Gd(III) complexes:

basic relaxometric characterization

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Relaxivity: definitions
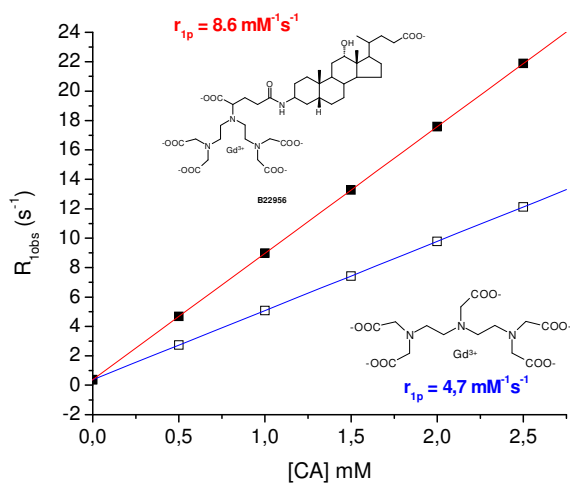
Diamagnetic contribution

$$R_{iobs} = [CA]r_{ip} + R_{iw} \qquad i = 1,2$$

Observed Relaxation Rate

relaxivity – r_{1p} [$s^{-1}mM^{-1}$]

Contribution to the observed water proton relaxation rate that a paramagnetic complex gives to a solution in which it is solved in 1 mM concentration

It is function of many parameters
⇩
B , T , pH, q , τ_R , τ_M , r, $T_{e1,2}$

Check the absence of free Gd(III) ion by Xylenol Orange spectrophotometric method

70 µl of the Gd-L solution + 700 µl of Xylenol Orange solution (3 mg/ 250 ml Acetic buffer)
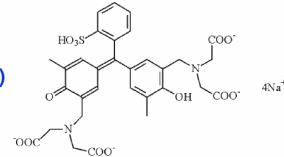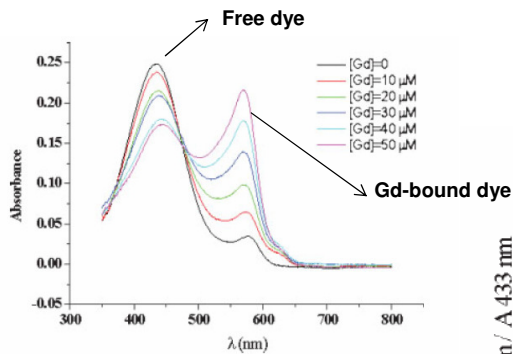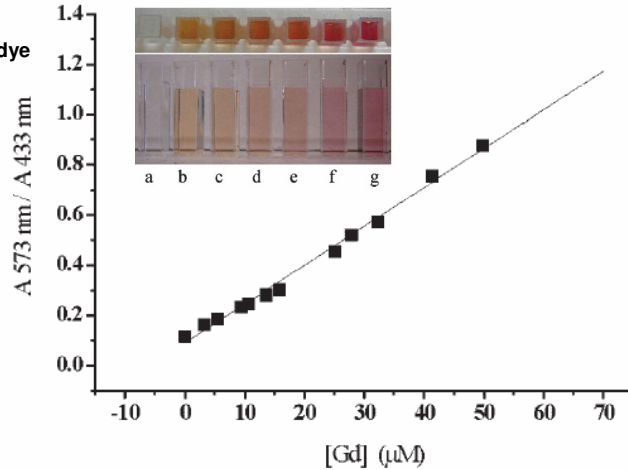
Free dye

Gd-bound dye

Figure 1. Chemical structure of Xylenol Orange.

$$[Gd^{3+}] = A + B \frac{Abs^{573}}{Abs^{433}}$$

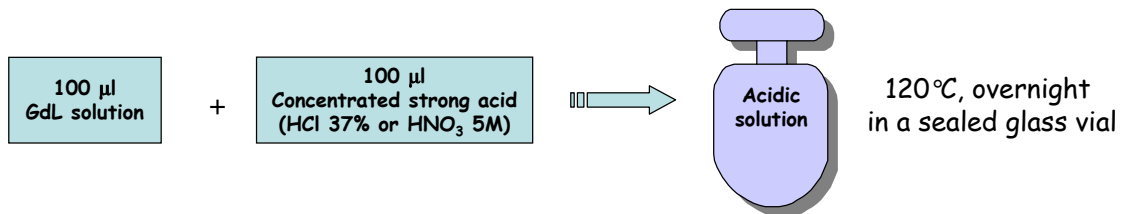A. Barge et al. CONTRAST MEDIA & MOLECULAR IMAGING 1: 184–188 (2006)

$$r_{ip} = (R_{iobs} - R_{iw})/[CA]$$

How to determine the concentration of a paramagnetic complex

-ICP-MS

-Relaxometric Procedure:

