complexes for use in medical diagnostics. Ger. Patent DE 19525924 A1, 1997.

(23) Wiener, E. C.; Brechbiel, M. W.; Brothers, H.; Magin, R. L.; Gansow, O. A.; Tomalia, D. A.; Lauterbur, P. C. Dendrimer-based metal chelates: a new class of magnetic resonance imaging contrast agents. *Magn. Reson. Med.* **1994**, *31*, 1−8.

(24) Wiener, E. C.; Konda, S.; Shadron, A.; Brechbiel, M.; Gansow, O. Targeting dendrimer-chelates to tumors and tumor cells expressing the high-affinity folate receptor. *Invest. Radiol.* **1997**, *32*, 748−754.

(25) Sipkins, D. A.; Cheresh, D. A.; Kazemi, M. R.; Nevin, L. M.; Bednarski, M. D.; Li, K. C. P. Detection of tumor angiogenesis in vivo by alpha(v)beta(3)- targeted magnetic resonance imaging. *Nat. Med.* **1998**, *4*, 623−626.

(26) Bulte, J. W.; de Cuyper, M.; Despres, D.; Frank, J. A. Short- vs long-circulating magnetoliposomes as bone marrow-seeking MR contrast agents. *J. Magn. Reson. Imaging* **1999**, *9*, 329−335.

(27) Wagner, H. N., Jr.; Hosain, F.; DeLand, F. H.; Som, P. A new radiopharmaceutical for cisternography: chelated ytterbium 169. *Radiology* **1970**, *95*, 121−5.

(28) Gilday, D. L.; Reba, R. C.; Hosain, F.; Longo, R.; Wagner, H. N., Jr. Evaluation of ytterbium-169 diethylenetriaminepentaacetic acid as a brain-scanning agent. *Radiology* **1969**, *93*, 1129−1134.

(29) Hosain, F.; Reba, R. C.; Wagner, H. N., Jr. Ytterbium-169 diethylenetriaminepentaacetic acid complex. A new radiopharmaceutical for brain scanning. *Radiology* **1968**, *91*, 1294.

(30) Hosain, F.; Wagner, H. N., Jr. Measurement of extracellular fluid volume with 169Yb-diethylenetriaminepentaacetate. *J. Lab. Clin. Med.* **1971**, *77*, 699−704.

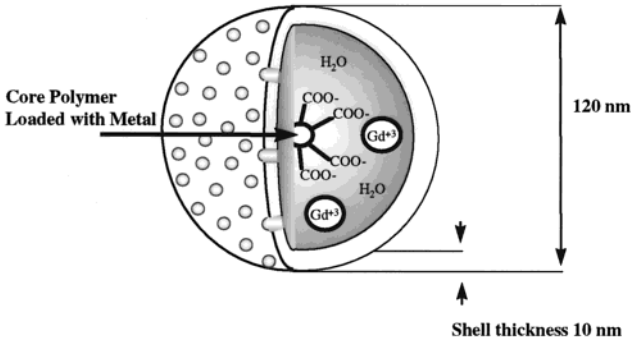

Figure 1. Schematic representation of the Gd^{3+}-loaded core−shell nanoparticle.

Table 1. Percent Monomer Composition (by weight) of Polymeric Nanoparticles

monomer	core	shell
styrene		20
ethylacrylate	48	70
methacrylic acid	48	9
butylacrylate		
allylmethacrylate	4	1

(MAA) monomers (Table 1), that form strong complexes with Gd^{3+}, or some other suitable high-spin metal. The Gd^{3+} complexed core polymer is encapsulated within a polymeric shell that ensures that Gd^{3+} is sequestered in the core and helps provide the desired pharmacokinetic properties. At the same time, the shell is porous to water allowing for rapid exchange of water into and out of the nanoparticle. This rapid exchange of water is necessary to produce the desired relaxivity (decreased T_1) in water. The 120 nm nanoparticles have a very narrow particle size distribution and are small enough to pass freely throughout the body. If necessary polymers can be made with a range of particle sizes and surface characteristics using the chemistry described in this paper. For example, we have made particles that range in size from 70 to 120 nm.

