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Multinuclear Magnetic Resonance Characterization of Paramagnetic Contrast Agents

The Manifold Effects of Concentration and Counterions

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RATIONALE AND OBJECTIVES. **Proper fitting of the nuclear magnetic resonance dispersion (NMRD) profiles to the numerous factors governing nuclear relaxation in paramagnetic systems requires knowledge of some parameters usually obtained by other techniques. The rotational correlation time (τ_R) for example can be measured by carbon-13, hydrogen-2, or oxygen-17 NMR. Discrepancies between values reported in the literature might be attributed to the different concentration ranges used so far in these modalities. In the present work focussing on commercial nonspecific contrast agents, the influence of the solution composition (type and concentration of the complexes and of the counterions) has been examined with regard to the water proton relaxation enhancement and molecular dynamics.**

METHODS. **The proton relaxation rate enhancement of Magnevist, Dotarem, Omniscan, and ProHance was measured in aqueous solution up to a concentration of 0.5 M. In the same concentration window, the rotational correlation times were obtained from the study of deuterium relaxation rates of the diamagnetic deuterated analogs (lanthanum complexes) of the gadolinium chelates.**

RESULTS. **Above 50 mM, the relaxation rate enhancement versus concentrations strongly deviates from linearity. Magnevist, a clinical formulation containing two meglumine counterions per molecule of paramagnetic complex, exhibits the largest concentration effect. A slowing down of the molecular dynamics accounts for this behavior as confirmed by the analysis of the rotational correlation times obtained by deuterium relaxometry. At low concentrations ($\leq$ 50 mM), τ_R values obtained by proton NMRD analysis and by deuterium relaxation are in very good agreement.**

CONCLUSIONS. **This study shows that NMR analyses of small molecular weight complexes should be carried out on solutions containing no more than 50 mM to avoid the biaising effects of concentration. On the other hand, the benefitting relaxivity enhancement induced by highly concentrated solutions has to be taken into account in the context of bolus injection or vesicular entrapment.**

KEY WORDS. **Gadolinium contrast agents; relaxivity.**

THE EFFICACY OF A MAGNETIC RESONANCE contrast agent usually is defined by its proton relaxivity (r_1), which is the increase of relaxation rate of water protons induced by 1 mmol of the compound per liter of solvent. Proton relaxivity, which usually is measured on solutions containing less than 10 mM of contrast agent, is a field dependent property arising from inner sphere and outer sphere magnetic interactions. The models describing those relaxation mechanisms are well understood and documented[1–3] but both contain a large number of independent parameters. For gadolinium (Gd)-DTPA, Gd-DOTA, Gd-DTPA-BMA, and Gd-HP-DO3A, which are the components of the four commercially available formulations (Magnevist, Dotarem, Omniscan, and ProHance), inner sphere and outer sphere mechanisms contribute to the same extent to the global relaxivity. Consequently, the determination of all the parameters involved in the theoretical models from the sole nuclear magnetic relaxation dispersion (NMRD) profiles is difficult and ambiguous. Some of these parameters can, however, be

