

Critical examination of Fe₈ as a contrast agent for magnetic resonance imaging

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Received 6 October 2004; accepted 15 December 2004

Available online 11 July 2005

Abstract

The compound Fe₈ (C₃₆H₁₂₀O₂₃N₁₈Fe₈Br₈) was examined for its potential as a contrast agent for magnetic resonance imaging. The compound was prepared, isolated, and shown to exhibit superparamagnetism ($S = 10$). The structure of Fe₈ in aqueous solution was probed by several NMR techniques. Relaxivity of aqueous solutions of Fe₈ as well as that of comparison compounds FeCl₃, FeCl₃(tacn) (tacn = 1,4,7-triazacyclononane), and Gd(DTPA) (DTPA = diethylenetriamine pentaacetic acid) were determined by T_1 and T_2 inversion recovery and CPMG sequences. In contrast to a previous report, the relaxivity of Fe₈ solutions was found to be weak compared to Gd(DTPA). However, an interesting dependence upon pH was observed. The influence of environmental factors on relaxivity is of interest in the burgeoning field of molecular imaging.

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Keywords: Single-molecule magnet; Magnetic resonance imaging; Superparamagnet; Relaxation; Contrast agent

1. Introduction

Magnetic resonance imaging (MRI) is a non-invasive technique that takes advantage of the endogenous differences in the spins of different regions of a water-containing specimen. A key advantage of MRI is that it provides the ability to visualize and diagnose abnormalities in vivo in a non-invasive fashion. It provides obvious benefits over techniques that employ radioactive exposure and radiation.

The diagnostic power of MRI images is frequently enhanced by the use of contrast agents [1]. These are usually paramagnetic substances, which enhance the relaxation of nuclear spins to their equilibrium states.

The bulk relaxation properties of proton spins in water molecules are described phenomenologically by $R_1 = 1/T_1$ and $R_2 = 1/T_2$ (in units of s⁻¹), which indicate the rate at which the longitudinal magnetization reaches equilibrium, and the rate at which transverse magnetization decays, respectively. MRI images can be tailored to achieve image contrast based on both the R_1 and the R_2 mechanisms [1]. In the former case positive contrast is obtained, namely an increase of pixel intensity where R_1 relaxation is rapid, and in the latter case negative contrast is found, i.e. a drop in pixel intensity where R_2 relaxation is rapid [1].

Many factors contribute to the relaxation mechanisms, but paramagnetic relaxation is known to be extremely effective in this arena [1]. This phenomenon relies on the presence of fluctuating magnetic fields induced by the motion of paramagnetic species with respect to the water molecules. It depends on the spin

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value of the paramagnetic center (roughly as $S(S+1)$), as well as on the concentration of the paramagnetic agent in the media. At low concentrations there is a linear dependence of R_1 and R_2 on the concentration.

The notion of relaxivity is used to allow one to compare the effectiveness of different relaxation agents (contrast agents) independently of the concentration used. It is defined as $r_{1,2} = R_{1,2}/c$, where c is the concentration. A competitive value in medical diagnostics is $4\text{--}5 \text{ s}^{-1} \text{ mM}^{-1}$, recorded at magnetic fields of 2–3 T [1].

Recently, superparamagnetic iron oxide particles have attracted much attention as contrast agents, due to their extremely large spin values [2]. Single-molecule magnets, by contrast, offer superparamagnetism in uniquely small particles [3,4], that would be very powerful as contrast agents. Our attention was attracted to a report that the classic compound “ Fe_8 ” provided contrast similar to that offered by the commercial compound Magnevist (GdDTPA) [5], and we report here a critical examination of its usefulness.

2. Experimental

2.1. Synthesis

The intermediate complex $\text{Fe}(\text{tacn})\text{Cl}_3$ and crystals of Fe_8 ($\text{C}_{36}\text{H}_{120}\text{O}_{23}\text{N}_{18}\text{Fe}_8\text{Br}_8$) were obtained according to the method of Wiegardt [6,7]. The bright yellow precipitate formed from reacting tacn (1,4,7-triazacyclononane) ligand, purchased from Aldrich, and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in ethanol, and was isolated and dried. The product was dissolved in a mixture of H_2O and pyridine. After 15 min, NaBr was added to the solution and the mixture was cooled to 200 °C and allowed to stand for 24 h. The brown crystals were isolated, washed and subjected to physical studies.