The Gd^{3+}-loaded nanoparticles are synthesized in three distinct steps. First the core polymer is constructed using emulsion polymerization. The core particle contains the functionality necessary for metal binding, in this case free carboxylic acid groups. This core polymer is then loaded by adding gadolinium nitrate to the polymer emulsion. In the final step, the shell is added during a second round of polymerization using the metal-loaded core polymer as a seed.

Other examples of polymeric contrast agents exist, and can be grouped into roughly four classes. These are polymer conjugates of metal chelates,[31−40] metal-loaded ion-exchange resins,[7,9,12] dendrimers,[21−24] and liposomes.[17−20] However, none of these exhibit the unique core−shell morphology described herein.

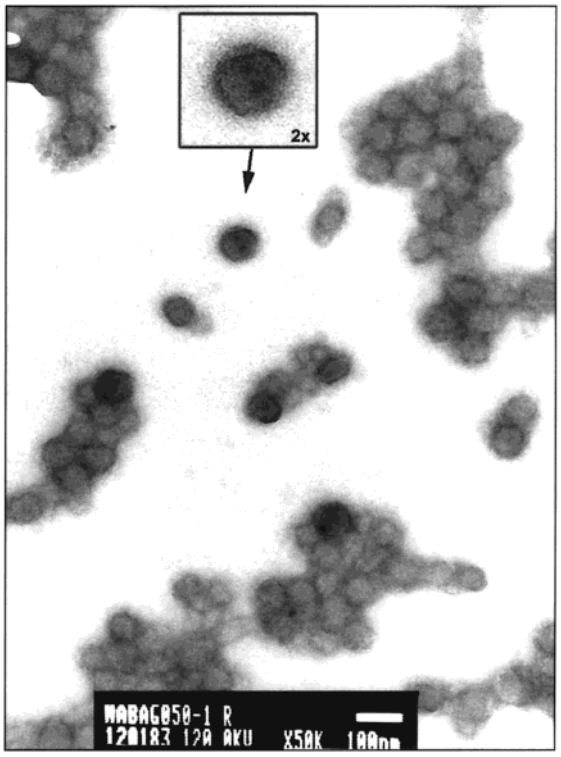

Figure 2. Transmission electron micrograph for the Gd^{3+} nanoparticles.

Table 2. NMR Relaxation Times T_1

sample	g Gd/g polymer	g Gd/L	T_1 (ms)
ultrapure H_2O			2200
10% latex polymer	0	0	2054
core with Gd^{3+}	0.005	0.5[a]	97
core with Gd^{3+}	0.015	1.5[a]	36
core with Gd^{3+}	0.03	3.0[a]	16
core with Gd^{3+}	0.045	4.5[a]	11
core−shell polymer	0.026	1.48[b]	29
core−shell polymer	0.031	1.98[b]	22

[a] Estimate based on average value of 10% solid content for core polymer emulsion. [b] Estimated based on a solid content of 6.4% for the core−shell polymer. The imaging studies were carried out with the 0.031 gGd/g polymer material.

Experimental Section

The T_1 measurements were performed on a DMX400 spectrometer using a standard inversion recovery (IR) pulse sequence. The recycle delay time was set to five times the T_1 value. Typically 12 points were taken for each T_1 measurement. The probe matching was adjusted for minimal radiation damping from the dominating water signal.

Core Polymer. A monomer emulsion was made from a mixture of 200 g deionized water, 8.13 g of 28% w/w solids ammonium lauryl

(31) Armitage, F. E.; Richardson, D. E.; Li, K. C. P. Polymeric contrast agents for magnetic resonance imaging: synthesis and characterization of gadolinium diethylenetriaminepentaacetic acid conjugated to polysaccharides. *Bioconjugate Chem.* **1990**, *1*, 365−374.