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obtained reliably by alternative NMR techniques. The number of water molecules coordinated to the paramagnetic ion (q) can, for instance, be obtained from the chemical shift of water oxygen-17 (O-17) induced by the dysprosium complexes.[4] The measurement of the nuclear relaxation rates and the chemical shifts of water O-17 in solutions of Gd-complexes at different temperatures allows the determination of: (a) the residence time of coordinated water molecules (τ_M), (b) the longitudinal electronic relaxation rate (τ_{S1}), and (c) the rotational correlation time of the hydrated complex (τ_R).[5,6] Analysis of the carbon-13 (C-13) NMR relaxation rates of the diamagnetic lanthanum or yttrium complexes also has been used to obtain the rotational correlation time τ_R.[7–10] Strikingly, the τ_R values obtained by the above-mentioned methods are sometimes rather different. For example, τ_R of Gd-DTPA obtained by O-17 NMR is reported to be 103 ± 10 ps at 298 K in reference,[5] whereas both the C-13 NMR analysis[7] and the proton NMRD[11] give values of 73 ps at the same temperature. τ_R calculated at 310 K using the O-17 data from reference[5] is 78 ps, whereas it is 56 ps from experimental proton NMRD data.[12] An even more pronounced discrepancy appears between the values of τ_R of Gd-DTPA-BMA obtained by O-17 NMR at 298 K (167 ± 5 ps)[6] and by fitting the proton NMRD curve (72 ps).[13] It is likely that these apparent inconsistencies can reflect the differences in the concentration ranges used by the various authors: 0.5 to 5 mM for proton relaxometry, 40 mM to 200 mM for C-13 NMR and 50 mM to 360 mM for O-17 NMR. It is well known that an increase of the concentration enhances intermolecular interactions with straightforward consequences on the τ_R values.

The aim of this work is threefold: first, to demonstrate and quantify the influence of the concentration of paramagnetic compounds on the proton relaxation rates of the solvent and on the rotational correlation times of the complexes; second, to determine a concentration range in which the measurements should be performed; and, third, to clarify the contradictory results reported in the literature. The four Gd complexes and their clinical formulations mentioned above were investigated. The rotational correlation times (τ_R) were directly estimated by the analysis of the longitudinal deuterium relaxation rates of their analogous diamagnetic lanthanum complexes.

Materials and Methods

Chemicals

The contrast agents studied were Gd-DTPA dimeglumine salt, Gd-DOTA meglumine salt, Gd-DTPA-BMA, and Gd-HP-DO3A. These compounds were obtained as their 0.5 M clinical formulations: Magnevist (Schering, Berlin, Germany), Dotarem (Guerbet, Aulnay-sous-Bois, France), Omniscan (Nycomed, Oslo, Norway), and ProHance (Bracco, Milano, Italy). Dilutions were performed by addition of distilled water to the commercial solutions. The error on the concentration is assumed to be equal to ± 5%. DTPA was purchased from Fluka (Bornem, Belgium), and DOTA, DTPA-BMA, and HP-DO3A were provided, respectively, by the Laboratoire Guerbet, by Nycomed Imaging, and by Bracco. $GdCl_3.6H_2O$, D_2O (99.9%), H_2O deuterium depleted (99.999%), La_2O_3, meglumine (N-methyl-D-glucamine) and TTHA were purchased from Aldrich (Bornem, Belgium). K_2CO_3 (analytical grade) was obtained from Merck (Overijse, Belgium).

The deuterated analogs labeled on the α carbons of the carboxylic groups were synthesized according to the procedure described by Wheeler and Legg[14]: the ligand was dissolved in D_2O and the pD was adjusted to 10.6 by addition of K_2CO_3. The mixture was refluxed under stirring for 24 hours. The pD was then adjusted to 2 with hydrochloric acid, the solution was partly evaporated, and the solid KCl salt was discarded. After addition of acetone, the deuterated ligand was recovered by filtration. No hydrolysis of the amide bonds of DTPA-BMA was noticed as shown by the H-1 NMR spectra. The deuteration was nearly complete (< 95%) for all ligands.

The deuteration was confirmed by H-1 NMR spectroscopy at 300 MHz (solvent D_2O, pH = 10):

DTPA: δ(ppm): 4 (8H, 4 × CH_2, s), 3.7 (2H, CH_2, s), 3.5 (4H, 2 × CH_2, t), 3.2 (4H, 2 × CH_2, t); DTPA-d_{10}: δ(ppm): 3.5 (4H, 2 × CH_2, t), 3.2 (4H, 2 × CH_2, t);

DOTA: δ(ppm): 3.7 (8H, 4 × CH_2, s), 3.3 (16H, 8 × CH_2, s(b)); DOTA-d_8: δ(ppm): 3.3 (16H, 8 × CH_2, s(b));