The procedure consists of:

1) mineralizing the complex solution at very acidic pH, at high temperature for 24h in order to cause the release of all the metal ion from the complex

| 100 µl GdL solution | + | 100 µl Concentrated strong acid (HCl 37% or HNO$_3$ 5M) |

Acidic solution

120°C, overnight in a sealed glass vial

2) Measuring the observed 1H-longitudinal relaxation rate of the acidic solution:

$$R_{1obs} = [Gd]r_{1p} + R_{1dia}$$
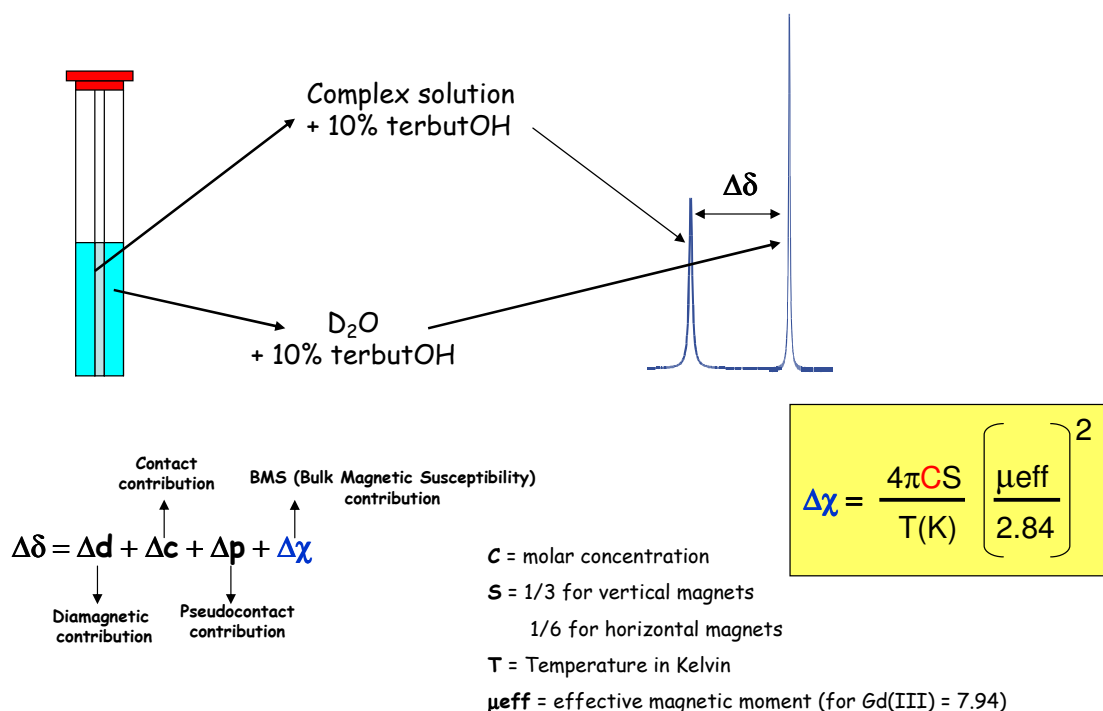
In acidic conditions, at 20 MHz and 298K:

R_{1dia} = 0.5
r_{1p} (Gd-aquoion)= 13.5 mM^{-1}s^{-1}

$$[Gd(III)] = \frac{R_{1obs} - 0.5}{13.5}$$

[GdL] = [Gd(III)]×2

How to determine the concentration of a paramagnetic complex

-Lanthanide ion induced paramagnetic shift (Evans Method):

Complex solution
+ 10% terbutOH

$\Delta\delta$

D_2O
+ 10% terbutOH

$$\Delta\delta = \Delta d + \Delta c + \Delta p + \Delta\chi$$

Contact contribution
BMS (Bulk Magnetic Susceptibility) contribution
Diamagnetic contribution
Pseudocontact contribution

$$\Delta\chi = \frac{4\pi CS}{T(K)} \left[\frac{\mu eff}{2.84}\right]^2$$

C = molar concentration

S = 1/3 for vertical magnets

      1/6 for horizontal magnets

T = Temperature in Kelvin

μeff = effective magnetic moment (for Gd(III) = 7.94)

Techniques for a basic relaxometric characterization of Gd-L

-^{1}H-water longitudinal relaxation rate at a fixed field as a function of:

➔ Temperature
➔ pH
➔ Concentration of an interacting substrate
➔ time
➔ ....

