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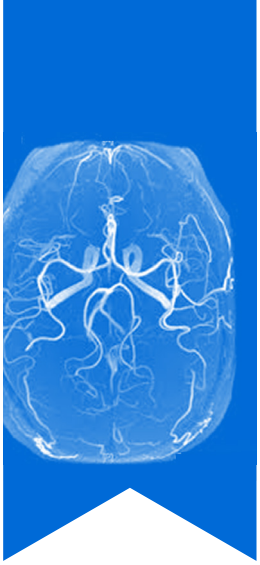

FFC NMR APPLICATION NOTE: CONTRAST AGENTS

# Fast Field Cycling NMR application: MRI contrast agents

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## Introduction

Contrast agents (CAs) are commonly injected in patients undertaking Magnetic Resonance Imaging (MRI) scans as they accelerate the relaxation rate of water molecules in the tissues, enhancing the signal contrast between different tissues in the body. The contrast agents currently available consist of paramagnetic complexes, most of them gadolinium (Gd(III)-based) (see **FIG. 1**), or superparamagnetic iron oxide particles. The efficacy of the CA is given by its "relaxivity" ($r_1$), which is the enhancement of the longitudinal relaxation rate ($R_1$) of the water protons normalized to a 1 mM concentration of the paramagnetic ions present.
Proton NMRD profiles are commonly used to characterize a CA by measuring the relaxation rate of the water protons at different magnetic field strengths. With translation to the clinical setting in mind, it is essential to measure and analyze the NMRD profiles of any potential CA. Relaxation depends on several structural and dynamics parameters of the metal system and understanding how these parameters influence the shape of the NMRD profile is important for optimizing relaxivity. [1-8]

**FIG. 1**

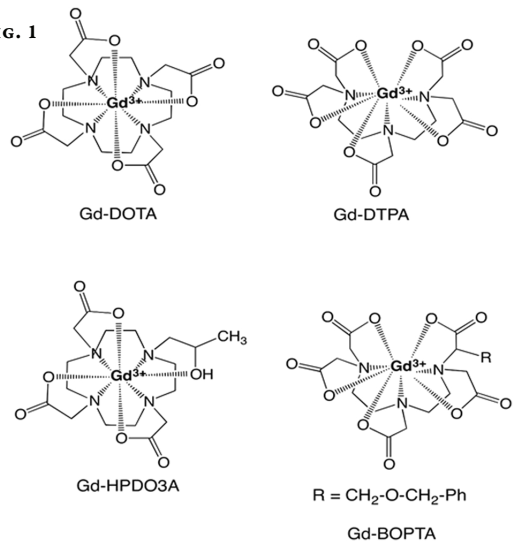


**FIG. 1:** *Structure of some selected Gd(III) complexes approved for clinical use.*

## Fast Field Cycling NMR versus Fixed Field NMR

Fast Field Cycling [9] is the only technique that allows acquisition of a full NMRD profile of a CA from very low fields, such as 10 kHz, up to 125 MHz continuously.

### Why is it important to measure relaxivity at very low magnetic fields?

Measuring the $R_1$ of a CA at a single high magnetic field, for example at 1.5 Tesla (i.e. around 60 MHz), which is the magnetic field at which many MRI scanners work, only gives information of the relaxivity of the CA at that specific field which can be compared with clinical CA's but does not help in understanding and improving the magnetic properties of the CA.
To effectively design a new CA or develop and study a relaxation model, the acquisition of a full NMRD profile is required. **FIGURE 2** reports an example of two common Gd(III)-complexes, with identical hydration states and very similar structures. At high magnetic fields (>10 MHz) the NMRD profiles of these complexes overlap, whereas at magnetic fields lower than 10 MHz the relaxivity of the two complexes is well differentiated. The differences are strictly related to different structural and dynamic properties of the CAs.