(32) Bishop, J. F.; Mourey, T. H.; Texter, J. Adsorption of triblock copolymers on nanoparticulate pharmaceutical imaging agents. *ACS Symp. Ser.* **1995**, *615*, 205−216.

(33) Brasch, R. C.; Mann, J. S.; Nitecki, D. E. Macromolecular contrast media for magnetic resonance imaging. WO 9427498 A1, 1994/1995.

(34) Braybrook, J. H.; Hall, L. D. Carbohydrate contrast agents for nuclear magnetic resonance imaging: enhancement of the relaxation of water by paramagnetic metal complexes of polysaccharides. *Carbohydr. Res.* **1989**, *187*, C6−C8.

(35) Butterfield, D. E.; Fujii, D. K.; Ladd, D. L.; Snow, R. A.; Tan, J. S.; Toner, J. L. Segmented chelating polymers as imaging and therapeutic agents. WO 9526754 A1, 1995.

(36) Hollister, K. R.; Keller, K. E.; Wei, D.; Peng, X.; Ladd, D. L.; Snow, R. A. Chelating polymers as contrast agents for medical imaging. WO 9640274 A2, 1996.

(37) Loubeyre, P.; Canet, E.; Zhao, S.; Benderbous, S.; Amiel, M.; Revel, D. Carboxymethyl-dextran-gadolinium-DTPA as a blood-pool contrast agent for magnetic resonance angiography. Experimental study in rabbits. *Invest. Radiol.* **1996**, *31*, 288−293.

(38) Matthews, S. E.; Pouton, C. W.; Threadgill, M. D. Macromolecular systems for chemotherapy and magnetic resonance imaging. *Adv. Drug Delivery Rev.* **1996**, *18*, 219−267.

(39) Schmitt-Willich, H.; Platzek, J.; Muehler, A.; Adam, G.; Guenther, R. Use of polymeric contrast agents of medium molecular weight for differentiation of benign and malignant tumors by modern imaging techniques. German Patent DE 19518222 A1, 1996.

(40) Torchilin, V. P. Novel polymers in microparticulate diagnostic agents. *CHEMTECH* **1999**, 27−34.

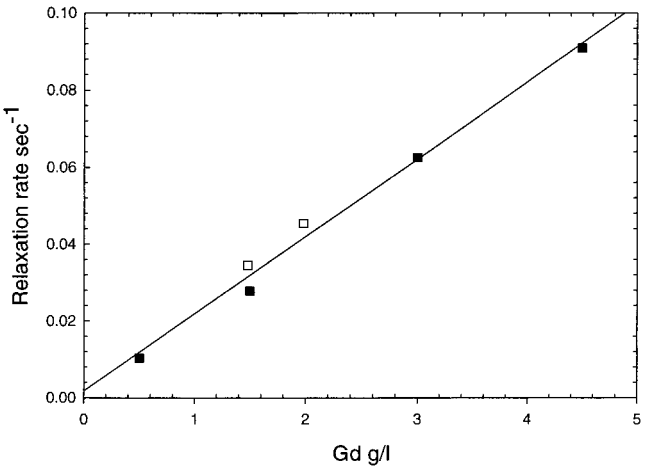

Figure 3. Plot of relaxation rate versus Gd concentration. Core−shell particles at two different Gd loadings are shown as open squares. Particles without shells are shown as filled squares.