– **Magnetic field dependence of ^{1}H-water longitudinal relaxation rate**

(NMRD Profiles)

Informations about
$\left\{ \begin{array}{l} \tau_R \\ q \\ \text{Electronic relaxation time} \end{array} \right.$

- **Temperature dependence of the ^{17}O-water transverse relaxation rate**

Informations about
$\left\{ \begin{array}{l} \tau_M \\ q \\ \text{Electronic relaxation time} \end{array} \right.$

# -^{1}H-water longitudinal relaxation rate at a fixed field

T_1 (1/R_1) measurement through the Inversion Recovery sequence

2.2 Measurement of T_1

180   90   τ   Acquisition

$$M_z(t) = M_{z,\text{eq}} \left(1 - 2e^{-t/T_1}\right)$$
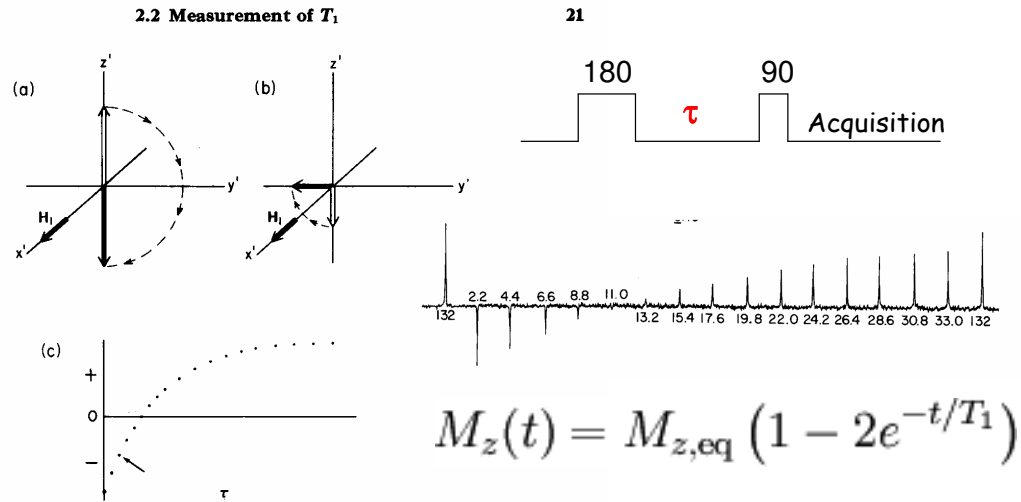
Figure 2.3. Determination of T_1 by 180°, τ, 90 sequences. (a) **M** is inverted by a 180° pulse at time 0. (b) After a time τ a 90° pulse rotates **M** to the y' (or −y') axis. (c) The initial amplitude of the FID after the 90° pulse, which is proportional to the value of **M** at time τ, is plotted as a function of τ. Note that each point results from a separate 180°, τ, 90° sequence. The point corresponding to (b) is indicated by the arrow.

Proton Relaxation Enhancement (PRE) Method

Exploits the water protons relaxation enhancement induced by the interaction of a low molecular weight Gd-complex with a macromolecular substrate