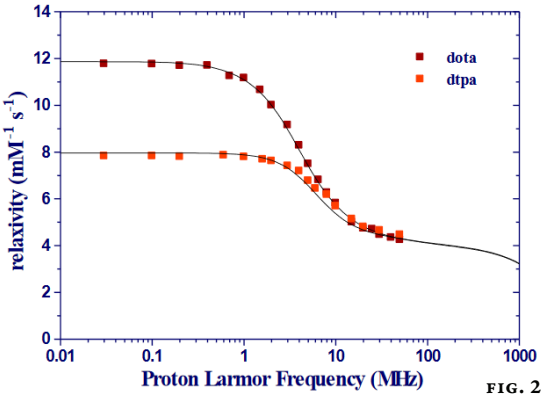


**FIG. 2**

**FIG. 2:** *$^1$H NMRD profiles of Gd-DOTA and GdDTPA are well differentiated at magnetic fields < 10 MHz (courtesy of Prof. M. Botta from the University of Eastern Piedmont).*

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It is very difficult to fit an NMRD profile accurately with very few field points (measurements of $R_1$). The smaller the range of frequencies considered, the less accurate the fitting. Applying FFC to obtain the NMRD profile of a CA is essential for the following reasons:

1. It is the only way to obtain the R1 values at very low frequencies (there are no other fixed field instruments operating below 1 MHz).
2. Without a FFC relaxometer, a very complicated set up of several NMR instruments, each working at a specific magnetic field, would be required.
3. It is much less time-consuming using an FFC relaxometer than several fixed field NMR instruments.

## Importance of fitting the NMRD profile

The parameters that can be obtained from fitting the NMRD profile are pivotal in designing new CAs and increasing the performance of the existing CAs. The design and synthesis of new CAs that improve MRI sensitivity is an important research topic. State-of-the-art research aims at obtaining new CAs with a higher relaxivity and a higher specificity (molecular targeting), while limiting the potential toxicity of the metal ions, which are toxic to humans when not strongly bound to an organic chelate. Novel theoretical models are being studied to improve fitting of the FFC experimental data allowing estimation of the parameters involved in relaxation. From the NMRD profile of a CA and through analysis using simulation models, such as the SBM (vide infra), several parameters of the CA can be studied [**1**, **2**, **4** , **5**, **7**, **8**]:

- **r** is the distance between the proton of a metal-bound water molecule and the paramagnetic ion;
- **q** , or "***hydration number***", is the number of water molecules coordinated to the paramagnetic ion.
- $\tau_M$ is the mean residence (exchange) lifetime of a water molecule coordinated to the paramagnetic ion.
- $T_{1,2e}$ are the electronic relaxation times.
- $\tau_R$ is the rotational correlation (tumbling) time.

In general, for the CAs currently used in the clinic, **q**=1 and the value of r remains constant around the value 3.1 Å. However, the magnetic interaction between water protons and paramagnetic substances may be, to a certain extent, modified for optimizing the relaxivity [**1**, **2**, **4**, **5**].

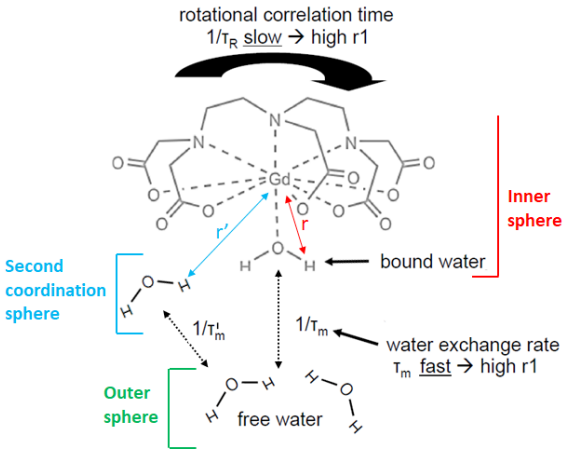


**FIG. 3:** *Sketch of the parameters involved in the relaxation of water due to a gadolinium complex. The inner sphere (IS), made up of nitrogen and oxygen atoms, contained within the organic ligands, which coordinate to the gadolinium ion and one coordinated water molecule, indicated in red. The second coordination sphere (SS) is indicated in blue. The distances r and r' from Gd to H of water in IS and SS are also depicted. Water molecules from the bulk water (Outer Sphere; OS) exchange with water molecules from IS and SS at the rates of $1/\tau_M$ and $1/\tau'_M$ respectively.*

In summary:

1. Through best fit of the NMRD profile to the equation of paramagnetic relaxation, the values of the molecular parameters of the CAs can be assessed.
2. Each parameter has an effect on the shape of the NMRD profile.
3. FFC relaxometry is used to interpret the behavior of CAs in aqueous solution, elucidate the role of the various molecular parameters and guide their further refinements through rational chemical design.
4. FFC indeed has a fundamental role in the continuous search for improved contrast-enhancing agents, by increasing the understanding of these agents at the molecular level.