DTPA-BMA: δ(ppm): 3.75 (4H, 2 × CH_2, s), 3.65 (4H, 2 × CH_2, s), 3.55 (2H, CH_2, s), 3.2 (4H, 2 × CH_2, t), 3.1 (4H, 2 × CH_2, t), 2.6 (6H, 2 × CH_3, s); DTPA-BMA-d_8: δ(ppm): 3.55 (2H, CH_2, s), 3.2 (4H, 2 × CH_2, t), 3.1 (4H, 2 × CH_2, t), 2.6 (6H, 2 × CH_3, s);

HP-DO3A: δ(ppm): 4.05-3.9 (1H, CH, m), 3.55 (2H, CH_2, s), 3.5 (2H, CH_2, s), 3.45 (2H, CH_2, s), 3.3-3 (16H, 8 × CH_2, m), 2.9 (2H, CH_2, s(b)), 1.05 (3H, CH_3, d); HP-DO3A-d_6: δ(ppm): 4.05–3.9 (1H, m, CH), 3.3–3 (16H, 8 × CH_2, m), 2.9 (2H, CH_2, s(b)), 1.05 (3H, CH_3, d).

The lanthanum complexes were synthesized by stirring an aqueous solution of two equivalents of ligand and one equivalent of lanthanum oxide (La_2O_3) at room temperature and pH 5.5 to 6.5. After 6 hours, acetone was added and the solid complex was filtered. Gd-TTHA trisosodium salt was synthesized at room temperature by mixing at pH 6 water solutions containing equivalent amounts of $GdCl_3$ and TTHA.

Spectroscopy and Relaxometry

The high resolution H-1 NMR spectra were recorded at 7.05 T on a Bruker AMX-300 spectrometer (Bruker, Karlsruhe, Germany). Proton and deuterium longitudinal relaxation rates were measured at 4.7 T on a Bruker MSL-200-15 spectrometer equipped with a broadband probe. The decoupling coil was used for proton observation. The very

high stability of the magnet made field-frequency lock unnecessary. The temperature (310 K) was controlled by a Bruker BVT-1000 temperature unit. 2 mL samples were contained in 10 mm outer diameter tubes. The 90° pulse for proton and deuterium was equal to 12 μs and 9 μs, respectively. Deuterium relaxation rates were measured on samples dissolved in deuterium depleted water (Aldrich). T_1 were obtained using an IRFT sequence with typically 10 to 15 time delays between the 180° and 90° pulses. A three parameters fit of the peak heights was performed. As a result of the small chemical shift difference (0.4 ppm) between the different CD_2 and the fast relaxation rate of deuterium, only one resolved resonance was observed for the different compounds.

The values of τ_M were calculated from the temperature dependence of O-17 NMR T2 of water in solutions containing the Gd complexes (concentration $\leq$ 50 mM, pH 6.0–7.0, 2 mL of samples contained in 10 mm o.d. tubes). T2 of Gd complexes solutions were obtained from linewidth measurements at 4.7 T (Bruker MSL-200) or 7.05 T (Bruker AMX-300). The spectra were proton decoupled to avoid any broadening due to chemical exchange of protons. R_2^{dia}, the O-17 transverse relaxation rate of distilled water (pH 6.5), was measured using a Carr-Purcell-Meiboom-Gill (CPMG) sequence and a subsequent two parameters fit of the peak heights. All the O-17 data were collected at natural isotopic abundance. The data were processed as described by Vander Elst et al.[15]

Proton NMRD profiles were recorded between 0.02 and 50 MHz on a field cycling relaxometer (Field Cycling Systems, Honesdale, PA). Samples (0.6 mL) were contained in 10 mm o.d. tubes. 200 MHz and 300 MHz proton relaxation data were obtained on the above mentioned spectrometers.