Low Relaxivity
R_{1obs}

High Relaxivity
$R_{1obs}{}^*$
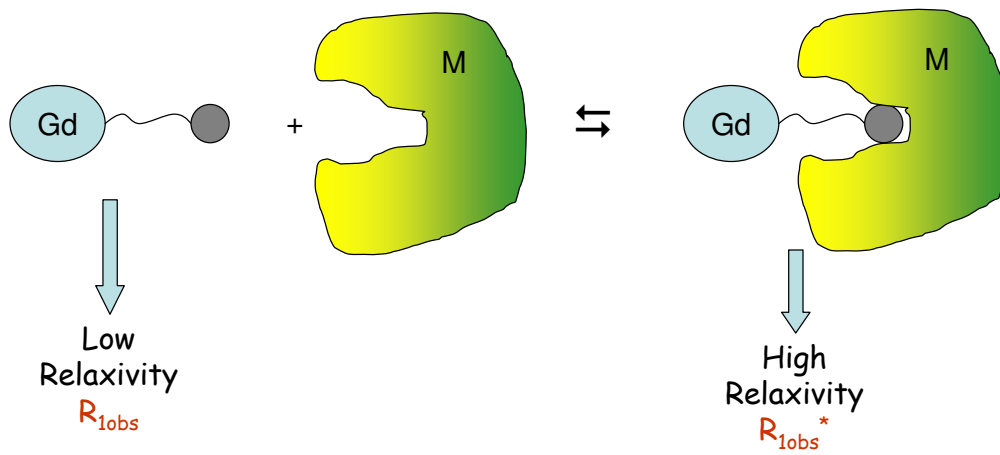
Relaxation Enhancement

$$\varepsilon^* = \frac{R_{1obs}{}^* - R_{1M}}{R_{1obs} - R_{1w}}$$

Proton Relaxation Enhancement (PRE) Method

Informations about
$\left\{\begin{array}{l}K_a \text{ (Association Constant)} \\ R_b \text{ (Relaxivity of the adduct)} \\ n \text{ (Number of independent binding sites)}\end{array}\right.$

$$\text{Gd-L} + \text{M} \rightleftarrows \text{Gd-L/M}$$

$$K_A = \frac{[Gd-L/M]}{[Gd-L][nM]}$$

$$R_{1obs} = r_1[GdL] + r_1^b[GdL-M] + R_{1d}$$

$$R_{1obs} = \frac{(\mathbf{K_A}[Gd-L]_T + \mathbf{nK_A}[M]_T + 1) - \sqrt{(\mathbf{K_A}[Gd-L]_T + \mathbf{nK_A}[M]_T + 1)^2 - 4\mathbf{K_A^2}[Gd-L]_T \mathbf{n}[M]_T}}{2\mathbf{K_A}}$$

$$- (\mathbf{r_1^b} - r_1 + r_1[Gd-L]_T)1000 + R_{1H}$$

Main limitation: the concentration of the species involved in the binding equilibrium can not be determined directly → the analysis is more difficult in the case of multi-site binding

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Proton Relaxation Enhancement (PRE) Method

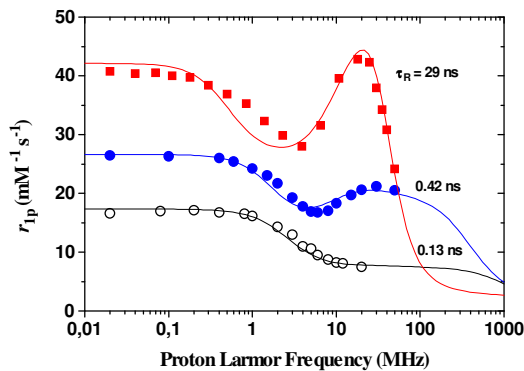The experimental procedure consists of carrying out two distinct titrations

E-titration

M-titration
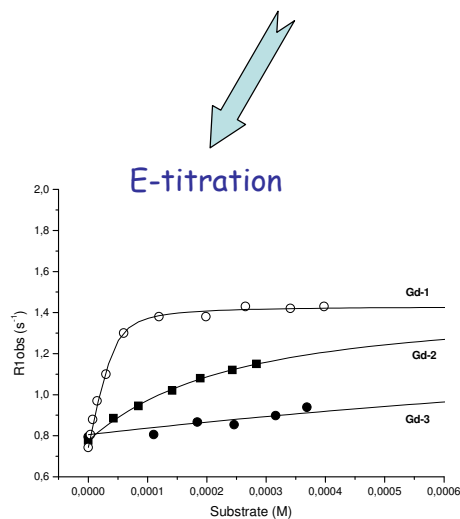
Accurate estimation of the relaxivity of the macromolecular adduct (R_b) and of nK_a
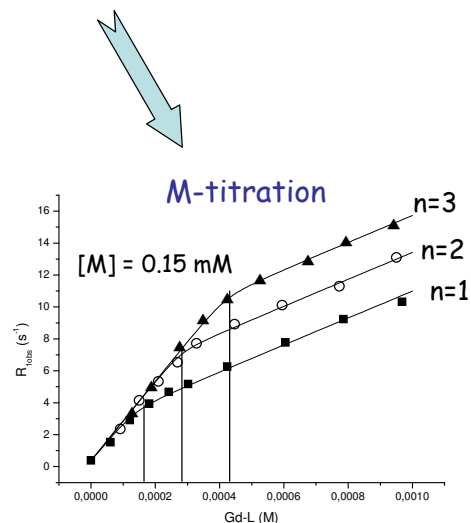
Accurate independent evaluation of n and K_a

