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Journal of Magnetism and Magnetic Materials 293 (2005) 520–525
Journal of magnetism and magnetic materials
www.elsevier.com/locate/jmmm

Proton NMR relaxivity of blood samples in the presence of iron, gadolinium and dysprosium compounds

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Available online 5 March 2005

Abstract

The longitudinal and transverse relaxation rates of some iron, gadolinium and dysprosium compounds have been measured on ^{1}H in aqueous solutions and in blood as a function of molar concentrations. The different relaxation characteristics were analyzed and certain explanations concerning the molecular sources of these variations were advanced. The evidenced properties of these compounds are promising for interesting applications in medicine.

Keywords: Relaxation rate; NMR; MRI; Contrast agent; Gadolinium; Iron; Dysprosium; Magnetic hyperthermia; Blood

1. Introduction

MRI tissue and blood characterization using relaxation times is not possible unless the molecular sources of variation are understood. Tissues and blood are complex molecular systems with complex NMR properties. A better comprehension of the molecular basis of relaxation offers the possibility to predict the changes expected for a given pathology. The purpose of this contribution is to demonstrate the different relaxation characteristics of some iron, gadolinium and dysprosium compounds in the presence and respectively in the absence of blood and to give a possible explanation about the molecular processes that cause the change's occurrence.

2. Experimental method

Some iron, gadolinium and dysprosium compounds such as: gadolinium citrate (Gd-CIT), dysprosium citrate (Dy-CIT), Dy-DTPA (DTPA—diethylenetriamine pentaacetic acid), iron oxide–gadolinium oxide–dextran and iron oxide–dysprosium oxide–dextran complexes were prepared. Citrates of gadolinium and dysprosium were prepared [1] starting from respective metal (99.9% purity,

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doi:10.1016/j.jmmm.2005.01.068

purchased from Chemical Corp, Sun Valley) firstly transformed in chlorides and then in citrates. The silver nitrate was added to establish the purity level, for the control of complete elimination of chlorine ion. Furthermore, the products were purified by recrystallization. The Dy-DTPA complex was obtained from respective citrate and afterwards purified by recristallization.

The preparation of the compounds ($5Fe_2O_3 + 3Gd_2O_3$)-dextran [2], and ($5Fe_2O_3 + 3Dy_2O_3$)-dextran were made using microemulsion method in the system of water/toluene. The starting materials $FeCl_3/GdCl_3$ or $FeCl_3/DyCl_3$ in molar ratio of 5:3, 420 ml of water, 120 ml of toluene and 1 g of dextran, were poured into a beaker. These solutions were stirred and then kept on a water bath about 10–12 h. The molecular weight of dextran was 40 000. The $5Fe_2O_3 + 3Gd_2O_3$ and $5Fe_2O_3 + 3Dy_2O_3$ core mean diameter was about 2.5–5.5 nm, whereas the median diameter of dextran-stabilized particles was distributed in the range 80–120 nm. These dimensions were estimated by X-ray diffraction and by transmission electron microscopy (TEM) methods.

All the studied compounds are soluble in water.

The longitudinal T_1^{-1} and transverse T_2^{-1} relaxation rates' measurements have been carried out in vitro, in aqueous solutions and in blood as a function of molar concentrations. The source of the blood was University's Department of Haematology, where it was controlled for HBs, HCV and HIV and heparinized. The blood was not centrifuged but the aqueous solutions and blood containing studied compounds were well stirred before starting measurements.

All measurements have been made at room temperature (about 25 °C) and the proton Larmor frequency $\nu_o = 90$ MHz. The pulsed NMR spectrometer used was a commercial Bruker SXP4/100 spectrometer. The T_1 relaxation times were determined with an inversion recovery sequence on 12–20 points and T_2 relaxation times were measured with a Carr–Purcell sequence with echo time 15 ms and repetition time 180 ms on nine to twelve points. All data exhibited single-exponential behavior. The experimental data were fitted by a least-squares procedure with the expression

$$Y_i(t_i) = A + B\exp(t_i/T_{1,2}),$$

where t_i represent the times at which the magnetization values Y_i was measured. The fitting errors were about 1% determined from computer fitting program.

R_1 and R_2 relaxivities, in $mM^{-1}s^{-1}$ were evaluated from the least-squares determination of the slopes of plots $1/T_{1,2}$ versus molar concentration of compound, using at least five independent measurements at several concentrations among 0 and 2 mM.