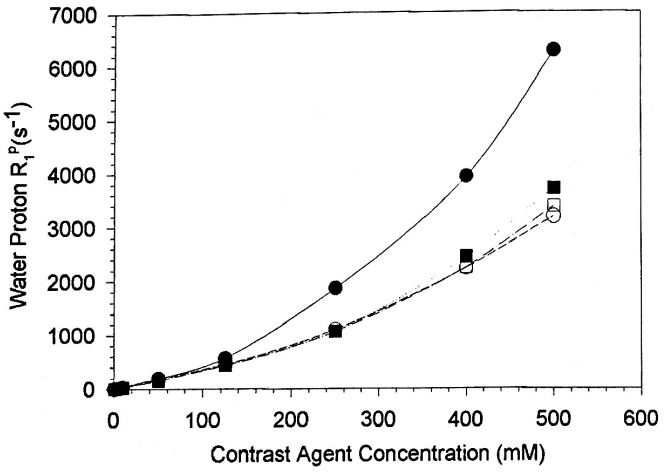


Figure 1. Paramagnetic proton relaxation rates of (●) Magnevist, (○) Omniscan, (■) Dotarem, and (□) ProHance at 310 K and 4.7 T for various dilutions of the clinical formulations.

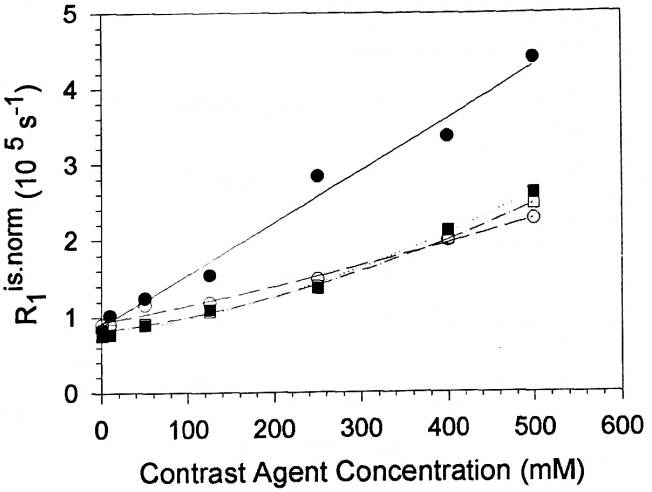


Figure 2. Variations of the $R_2^{is.norm}$ at 310 K and 4.7 T as a function of the concentration of contrast agents (●) Magnevist, (○) Omniscan, (■) Dotarem, and (□) ProHance.

Viscosimetry

Kinematic viscosities of the solutions were measured with a thermostated Cannon-Fenske viscosimeter (Herzog, Lauda, Germany). The densities of the solutions were measured by weighing known volumes of the liquids.

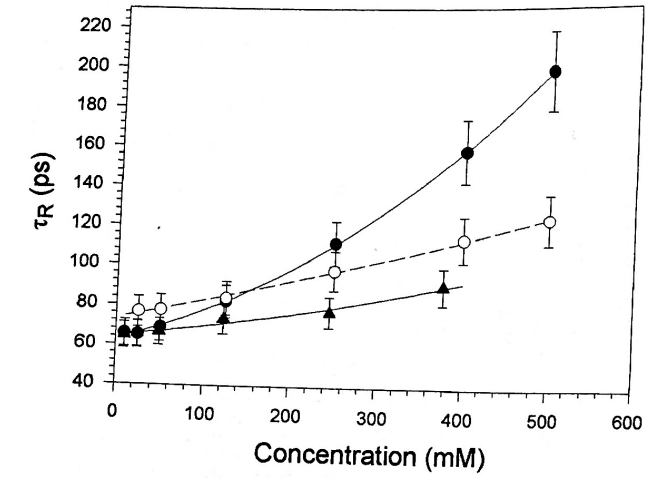
Results and Discussion

Water proton paramagnetic relaxation rates measured at 4.7 T for solutions containing 1 to 500 mM of the Gd complexes are represented in Figure 1. A clear deviation from linearity is observed for all the compounds when the concentration of the solutions is higher than 100 mM. Because the water content decreases in concentrated solutions, the water concentration of each sample was calculated from its measured density and from the content of the contrast agent reported by the manufacturer.