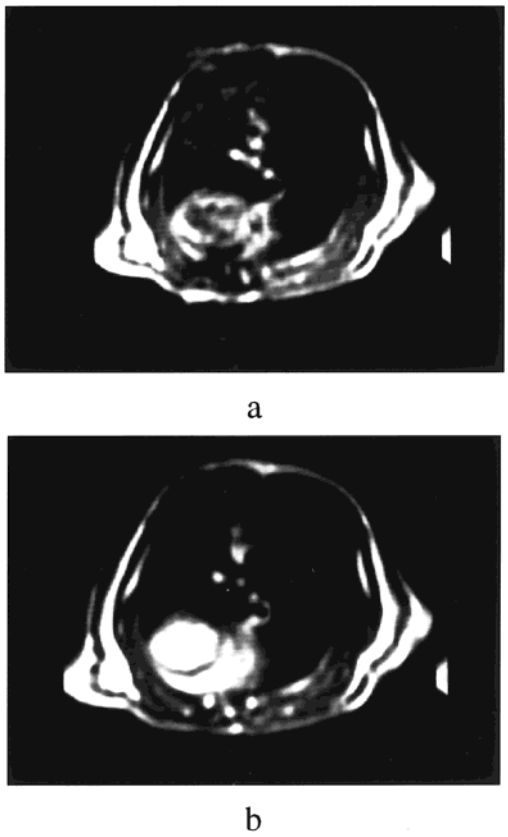

Figure 4. MR images of a rat heart before (a) and after (b) administration of the nanoparticles.

sulfate (ALS), 90.0 g ethyl acrylate (EA), 90.0 g methacrylic acid (MAA), and 7.5 g allyl methacrylate (ALMA) in a bottle. A reaction kettle was then prepared with 150 g deionized water, 34.0 g of the monomer emulsion, and 0.04 g ammonium persulfate in one mL of deionized water. The reaction kettle was heated to 80 °C while being purged with nitrogen. The rest of the monomer emulsion above and 32 g of a solution containing 0.20 g ammonium persulfate (APS) in deionized water were fed into the reaction flask (80 °C) at 4.0 g/min and 0.35 g/min, respectively. At the end of the feed, the temperature of the reaction flask was cooled to 75 °C, and then a solution of 0.10 g *tert*-butyl hydroxy peroxide (t-BHP) in 1 mL of deionized water was added. The reaction was cooled further to 55 °C. To this was added a solution of 0.060 g of sodium sulfoxylate formaldehyde (SSF) in 2 mL of deionized water. The reaction was cooled to ambient temperature, and the emulsion was filtered through 325 and 100 mesh sieves,

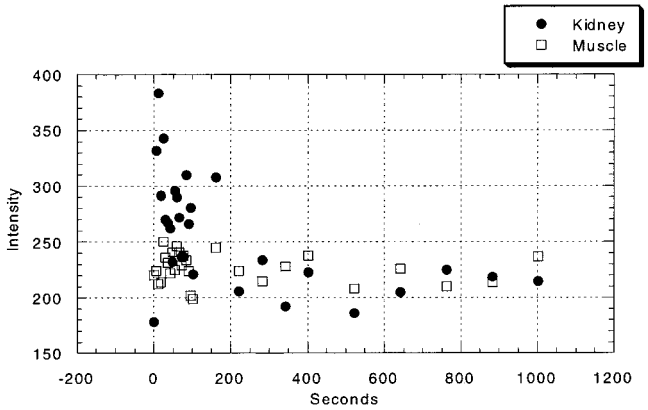

Figure 5. Plot of intensity versus time for a single pixel in kidney and a single pixel in muscle.

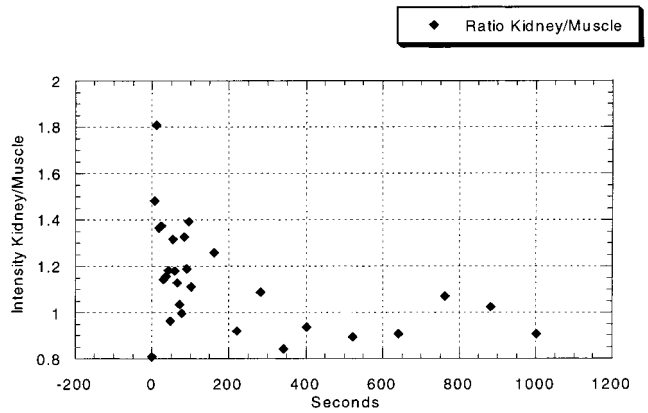

Figure 6. Ratio of kidney intensity divided by muscle intensity as a function of time.

respectively, to yield a polymer emulsion having an average particle size of 100 nm.