## Example 1: Effect of the parameter q on the relaxivity

**FIGURE 4** shows that increasing the number of IS coordinated water molecules, greatly increases the relaxivity.

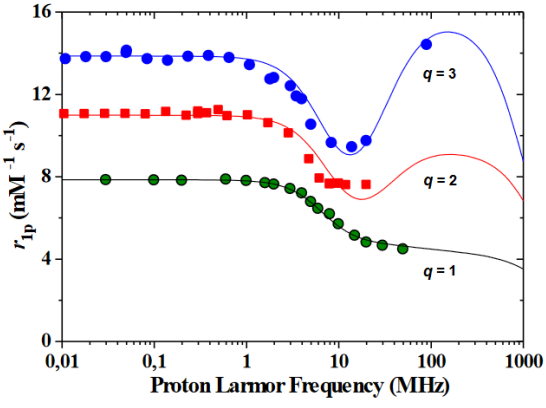


**FIG. 4**

**FIG. 4:** *1H NMRD profile at 25°C of three Gd(III) complexes differing in the number q of inner sphere water molecules. GdDTPA (green circles), GdHOPY (red squares), GdCalix[4]arene (blue circles). [Courtesy of Prof. M. Botta from the University of Eastern Piedmont].*

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## Example 2: Effect of the parameter $\tau_R$ on the relaxivity

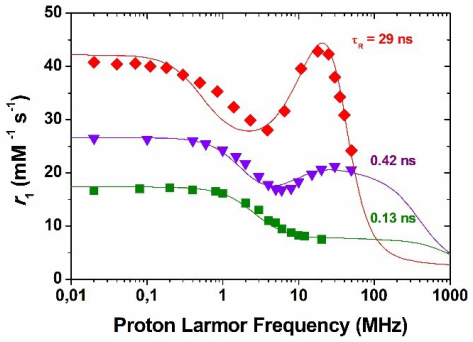


**FIG. 5:** *$^1$H NMRD profile at 25°C of three Gd(III) complexes. GdDOTA-BOM$_3$ (open circles), its inclusion complex with β-cyclodextrin (filled circles) and GdDOTA-BOM$_3$-HSA adduct (squares). The different shapes and amplitudes of the profiles are mainly due to the different $\tau_R$ of the paramagnetic complexes. [Courtesy of Prof. M. Botta from the University of Eastern Piedmont].*

As reported in **FIGURE 5**, slowing down the rotational correlation time produces a huge increase in relaxivity, especially at clinical magnetic fields below 2 Tesla.

## Example 3: Effect of the parameter $\tau_M$ on the relaxivity

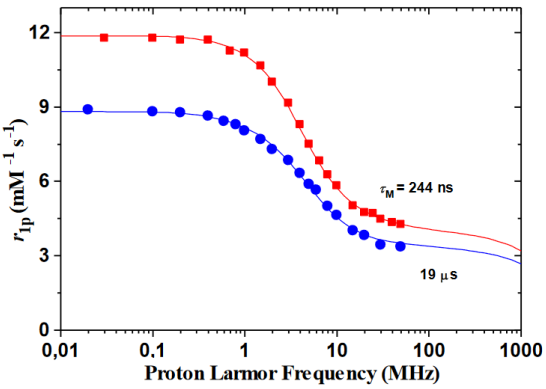


**FIG. 6:** *$^1$H NMRD profile at 25°C of [Gd(DOTA)]$^-$ (red squares) and [Gd(DOTAM)]$^{3+}$ (blue circles) showing the effect on relaxivity due to a slow rate of water exchange. [Courtesy of Prof. M. Botta from the University of Eastern Piedmont].*

**FIGURE 6** shows that an increase of the exchange lifetime limits the relaxivity attainable. This is particularly critical for macromolecular or nanosized derivatives.