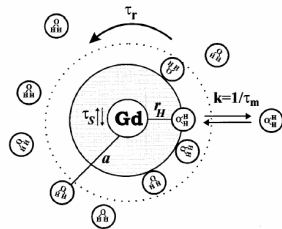## Magnetic field dependence of ^{1}H-water longitudinal relaxation rate

$$r_{1p} = r_{1p}^{IS} + r_{1p}^{OS} + r_{1p}^{SS}$$

$$r_{1p}^{IS} = \frac{[CA]q}{55.6} \frac{1}{T_{1M} + \tau_M}$$

$$\frac{1}{T_{1M}} = \frac{2}{15} \frac{\gamma_H^2 g^2 S(S+1)\beta^2}{r_H^6} \left[\frac{3\tau_{C1}}{1+\omega_H^2\tau_{C1}^2} + \frac{7\tau_{C2}}{1+\omega_S^2\tau_{C2}^2} \right]$$

$$\frac{1}{\tau_{Ci}} = \frac{1}{\tau_R} + \frac{1}{\tau_M} + \frac{1}{T_{ie}}$$

NMRD profiles

How to measure T$_1$ at different magnetic field strengths ?

- single NMR spectrometers operating at different fields
  (few data points, time (and money) consuming, difficulty to explore low magnetic fields)

- single NMR spectrometer tunable at different fields
  (better but still few data points at low fields and time-consuming)

- dedicated NMR spectrometer
  ("infinite" data points, very fast and automated acquisition)

Field Cycling Relaxometer

NMRD profiles

Field Cycling Relaxometer

Many NMR pioneers contributed significantly to the development of field-cycling NMR, including Purcell, Slichter, Hahn, Abragam, Redfield, and more recently Noack and Koenig.

FFC-2000 relaxometer by Stelar

Advantages:

-The use of low-inductance magnets allows the field to be switched very rapidly (in milliseconds) to any desirable value (currently in the 0.01-20 MHz range), maintaining the field stability and homogeneity required for the measurement.

- High sensitivity even at very low fields

- Only one acquisition field ➔ only one transmitter/receiver system

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NMRD profiles

How to measure T_1 in the Field Cycling Relaxometer ?

The equilibrium magnetization of a sample is directly dependent on the magnetic field

When the field is switched up or down M_0 changes accordingly with an exponential time dependence, whose time constant corresponds to the T_1 value at the final field
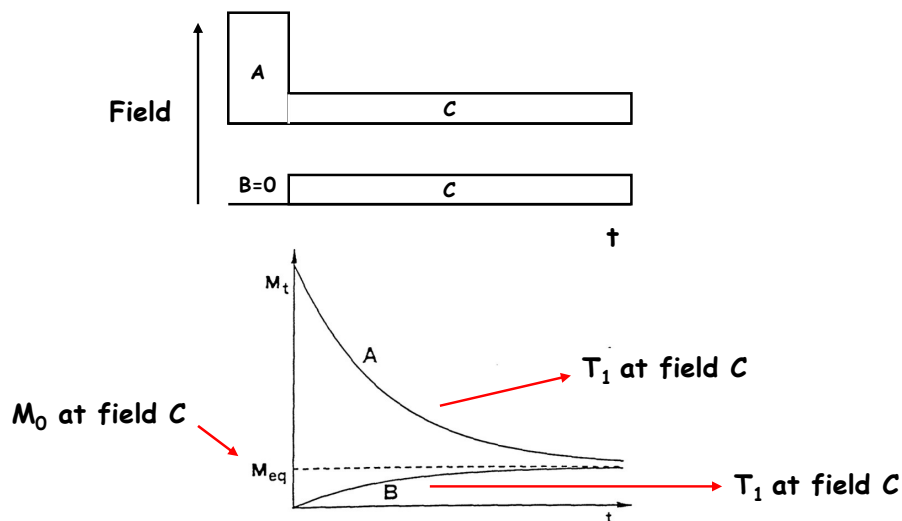
NMRD profiles