Metal-Loaded Core. This emulsion (265.84 g, 27.5% solids) was mixed with 200 g of deionized water and 10% ammonium hydroxide to produce a mixture having pH 5. To this was added dropwise, 59.85 g of a Gd(III) nitrate, hexahydrate solution (54,924 ppm in deionized water). The pH of the mixture was monitored and adjusted to 5 with ammonium hydroxide throughout the test, whenever necessary, during the addition of salt solution. Stirring was continued for 30 min at the end of salt addition. The resulting gadolinium-loaded emulsion polymer had a loading of 0.045 g of Gd/g of resin.

Core−Shell Polymer. The metal-loaded resin was encapsulated by forming a shell around the gadolinium-loaded core material (loading rate of 0.045 g Gd/g of core resin) using the following procedure. A reaction kettle was loaded with 200 g of deionized water, 3.0 g of 23% sodium benzyl laurate (siponate DS-4), 0.1 g of ammonium persulfate in 2 mL of deionized water, 0.2 g of sodium carbonate in 3 g of deionized water, and 200 g of the loaded core from Example 4 (10.1% solids). The kettle was heated to 80 °C while being purged with nitrogen.

Transmission electron microscopy study on the resulting material showed the resulting loaded, encapsulated resin to have a core−shell morphology (Figure 2).

Results and Discussion

***T_1* Relaxation and Contrast.** The Gd^{3+}-loaded nanoparticles described above were evaluated for their ability to modify the relaxation rate of water in vitro using a standard NMR spectrometer at 400 MHz. The results of these initial studies are given in Table 2. It is clear from Table 2 that the gadolinium-loaded nanoparticles significantly reduce the relaxation time relative to pure water or the unloaded polymer. Therefore, these

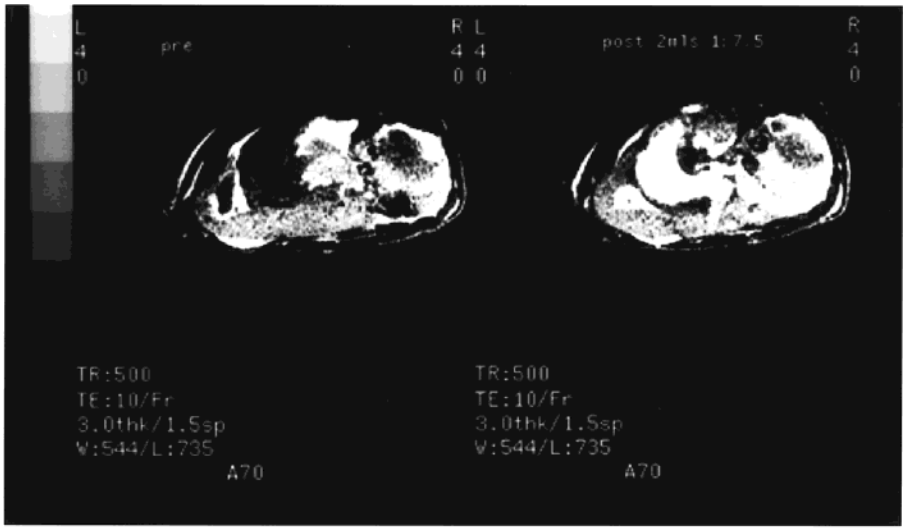

Figure 7. MR images of a rat GI tract before (a) and after (b) administration of the nanoparticles.

materials should act as contrast-enhancing agents. Metal loadings are reported in Table 2 as a ratio of weight of Gd per weight of polymer (g Gd/g polymer). At the 0.1 g Gd/g polymer loading T_1 is reduced by 3 orders of magnitude. Even at very low loadings of 0.005 g Gd/g polymer, the T_1 is reduced from 2200 ms for pure water to only 97 ms. Similar results were observed for in vitro experiments in a medical imager. To follow the change in T_1 with respect to total metal concentration, metal concentrations were calculated for the nanoparticles with and without a shell. These are reported in Table 2 with the T_1 values, and are plotted in Figure 3. Figure 3 shows that the shell has a small effect on the relaxivity of the particles in vitro, demonstrating that the shell allows good water exchange through the shell.