2.2. Physical measurements

Low temperature (0.4 K) magnetization versus field data were collected using a micro-Hall effect magnetometer which is described in [8].

Relaxivity measurements were conducted at 9.4 T on a Bruker AV 400 MHz narrow-bore NMR spectrometer in a standard 5 mm quartz tube. T_1 and T_2 were determined by routine inversion-recovery and CPMG sequences, respectively. The relaxation agents were dissolved in 99% D_2O to avoid both receiver saturation and radiation damping effects due to the appearance of a strong H_2O signal in the NMR measurements [9]. The water relaxation rates reported refer to the residual H_2O in the sample. All NMR measurements were taken at 25 °C.

The pH was adjusted in the samples by adding small amounts of NaOH to the solution. pH determinations

were conducted with a standard glass combination pH electrode. The values reported are uncorrected for the presence of D_2O as the main solvent ($\text{pD} = \text{pH} - 0.4$). Physical studies comparing the relaxivity of the compounds and their dependence on concentration were done in 0.1 M HEPES buffer at pH 7 (purchased from Sigma). The lyophilized sample of Fe_8 was prepared by dissolving the crystals in water and immediately freezing them using dry ice. The water was removed using a standard lyophilizer.

3. Results and discussion

3.1. Characterization of Fe_8

Crystals of Fe_8 gave magnetic behavior consistent with that previously reported [10]. Steps in the hysteresis curves were observed at regular field intervals consistent with the $S = 10$ ground state and the spin-Hamiltonian parameters reported for Fe_8 . We note that the hysteresis steps, while well defined, were broad, perhaps indicative of crystal defects.

3.2. Determination of relaxivity

As shown in Fig. 1, relaxivity was measured in aqueous solutions buffered at a pH of 7. Fe_8 gave a much

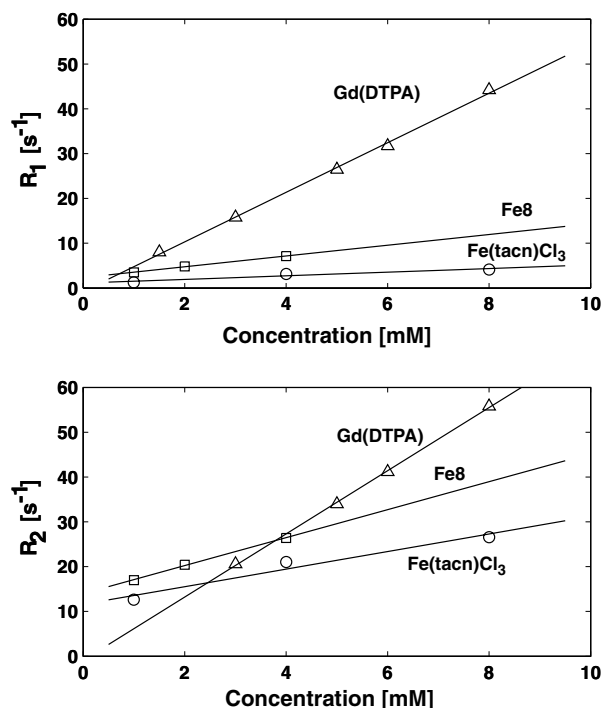


Fig. 1. Plot of R_1 and R_2 parameters vs. concentration of aqueous solutions of Gd(DTPA), $\text{Fe}(\text{tacn})\text{Cl}_3$, and Fe_8 , determined by inversion recovery and CPMG sequences on a 400 MHz NMR spectrometer.

lower T_1 relaxivity ($1.2 \text{ s}^{-1} \text{ mM}^{-1}$) than the commercially used reference, GdDTPA ($5.5 \text{ s}^{-1} \text{ mM}^{-1}$). Since this is in contrast to previous studies [5] we also investigated the relaxivity of the precursor complex $\text{Fe}(\text{tacn})\text{Cl}_3$ ($0.4 \text{ s}^{-1} \text{ mM}^{-1}$). The relaxation constants were monitored over a 24 h period, and were found to be approximately constant in solutions buffered at neutral pH. The r_2 parameters were measured as $3.1 \text{ s}^{-1} \text{ mM}^{-1}$ (Fe_8), $7.0 \text{ s}^{-1} \text{ mM}^{-1}$ (GdDTPA), and $2.0 \text{ s}^{-1} \text{ mM}^{-1}$ ($\text{Fe}(\text{tacn})\text{Cl}_3$).