If the real water content is considered and if the outer sphere contribution is estimated through the measured relaxivity of Gd-TTHA, a complex that has no water molecule in its first coordination sphere, a normalized inner sphere contribution $R_1^{is.norm}$ can be calculated. $R_1^{is.norm}$ is defined as the inner sphere paramagnetic relaxation rate divided by f the ratio of concentrations of coordinated and bulk water protons (Equation 1).

$$R_1^{is.norm} = \frac{(R_1^{obs} - R_1^{dia} - R_1^{os})}{f} \qquad (1)$$

where R_1^{obs} is the observed relaxation rate, R_1^{dia} is the diamagnetic relaxation rate of water (0.28 s^{-1} at 310 K) and $R_1^{os} = r_1(\text{Gd-TTHA}).c$, with r_1(Gd-TTHA) being the relaxivity of Gd-TTHA and c being the concentration of Gd-complex expressed in mM. The outer sphere relaxivity of Gd-TTHA at 4.7 T and 310 K is 1.5 $s^{-1}mM^{-1}$. As shown



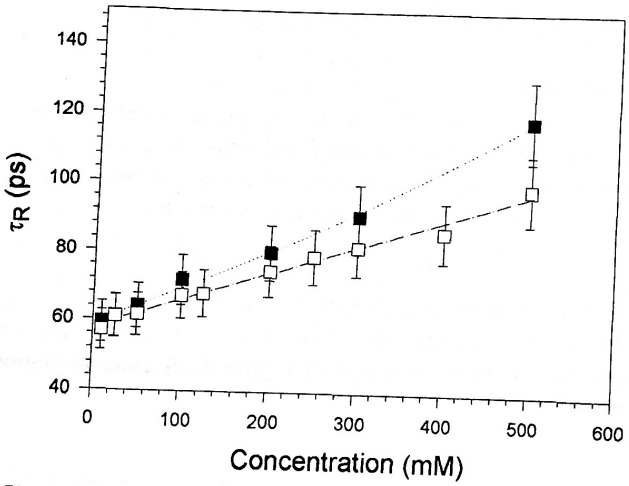


Figure 3. Evolution of τ_R calculated from deuterium relaxation rates measurements vs concentration (●) La-DTPA-d_{10}dimeglumine (pH = 8.5), (○) La-DTPA-BMA-d_8 (pH = 6.6), (▲) La-DTPA-d_{10} Na_2 (pH = 8.2), (■) La-DOTA-d_8 meglumine (pH = 6.8), and (□) La-HP-DO3A-d_6 (pH = 5.8) (T = 310 K).

later, this value is not significantly influenced by the concentration and viscosity of solutions at 4.7 T.

The normalized inner sphere contribution $R_1^{is.norm}$ is shown in Figure 2. As can be seen, the normalized inner sphere contribution is still concentration-dependent. Intermolecular interactions, viscosity increase and subsequent lengthening of the rotational correlation time of the complexes are likely to account for this behavior.

The foregoing hypothesis was quantitatively checked by the analysis of the rotational correlation times (τ_R) obtained from deuterium relaxation rates of the diamagnetic lanthanum complexes. In these experiments, meglumine is used as a counterion for La-DTPA-d_{10} and La-DOTA-d_8 like in the commercial formulations of their Gd analogs.