Intravenous Administration. To fully assess the potential of these materials as contrast agents, imaging experiments were carried out in a series of rats. In a typical experiment 0.5−1.0 mL of contrast agent was administered via the tail vein or femoral artery, with imaging beginning immediately after administration. This corresponds to approximately 1−2 mg of Gd for each 400 g (avg.) rat. Thus, the typical dose was 3.5 mg/kg of Gd or 112 mg/kg of the Gd^{3+}−polymer complex. This compares favorably to dosages for the small Gd^{3+}-chelates. Full body scans were taken at approximately 4 min intervals up to 45 min postinjection. Pre- and postcontrast images of the heart showed that the nanoparticles provided very significant enhancement of the intravascular space (Figure 4). The heart chamber was brightly lit by the contrast agent as were all of the major arteries leading into or out of the heart. Images of the kidneys also showed significant enhancement immediately postinjection, followed by a rapid decay in signal intensity.

The kidney uptake and clearance was deemed worthy of further study. A single 400 g rat was dosed with 0.7 mL of agent (3.5 mg/kg of Gd), and contrast intensities were monitored both in the kidney and adjacent muscle. This study showed a rapid rise in contrast intensity followed by rapid decay. These results are given in Figure 5. By contrast, the intensity in surrounding muscle remained relatively constant throughout the experiment. The ratio of intensities for kidney/muscle (Figure 6) jumped to a maximum soon after administration and then fell off slowly over a 10 min period. This suggests that the agent accumulated rapidly in the kidneys, perhaps during the first pass, and then was subsequently cleared.[41]

Oral Administration. In separate experiments the nanoparticles were administered orally by gastric lavage. In the case of oral dosing, 2−3 mL of three different dilute solutions of the contrast agent were administered. The dilutions were 1:1, 1:10, and 1:100. Full body scans were initiated 2 min postadministration and were continued in 2 min intervals for up to 1 h. Comparison of these images showed that the 1:10 dilution was optimal. This corresponds to approximately 0.6 mg of Gd, assuming a 3 mL dose. The most significant observation was that the gastrointestinal tract was easily visualized postadministration, and the enhancement persisted for the full hour. The stomach and intestines were clearly seen in at least two scan planes, indicating that this agent is quite effective as an oral contrast agent for MR at very small doses. Pre- and postadministration images are given in Figure 7 for comparison.

Conclusions

We have synthesized a novel class of Gd^{3+}-loaded nanoparticles that show potential as MR contrast agents. These nanoparticles consist of Gd^{3+} bound to a core polymer that is encapsulated within a thin polymeric shell. The shell allows efficient water exchange as evidenced by the measured relaxation times. In vitro and in vivo testing demonstrate the effectiveness of these nanoparticles as oral and IV contrast agents. When dosed orally they provide exceptional images of the gastrointestinal tract. These agents could be used to diagnose structural irregularities, disease, or simply to mark the GI tract in order to aid visualization of other structures within the abdomen. Further, they are not bioavailable and remain in the gastrointestinal tract, a fact that greatly reduces any potential toxicity concerns.

When administered intravenously, the Gd^{3+}-loaded nanoparticles remain in the intravascular space until removed primarily by the kidneys. As such they provide excellent visualization of the vasculature, particularly arterial flow. This makes them potentially attractive for diagnosing coronary artery disease and other conditions involving stenosis. Preliminary results indicate that the small 120 nm particles may be cleared by the kidney.

(41) Sarkar, S. K.; Rycyna, R. E.; Lenkinski, R. E.; Solleveld, H. A.; Kinter, L. B. Yb-DTPA, a Novel Contrast Agent in Magnetic Resonance Imaging: Applications to Rat Kidney. *Magnetic Resonance in Medicine* **1991**, *17*, 328−335.

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