3. Results and discussions

The measured R_1 and R_2 relaxivities of studied compounds in the presence and in the absence of the blood are shown in Table 1. Figs. 1a–c and Figs. 2a–c point out the dependence of the proton

Table 1
The R_1 and R_2 relaxivities for the studied compounds

Compounds	R_1 ($mM^{-1}s^{-1}$)	R_2 ($mM^{-1}s^{-1}$)
Gd-CIT	15	30.54
Gd-CIT and blood	3.624	32.635
Dy-CIT	0.452	1.023
Dy-CIT and blood	Coagulation of the blood	Coagulation of the blood
($5Fe_2O_3 + 3Gd_2O_3$)-dextran	61.78	201.39
($5Fe_2O_3 + 3Gd_2O_3$)-dextran and blood	11.86	188.725
($5Fe_2O_3 + 3Dy_2O_3$)-dextran	24.52	82.48
($5Fe_2O_3 + 3Dy_2O_3$)-dextran and blood	0.241	38.67
Dy-DTPA	0.79	1.61
Dy-DTPA and blood	0.42	93.72

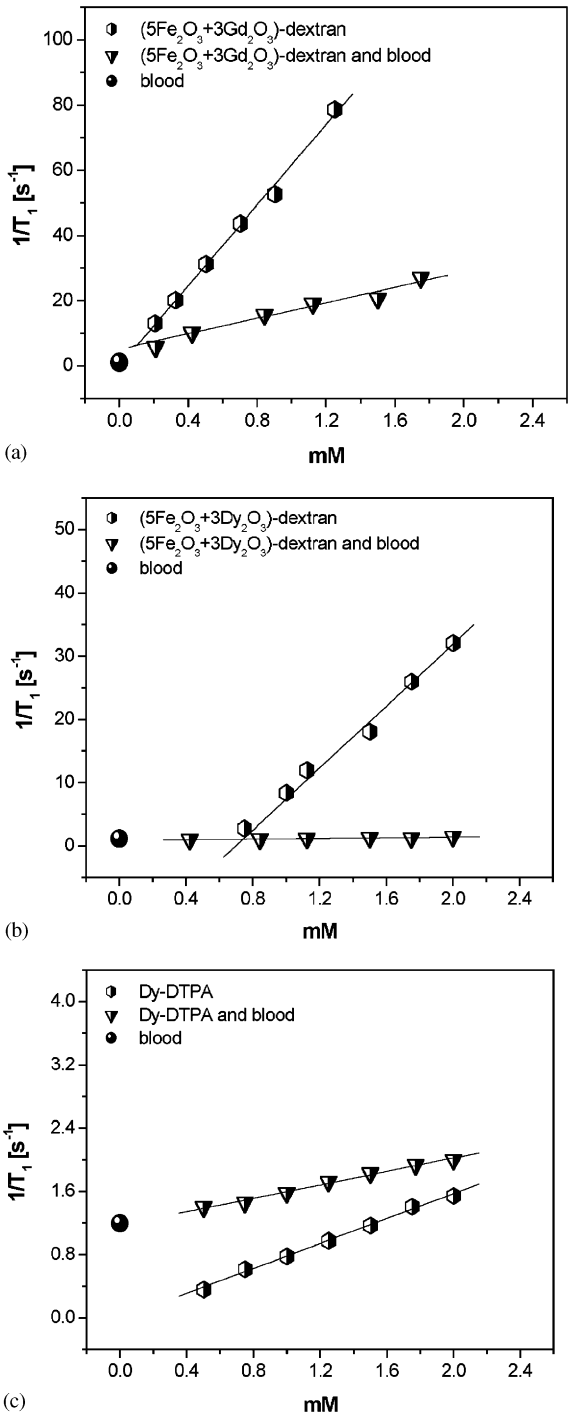

Fig. 1. a–c Proton longitudinal relaxation rates at 2.11 T and 25 °C, as a function of compounds′ concentration in the presence and the absence of the blood.

relaxation rates on the concentration of the compounds that exhibits a linear behavior.

The studied compounds involve both complexes of paramagnetic ions such Gd^{3+} and Dy^{3+} and superparamagnetic particles that combine the gadolinium and dysprosium oxides with iron oxide. That implies different spin-spin and spin-lattice relaxation mechanisms. Additional mechanisms become visible when blood is used. Although the blood contains different protein (hemoglobin, albumin and globulin), the major component is water (93% in plasma and 70% in red blood cells). The relaxation of blood is primarily a function of the specific water content and secondarily of the specific macromolecular characteristics. The relaxation of blood is visible only as a weighted average of its two fractions, red blood cells and plasma [3], due to fast exchange of water molecules. The studies of the mean residence time of water molecules in the erythrocyte emphasized that water exchange times between intracellular and extracellular compartments are on the order of 10 μs, considerably faster than T_1 and T_2 [4].