In diamagnetic systems the deuterium relaxation is fully controlled by quadrupolar interactions. In the extreme narrowing condition ($\omega\tau_R << 1$), it is given by Equation 2.

$$R_1 = \frac{3}{8}\left(\frac{e^2qQ}{\hbar}\right)^2 \tau_R \qquad (2)$$

The quadrupolar coupling constant (e^2qQ/h) depends on the degree of hybridization of the C-^{2}H bond.[16] The deuterium relaxation rate analysis, thus, enables an easy access to the rotational motion[16,17] because it only requires the measurement of T_1 (which usually is shorter than 100 ms) and the knowledge of the quadrupolar coupling constant, which is approximately 170 kHz for a $C_{(sp^3)}$-^{2}H bond.[16,18]

The study of the molecular dynamics of the lanthanum complexes clearly demonstrates the influence of the concentration (Fig. 3). As expected from the rather similar molecular weights of the four complexes, their rotational correlation times obtained at low concentration (10–25 mM) are very close. These τ_R values calculated from deuterium NMR of dilute solutions were compared to those obtained by the fitting of the proton NMRD profiles of the Gd-complexes recorded at the mmol concentration level (Fig. 4 and Table 1). Classic equations describing the inner and outer sphere interactions have been used.[1–3]. In the fittings, the number of water molecules in the first coordination sphere (q) was fixed to 1, τ_M was obtained from O-17 NMR and some geometric parameters were given fixed values: the distance between the proton of the coordinated water molecule and the Gd ion (r) was set to 3.1 Å, the distance of closest approach of outer sphere water molecules (d) was assumed to be equal to 3.6 Å,[11–13] and the relative diffusion constant (D) was fixed at 3.5 10^{-5} cm^2s^{-1}.[19] The values of τ_R obtained from the fittings are in very good agreement with those obtained by deuterium NMR at low concentration of the La complexes (Table 1

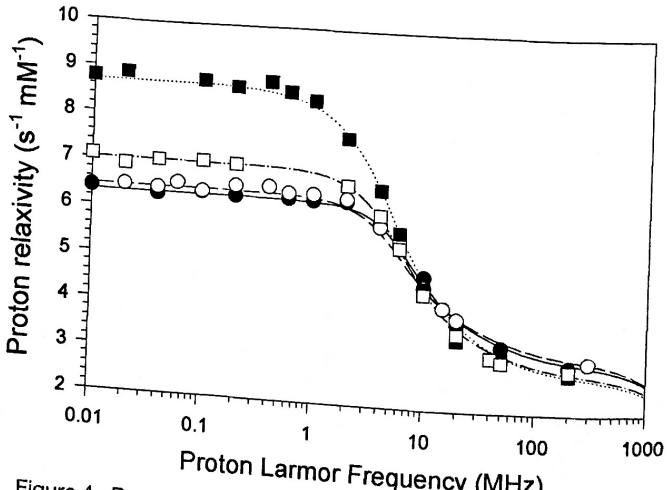


Figure 4. Proton NMRD profiles of the Gd-complexes (1 or 2 mM) in water at 310 K (●) Magnevist, (○) Omniscan, (■) Dotarem, and (□) ProHance.

TABLE 1. Parameters Obtained from the Analysis of the Proton NMRD Profiles of the Gd-Complexes (Concentration: 1 to 2 mM, T = 310 K)*

	Gd-DTPA dimeglumine	Gd-DTPA-BMA	Gd-DOTA meglumine	Gd-HP-DO3A
τ_R (ps)	59.0†	69.0†	55.0†	52.0†
	65.7 ± 6.6‡	75.7 ± 7.6‡	59.3 ± 5.9‡	56.9 ± 5.7‡
τ_M (μs)	0.14§	1.10§	0.12§	0.20§
τ_{SO} (ps)	82	107	440	140
τ_V (ps)	23	20	9.5	15

* τ_R values are compared to the results of the deuterium relaxation rate analysis of the La-complexes.
† Value obtained by fitting of the proton NMRD curve with q = 1, r = 3.1 Å, d = 3.6 Å, D = 3.5 10^{-5} cm^2s^{-1}.
‡ τ_R obtained by deuterium relaxation rate analysis of the La-complexes in 10 mM solutions except for La-DTPA-BMA-d_8 for which the concentration was equal to 25 mM.
§ τ_M obtained by oxygen-17 NMR on aqueous solutions of the Gd-complex (concentration ≤ 50 mM) (this work).

and Fig. 3) and indicate that, as expected, the segmental motion of the carboxylate arms is restricted by the complexation.