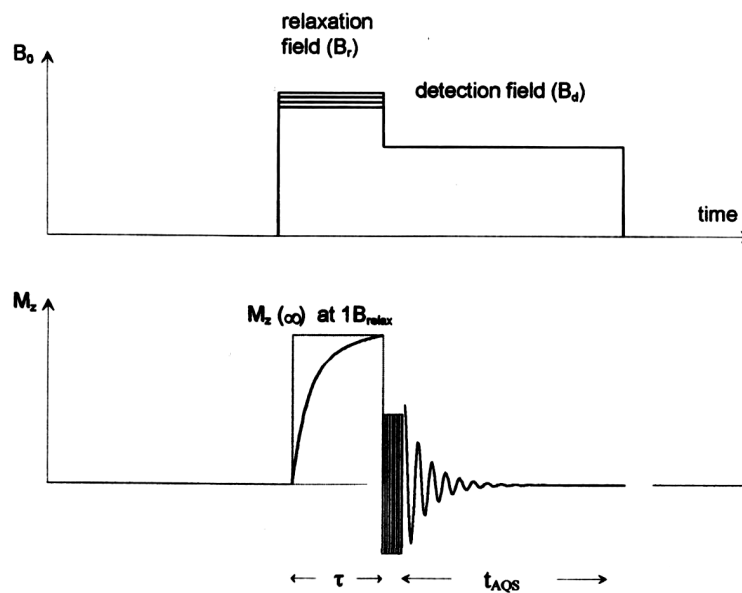How to measure T_1 by using the Field Cycling Relaxometer ?

- For fields **lower** than 8 MHz: Pre-polarised sequence
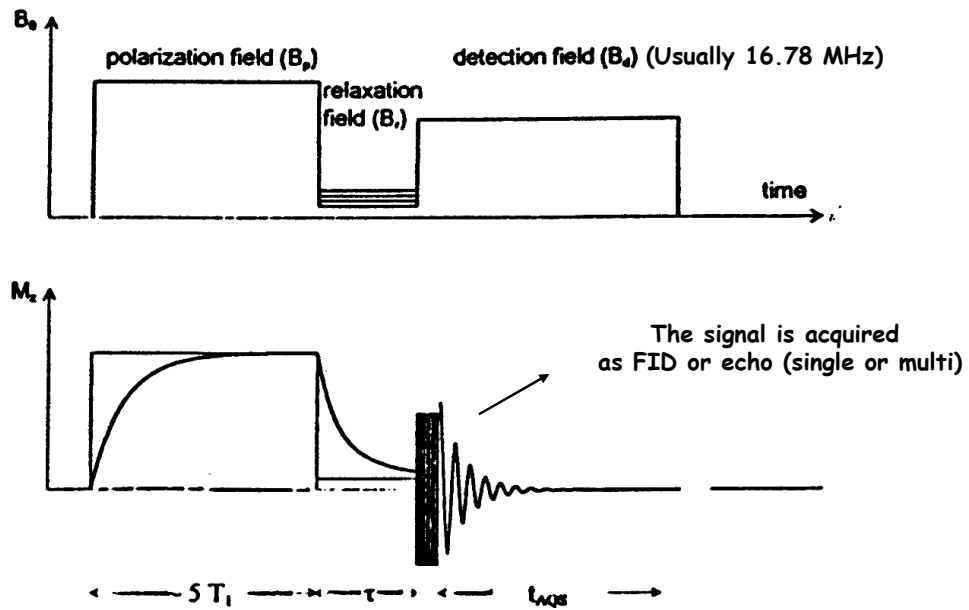
NMRD profiles

How to measure T_1 by using the Field Cycling Relaxometer ?