# The SBM model

The **SBM** (Solomon-Bloembergen–Morgan) **theory** provides an established model to fit the NMRD profile of paramagnetic compounds. This model and the corresponding equation(s) comprise all the parameters that can be changed to increase the relaxivity of a given paramagnetic CA. This equation is quite complex but is well reported and documented in several papers.[**1**, **2**, **4**, **5**, 7, 8] The model for a Gd(III) complex is shown in **FIG. 3** and is deemed to be composed of three separate coordination spheres:

1. The Inner Sphere (**IS**) consists of ligands and a water molecule coordinated to the Gd(III) ion.
2. The Second coordination Sphere (**SS**) consists of the hydrogen from a water molecule coordinated to the Gd(III) ion. It is represented by a mean residence time $\tau'_M$ that must be longer than the diffusion correlation time of free water. [**5**]
3. The Outer Sphere (**OS**) is instead a less organized structure which consists of bulk water that diffuses freely.[**10**, **11**]
4. Herein, the equations of the SBM model are reported. For further details and completeness, it is recommended to refer to the following specific literature: [**1**, **2**, **4**, **5**, 7, 8].

The total relaxivity due to the CA is given by the sum of the three relaxivities induced by the separate coordination spheres [**4**, 7, 8]:

$$r_1^{Total} = r_1^{IS} + r_1^{SS} + r_1^{OS} \qquad \text{eq.1}$$

The main contribution to the relaxivity is given by the **IS** coordination sphere and the corresponding relaxivity can be obtained by applying the following formulae:

$$r_1^{IS} = \frac{p_M}{T_{1M} + \tau_M} \qquad \text{eq.2}$$

$P_M$ is the mole fraction of water coordinated to the metal ion. It can be expressed in terms of the hydration number, **q**, which is the number of water molecules coordinated to the metal ion (**M**):

$$p_M = \frac{q[M]}{[H_2O]} \qquad \text{eq.3}$$

where square brackets denote concentration in millimolar ([$H_2O$]=55.6x10$^{-3}$). (55.6x10$^{-3}$ M)

$$\frac{1}{T_{1M}} = \frac{2}{15}\frac{\gamma_H^2 g_e^2 \mu_B^2 S(S+1)}{r^6}\left[\frac{3\tau_{c1}}{1+\omega_H^2\tau_{c1}^2} + \frac{7\tau_{c2}}{1+\omega_S^2\tau_{c2}^2}\right] \qquad \text{eq.4}$$

$$\frac{1}{\tau_{c\,1,2}} = \frac{1}{\tau_R} + \frac{1}{\tau_M} + \frac{1}{T_{1,2\,e}} \qquad \text{eq.5}$$

($1/\mathbf{T}(\mathbf{1,2\ e})$) are the longitudinal and transverse relaxation rates of the unpaired electrons; ($1/\mathbf{T}_{\mathbf{1M}}$) is the relaxation rate of the coordinated **IS** water protons; $\boldsymbol{\tau}_{\mathbf{R}}$ is the rotational correlation time; **S** is the spin quantum number; **r** is the ion-proton distance; $\boldsymbol{\omega}_{\mathbf{H}}$ is the Larmor frequency of the proton; ($\boldsymbol{\omega}_{\mathbf{S}}$) is the Larmor frequency of the electron; $\boldsymbol{\gamma}_{\mathbf{H}}$ is the proton gyromagnetic ratio; **g_e** is the electronic g-factor [$\mathbf{g}_{\mathbf{c}}$=2 for Gd(III) and Mn(II)]; $\boldsymbol{\mu}_{\mathbf{B}}$ is the Bohr magneton; $\boldsymbol{\tau}_{\mathbf{M}}$ is the mean residence (exchange) lifetime of a water molecule bound to the paramagnetic ion.[**1**, **2**, **4**, 7, 8]