From 10 to 50 mM, the variation of the τ_R values is small (less than 8%) but a marked increase happens beyond 50 mM. For example, a threefold increase of τ_R is observed when the concentration of La-DTPA-d_{10} dimeglumine varies from 50 mM (τ_R = 68.9 ± 7 ps) to 500 mM (τ_R = 198.8 ± 20 ps). On the other hand, when sodium is used instead of meglumine as counterion, the influence of concentration on the rotational correlation time of La-DTPA-d_{10} is much lower (Fig. 3). The effect of meglumine on τ_R was confirmed by the study of a 250 mM solution of La-DTPA-BMA-d_8. Addition of one and two equivalents of meglumine increases τ_R from 99 ps to 121 and 134 ps respectively (results not shown). Meglumine clearly affects the viscosity* and can thus induce marked changes in the molecular rotation of the lanthanide complexes. Nevertheless, even for La-DTPA-BMA-d_8 and La-HP-DO3A-d_6, solutions that do not contain counterions, τ_R still grows when the concentration is increased, a fact that indicates an increase of intermolecular interactions and can be linked to the increase of viscosity.

The τ_R lengthening should be reflected also in the proton NMRD profiles of highly concentrated solutions. Because of technical limitations of the field cycling instrument, however, it was not possible to accurately measure relaxation rates higher than 35 s^{-1}. Therefore, the proton NMRD profile of a solution mimicking 500 mM of Gd-DTPA was measured: an aqueous solution of 499 mM of La-DTPA dimeglumine and 1 mM of Gd-DTPA dimeglumine. The influence of meglumine also was investigated by analyzing the proton NMRD profile of a solution containing 1 M of meglumine and 1 mM of Gd-DTPA. Compared with the data obtained for 1 mM of Gd-DTPA dimeglumine in water, the proton relaxation rates of both these "model" solutions were much higher (Fig. 5).

Without additional information about the outer sphere contribution at these concentration levels, the fittings of these curves could be quite difficult. It was therefore assumed that the outer sphere contribution could be estimated through the proton NMRD profile of 1 mM of Gd-TTHA in a solution containing 1 M of meglumine. Amazingly, the addition of meglumine to the solution does not markedly affect the outer sphere high field relaxivity of Gd-TTHA, whereas it induces a slight decrease of the low field part of the NMRD profile (Fig. 5). After subtraction of the outer sphere component, the inner sphere contribution was then fitted using the Solomon Bloembergen equations[1,2] and fixing the concentration of water to its real value (40 M). In these fittings, τ_M was set to 130 ns, a value estimated from O-17 NMR data of a solution containing 50 mM of Gd-DTPA and 1 M of meglumine. The results of the fittings are shown in Fig. 5 and Table 2. The τ_R value of the Gd-DTPA/La-DTPA solution (160 ps) is in reasonable agreement with