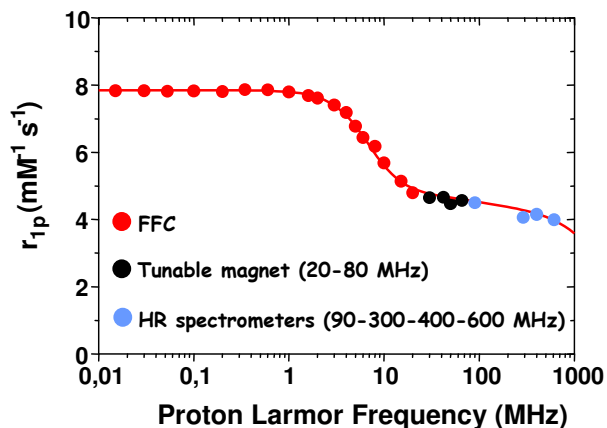- For fields **higher** than 8 MHz: Non-polarised sequence

NMRD profiles

To improve the data analysis, the T_1 values acquired with the Field Cycling Relaxometer can be implemented with data measured at any other magnetic field by means of conventional or tunable magnets

Typical NMRD profile from our lab…

FFC

- 10 mm NMR tube
- min. sample volume ca. 500 μL
- acquisition time (20 data points) ca. 15'
- min. R_1 measurable 0.8 – 1 s^{-1}

Tunable magnet

- 5 mm NMR tube
- min. sample volume ca. 50 μL
- acquisition time ca. 5' for each field

High Resolution spectrometers

- 5 mm NMR tube
- min. sample volume ca. 50 μL (use of coaxial capillaries for avoiding radiation dumping effects)
- acquisition time ca. 10' for each field (including locking and shimming)

Temperature dependence of the ^{17}O-water transverse relaxation rate

- It is the technique of election for determining the exchange rate of metal bound water molecules in Gd(III) complexes

- It is also very sensitive to the hydration state (q) of the metal ion

Swift-Connick Equations

$$R_{2p}^O = P_M k_{ex}^O \frac{R_{2M}^{O\,2} + k_{ex}^O R_{2M}^O + \Delta\omega_M^{O2}}{\left(R_{2M}^O + k_{ex}^O\right)^2 + \Delta\omega_M^{O2}} \qquad P_M = \frac{q \times [GdL]}{55.6}$$

$\Delta\omega_M^O = {}^{17}O$ chemical shift difference between coordinated and bulk water molecule

$$R_{2M}^O = \boxed{R_{2M}^{O\,sc}} + R_{2M}^{O\,dip} + R_{2M}^{O\,quad} + R_{2M}^{O\,Cur} \qquad R_{2M}^{O\,sc} = \frac{1}{3}\left(\frac{A}{\hbar}\right)^2 S(S+1)\left(\tau_{E1} + \frac{\tau_{E2}}{1+\omega_s^2\tau_{E2}^2}\right)$$

⇩ **Transverse relaxation rate of the coordinated water oxygen**

$$\tau_{Ei}^{-1} = \tau_M^{O-1} + T_{iE}^{-1}$$

Most of the parameter determining R_{2p}^O are exactly identical to those that control the proton relaxivity

Almost all the relevant parameters are temperature dependent (τ_M, τ_R, τ_V, $\Delta\omega$)

- The temperature dependence of the correlation times may be expressed in terms of Arrhenius or Eyring laws

$$\tau_j = \tau_j^{298} \, e^{\left[\frac{E_j}{R}\left(\frac{1}{T} - \frac{1}{298.15}\right)\right]}$$

$$\tau_j^{-1} = \frac{k_B T}{h} e^{\left[\frac{\Delta S_j}{R} - \frac{\Delta H_j}{RT}\right]} = \frac{\left(\tau_j^{-1}\right)^{298.15} T}{298.15} e^{\left[\frac{\Delta H_j}{R}\left(\frac{1}{298.15} - \frac{1}{T}\right)\right]}$$

Arrhenius-type dependence

Eyring-type dependence

- The temperature dependence of the resonance frequency difference between free and metal bound ^{17}O-water has a different behaviour:

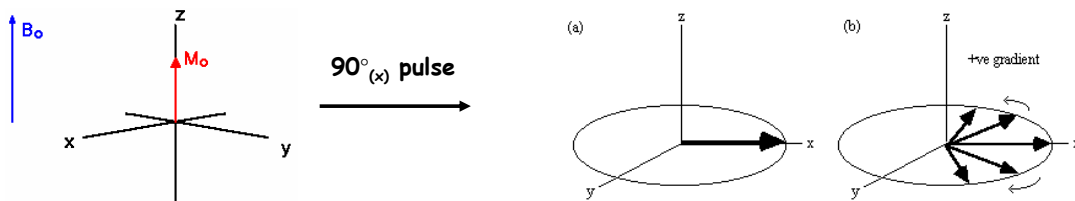$$\Delta \omega_M^O = \frac{g_e \mu_B S(S+1)B}{3\,k_B T} \frac{A}{\hbar}$$