The **SS** contribution can be described by the same mechanism as the **IS** [**4**, 7, 8]:

$$r_1^{SS} = \frac{p'_M}{T'_{1M} + \tau'_M} \qquad \text{eq.6}$$

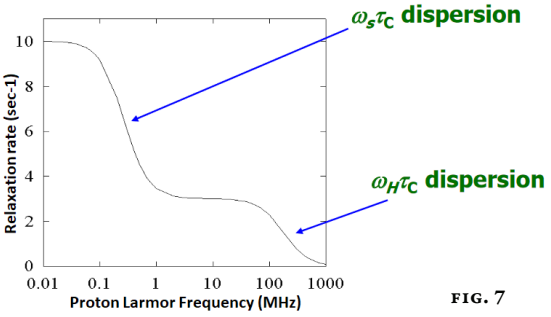


**FIG. 7**

**FIG. 7:** *Typical NMRD profile of a paramagnetic complex of gadolinium or manganese. The dispersion of ($1/T_{1M}$) depends on the two terms in square brackets in EQ. 4. As shown in this picture, at lower frequencies the electronic contribution ($\omega_S$) is dominant. At higher frequencies instead, the proton contribution ($\omega_H$) is more relevant. [Courtesy of Prof. M. Botta from the University of Eastern Piedmont].*

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The **OS** contribution to relaxivity is dominated by the diffusion time of water [**10**, **11**] and cannot be easily changed by modifying the CA. This contribution often remains a constant and in the literature a typical reported value of around 2-2.5 $mM^{-1} s^{-1}$ at high fields. [**8**]

## FFC technique

The Stelar relaxometer works by fast electronic switching of the magnetic field from an initial polarizing magnetic field ($\mathbf{B_{POL}}$), where the equilibrium of nuclear magnetization is attained in about $4\mathbf{T_1}$, to a field of interest (relaxation field; $\mathbf{B_{RELAX}}$) at which the nuclear spins relax to the new equilibrium state with a characteristic relaxation time constant $\mathbf{T_1}$. After a delay time, $\tau$, the $\mathbf{B_{RELAX}}$ is switched to the field of acquisition ($\mathbf{B_{ACQ}}$) and the NMR signal is detected after a $\pi/2$ RF pulse (**Fig. 8**).

The magnetic field dependence of $1/\mathbf{T_1}$ is shown in the graphical form as a Nuclear Magnetic Resonance Dispersion (NMRD) profile (**Fig. 9**).

Each point of the NMRD profile (i.e. a certain $B_{RELAX}$) is obtained detecting the NMR signal using a number of different delay times $\tau$.

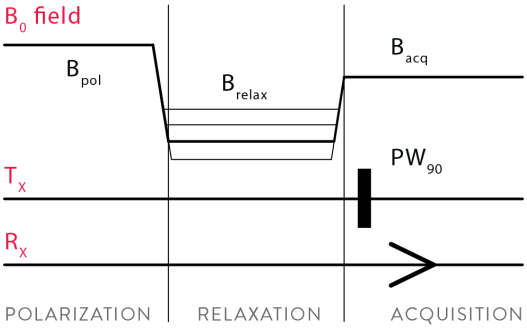


**FIG. 8**

*Fig. 8 (left): Fast Field Cycling NMR method.*

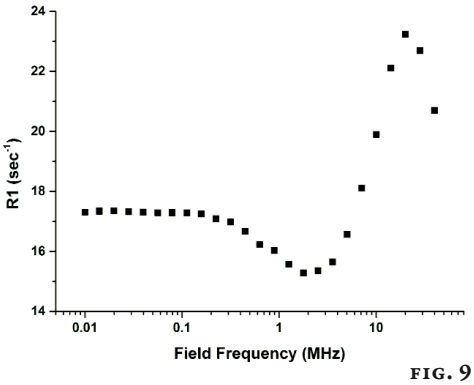


**FIG. 9**

*Fig.9 (right): Example of NMRD profile. A Gadolinium-based contrast agent measured from 0.01MHz to 40MHz (from in-house data).*

ACKNOWLEDGEMENTS

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