3.3. Examination of solution species

Aqueous solution equilibria of iron complexes can be complex, and it would be appropriate to question the nature of solution species that would form upon dissolving the Fe_8 complex [11,12]. The relaxivity data indicate that Fe_8 does not decompose fully and exclusively to form $\text{Fe}(\text{tacn})\text{Cl}_3$. If complete decomposition took place its relaxivity would be at least six times larger than the one for $\text{Fe}(\text{tacn})\text{Cl}_3$ (considering the presence of only six ligands in Fe_8). Indeed, Fe(III) itself can show appreciable relaxivity in aqueous solution (for a review, see [13]). The fairly weak relaxivity shown for the solution prepared from Fe_8 suggests that some clustering of Fe persists in solution, resulting however in antiferromagnetic coupling, lower overall spin, and therefore lower contrast.

Among the many experiments that were attempted to shed light on solution speciation, perhaps the most definitive was that the Fe_8 was dissolved in water at a concentration of 1 mM and immediately frozen, then lyophilized to remove the water in the gentlest manner possible. A brown powder was obtained that was examined for superparamagnetism. The data indicated that

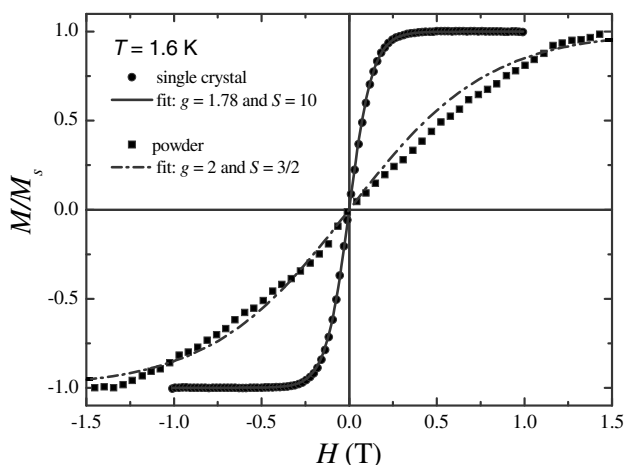


Fig. 2. Hysteresis curves for a single crystal of Fe_8 and grains/powder resulting from lyophilization of an aqueous solution of Fe_8 at 1.6 K. The powder appeared to be a low-spin $\sim 3/2$ at 0.4 K with no (or very small) hysteresis.

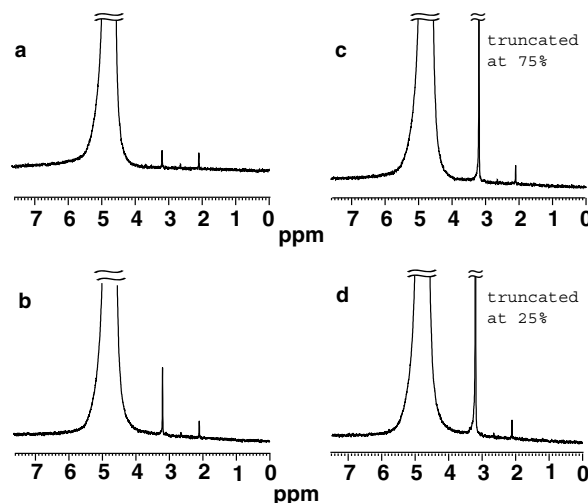


Fig. 3. ^1H NMR spectra of Fe_8 (at $c = 4 \text{ mM}$) in 99% D_2O , at (a) immediately after dissolving, (b) 30 min (c) 120 min, and (d) 240 min after dissolving.

the powder did not have a significant amount of Fe_8 (Fig. 2). It appeared to be a low-spin substance, with spin $\sim 3/2$. At 0.4 K there was no (or very small) hysteresis. The data were noisy because of the small amount of material.

The ^1H NMR spectra of the solutions of the Fe_8 complexes were examined to assess whether a solution species could be assigned. The single peak at $\delta 3.2$ increased substantially in intensity over 3 h as illustrated in Fig. 3. This peak coincides in shift with the one observed for $\text{Fe}(\text{tacn})\text{Cl}_3$ and we therefore conclude that at least partial decomposition into this precursor compound occurs. Signals corresponding to that of free ligand ($\delta 2.8 \text{ ppm}$) were not observed.