The differences in the R_1 and R_2 relaxivities of dysprosium and gadolinium compounds in both water and blood mainly comes from their distinct electron configuration and effective magnetic moment of the respective metal ions ($\mu_{Gd} = 7.55\,\mu_B$, $\mu_{Dy} = 10.2\,\mu_B$).

In the case of citrates, the differences in the R_1 and R_2 relaxivities should be justified in the first approximation through different electron spin relaxation times (T_{1e}) about 0.1–1 ns for gadolinium and 0.1–1 ps for dysprosium [5]. For metal ions with relatively long T_{1e}'s the magnitude of the outer-sphere relaxivities scales roughly with the square of the effective magnetic moment of the metal ion. Scalar interactions rule over relaxation, therefore T_2 relaxation enhancement exceeds T_1 enhancement [6].

Solvent relaxation in the presence of superparamagnetic particles like the compounds ($5Fe_2O_3 + 3Gd_2O_3$)-dextran and ($5Fe_2O_3 + 3Dy_2O_3$)-dextran, chiefly differs from that in the presence of paramagnetic solutes due to much greater weighting of the magnetic moment contribution that dominates other possible factors. The size and the

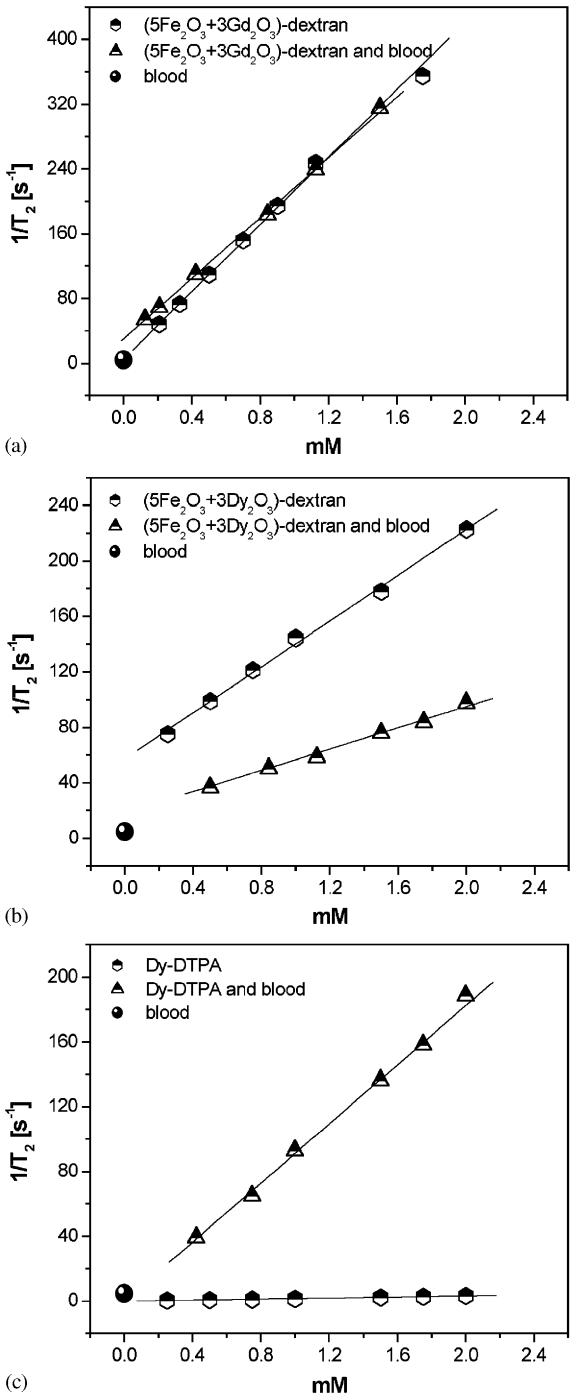

Fig. 2. a–c Proton transverse relaxation rates at 2.11 T and 25 °C, as a function of compounds' concentration in the presence and the absence of the blood.

composition of these particles represent the essential parameters. The very large magnetic moments in the presence of a static magnetic field and dipolar interactions between superparamagnetic cores and surrounding solvent protons result in increasing both longitudinal and transverse relaxation rates, especially for *small size domain* particles (below 10 nm). Smaller particles are adequately described by the microscopic outer-sphere theory [7] which predicts that the transverse relaxivity R_2, of water will increase with the radius of the particle, whereas the longitudinal relaxivity R_1, will first increase then reach a maximum for $\omega_I \tau_R \approx 1$ and finally decrease. ω_I is the proton Larmor frequency and τ_R is the time required for a water molecule to diffuse over a distance equal with the radius of the particle.