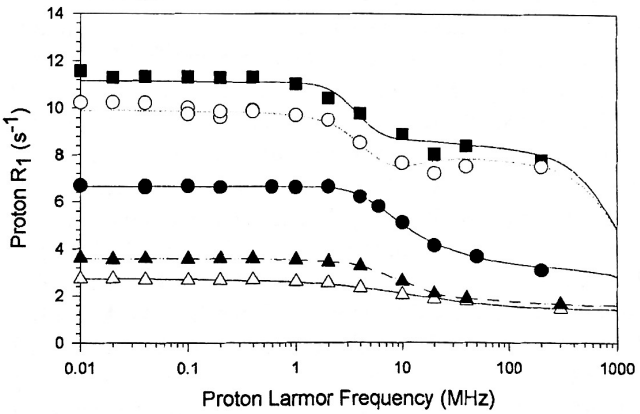


Figure 5. Proton NMRD profiles of model solutions for the study of 500 mM of Gd-DTPA at 310 K: (■) 1 mM of Gd-DTPA dimeglumine + 499 mM of La-DTPA dimeglumine, (○) 1 mM of Gd-DTPA dimeglumine + 998 mM of meglumine, (●) 1 mM of Gd-DTPA dimeglumine, (△) 1 mM of Gd-TTHA trisosium salt + 1 M of meglumine, and (▲) 1 mM of Gd-TTHA trisodium salt.

*At 310 K and pH 7.05 the viscosities of solutions containing 250 mM, 500 mM and 1 M of meglumine are increased by 15, 36, and 89%, respectively, with respect to water.

TABLE 2. Parameters Obtained from the Analysis of the Proton NMRD Profiles of the Model Solutions at 310 K*

	1 mM of Gd-DTPA dimeglumine + 499 mM of La-DTPA dimeglumine	1 mM of Gd-DTPA dimeglumine 1 mM + 1M of meglumine
τ_R (ps)	160	147
τ_{SO} (ps)	97	78
τ_V (ps)	52	32

* The values of r and τ_M were fixed to 3.1 Å and 130 ns respectively.

the result obtained by deuterium relaxation rate analysis at the same concentration (198.8 ± 20 ps). Similarily, τ_R of the other model solution (1 mM of Gd-DTPA + 1 M of meglumine) was found to be 147 ps. The relaxation rate enhancement of these model solutions can thus be explained by the lengthening of τ_R subsequent to the increase of intermolecular interactions and/or viscosity.

From the foregoing, it appears that care should be taken in the comparison of parameters describing the molecular dynamics and relaxivity of paramagnetic complexes when they are obtained through various approaches involving different concentration ranges. The same caution should be observed in the context of the calculation of molecular structures or dynamics using those parameters. This caveat is particularly justified when the solutions contain more than 50 to 100 mmol/L of substrate because, on the one hand, their water content decreases and, on the other hand, intermolecular interactions and viscosity may be significantly enhanced. These effects also influence the values of τ_R measured by O-17 NMR and probably account for part of the inconsistencies reported in the literature, like for Gd-DTPA-BMA. High concentrations (> 200 mM) were used in an early O-17 NMR study of the compound yielding a τ_R of approximately 120 ps at 310 K, a value that is overestimated[6]. Water depletion can be invoked to explain this abnormality but another reason can account for the overestimation of τ_R of the Gd(III) complexes studied by O-17 NMR: the use of the oxygen quadrupolar coupling constant and asymmetry parameter measured on acidified water. These parameters can be very different from their values obtained at physiological pH. The latter can contribute to the discrepancies between the τ_R obtained by O-17[5–6] and by other techniques not only for Gd-DTPA-BMA but also for Gd-DTPA and Gd-DOTA.[7,11–12] In a later work,[20] it has been acknowledged that the estimation of τ_R from O-17 NMR longitudinal relaxation rates probably was not reliable due to the uncertainties of the O-17 quadrupolar coupling constant or the Gd-O distance.

Most of the reported τ_R values deriving from C-13 NMR data have been obtained on solutions of 100 mM and less,[7–10] it is therefore not surprising that they better agree with those obtained by proton NMRD. In this context, it is worth noting that, contrary to deuterium studies, C-13 relaxation rates analysis requires Nuclear Overhauser Effect (NOE) measurements and a precise knowledge of the C-H distances. As shown in the present work, the analysis of deuterium relaxation rates measured on 10 to 50 mM solutions of the labeled diamagnetic complex appears as an easy and accurate way to determine τ_R.

Finally, the relaxivity enhancement induced by high concentration is a factor to be taken into account in situations like entrapment of highly concentrated solutions of contrast media in vesicles.

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