3.4. Environmental dependence of relaxivity

During the course of examination of the solution species formed by Fe_8 , a very strong dependence on pH was observed. As shown in Fig. 4, an increase of pH from neutral to 11 resulted in more than a 5-fold increase in R_1 for the Fe_8 complex. In contrast, the R_1 for GdDTPA was nearly constant over this range, and decreased for $\text{Fe}(\text{tacn})\text{Cl}_3$. This data provides additional evidence for decomposition/rearrangement processes of Fe_8 in solution. The R_2 parameters, however, did not change as dramatically over this pH range.

The increase in pH of the solutions of Fe_8 were accompanied by complex changes in ^1H NMR spectra. At neutral pH, a single peak at $\delta 3.2$ dominated the spectrum, while a smaller peak at $\delta 2.2$ was present. At pH 7.6 a peak at 2.9 ppm appeared. The peak at 2.2 ppm grows larger up to a pH of 9 and becomes smaller again when approaching a pH of 10.7. At pH 11.3 small peaks appear at 3.0, 2.9, and 2.8 ppm (apparently

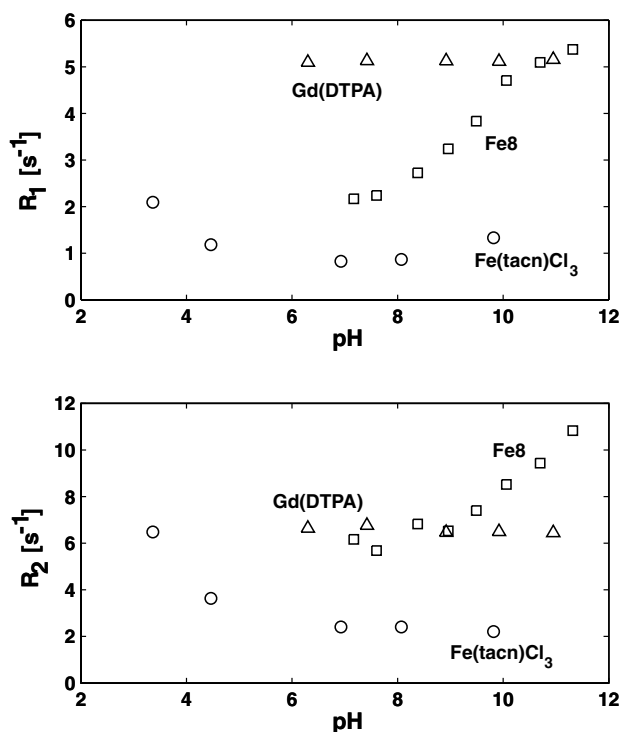


Fig. 4. pH-dependence of the relaxation parameters R_1 and R_2 for Fe₈ (squares), Fe(tacn)Cl₃ (circles), and Gd(DTPA) (triangles) (pH values are uncorrected for the presence of the large D₂O concentration).

corresponding to free ligand). In addition, a large peak is visible at 2.23 ppm. Thus, significant changes in solution speciation occurred with increasing pH, and some of these new species are likely to be responsible for the increased relaxivity.

Dependence of relaxivity on pH is of interest in the burgeoning field of molecular imaging in which “smart” contrast agents are desired as reporters of molecular processes in living systems [14,15]. However, further work will be required to determine whether Fe clusters may be of use for such purposes.

4. Conclusion

In contrast to the earlier report [5], Fe₈ in neutral, aqueous solution did not show stronger relaxivity than GdDTPA. This is corroborated by measurements of the magnetic properties before and after dissolving

Fe₈, as well as solution NMR spectra and measured R_1 and R_2 parameters under various conditions. However, we found the relaxation properties of this compound to be highly dependent upon pH, a behavior that is of interest in the development of environment-sensitive contrast agents.

Acknowledgements

This work was supported by the National Science Foundation, Grant #NSF-DMR-0103290, and the NYU Whitehead Fellowship for Junior Faculty in the Biomedical and Biological Sciences. New York University is a member institution of the New York Structural Biology Center supported by NIH Grant 1P41 GM66354. NYU’s NMR facilities were supported by a NSF grant MRI-0116222. We acknowledge Dr. Chin Lin and the Molecular Imaging and Contrast Agent Working Group at NYU.

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