R_2 relaxivity obtained for $(5Fe_2O_3 + 3Gd_2O_3)$-dextran nanoparticles are two times greater than that measured for SPIO at 37 °C and at 20 MHz in plasma [8], while R_1 relaxivity is three times smaller. This result implies some explanations particularly because the size of both of them is in the same range. The involved parameters are magnetic susceptibility of the compounds, temperature, Larmor frequencies and the solvent. Magnetic susceptibility of the $(5Fe_2O_3 + 3Gd_2O_3)$-dextran nanoparticles [9] is higher than SPIO's magnetic susceptibility. Studies of the temperature dependence of transverse relaxation rates [10] pointed out that an increase in temperature from 25 to 37 °C generates a decrease of only 3–4% of these rates. A higher Larmor frequency and a solvent containing not only plasma but also red blood cell could be responsible for smaller value of R_1 relaxivity of the $(5Fe_2O_3 + 3Gd_2O_3)$-dextran nanoparticles. It must be added that both relaxivity processes, R_1 and R_2, are mainly influenced by regional changes in magnetic susceptibility that generate strong magnetic field gradients and by resultant diffusion of water molecules across such regions. Diffusion of protons leads to an irreversible dephasing of the transverse magnetization that cannot be completely rephased by application of a 180° radio-frequency pulse. Thus, in a Carr–Purcell sequence, the estimated transverse relaxation rate is considerably increased.

These facts should be considered in other situations too. For example, although Dy-DTPA relaxes strongly through dipole–dipole interactions with water within hydration sphere of each ion, it can also introduce susceptibility effects that may account for its greater efficacy on blood relaxation [11]. These susceptibility effects should be explained by PRE effect [12]. The PRE effect is operative when metal ion or intact compound is covalently or noncovalently attached to a macromolecule (for example to protein amino acid residues). The largest gain in R_2 relaxivity seems to involve a noncovalent binding to proteins (common globular protein, possible β-globulin) and an anisotropic motion. The greater part of the relaxivity probably stems from hydrogen-bonded water molecules in the second co-ordination sphere.

It is important to specify that the protein protons dominate the relaxation of the solution only for resonant frequencies below roughly 1 MHz [13]. At resonant frequencies above 1 MHz (our case 90 MHz) the motion of bound water molecules is of primary importance for the relaxation characteristics.

A plausible explanation of the coagulation of the blood in the presence of the Dy-CIT should be that the dysprosium ions activate in vitro the Hageman factor [14] of coagulation. A similar evolution occurs in vitro in the presence of the dysprosium phosphate. Another explanation, more probable, could be based on first experimental investigations on the relations between electron transfer processes and thrombogenesis by Sawyer et al. [15] and on the theory describing the contact activation of fibrinogen in terms of exchange currents proposed by Brauerschmidt [16]. According to this model, an electron transfer between the semiconductor protein and a solid induces fibrin formation. The dysprosium compounds represent the solid in our case. Thus the degree of antithrombogenicity of a given material is dependent on its electronic structure. The dramatic decrease of the R_1 and R_2 for $(5Fe_2O_3 + 3Dy_2O_3)$-dextran compound in the presence of the blood certainly implies its specific electronic structure together with all parameters that usually govern the nanoparticles relaxation.

4. Conclusions

Several iron, gadolinium and dysprosium compounds were prepared and their R_1 and R_2 relaxivities were measured in the presence and in the absence of the blood. The increase or decrease of R_1 and R_2 relaxivities in the presence of blood depend on a number of factors such as mass, size, composition, and distinct electron configuration of compounds and are the result of more than one type of relaxation processes.

Possible applications of the dysprosium compounds include implementation of new laboratory tests for haematological pathology. The $(5Fe_2O_3 + 3Gd_2O_3)$-dextran compound should be considered not only as MR contrast agent, but also for magnetic fluid hyperthermia (MFH) method [17], a promising approach for cancer treatment. In view of the fact that gadolinium compounds have higher pyromagnetic coefficients than other compounds [18], this fact seems reliable. Magnetic separation technologies applied in diverse fields of biology [19–21], including cellular medicine, represent another possible application area.

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