Transverse relaxation time, T_2, and its measurement

T_2 is the time-constant of the magnetization decay in the x-y plane after a perturbation pulse

The spin-dephasing in the x-y plane is caused by T_2 relaxation mechanisms plus magnetic field inhomogeneity

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_2^{inh}}$$

- T_2 can be measured by multi-echo pulse sequence (e.g. CPMG)
- T_2^* can be simply measured by determining the linewidth at half height of the NMR signal of interest

Temperature dependence of the ^{17}O-water transverse relaxation rate

When T_2 values are extremely short (ms range), as in the case of ^{17}O-water in an aqueous solution of a Gd(III) complex, the CPMG sequence does not give accurate results.
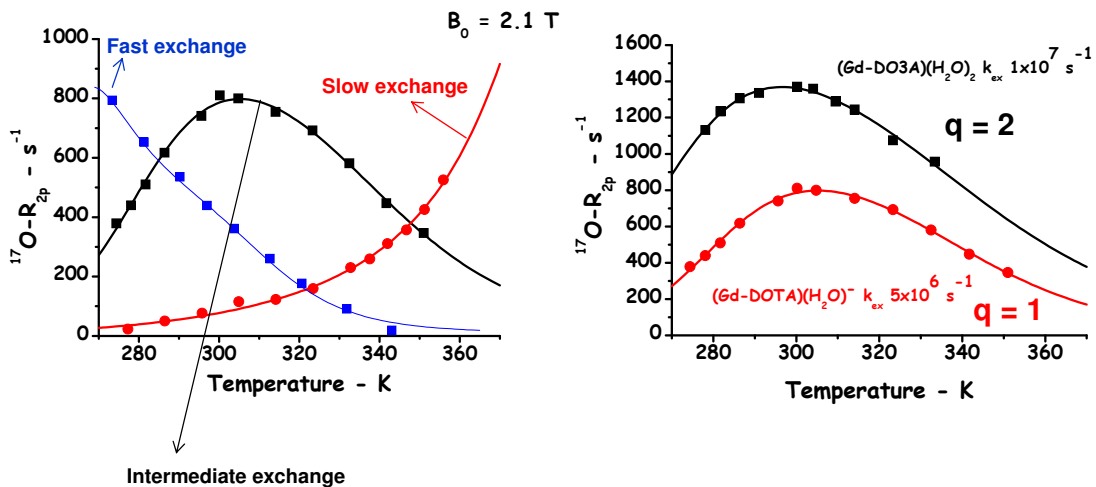
In this case, T_2^* is measured, and, upon good shimming conditions, $T_2 = T_2^*$.

$$\frac{1}{T_2^*} = \frac{1}{T_2} = R_2 = \pi \times \Delta \upsilon_{1/2 \ (in \ Hz)}$$
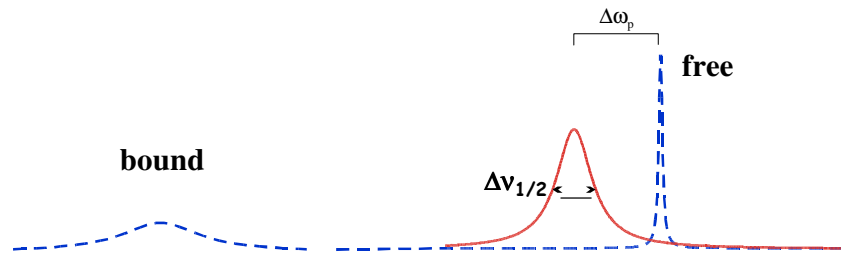
Temperature dependence of the ^{17}O-water transverse relaxation rate

Qualitatively, the shape and the efficacy (normalized to the Gd(III) content) of the paramagnetic contribution of water ^{17}O-R_{2p} values provides an indication about **k_{ex}** and **q**.

Temperature dependence of the ^{17}O-water transverse relaxation rate

Some practical info...

- High resolution NMR spectrometer are used and particular attention has to be paid for maximizing the field homogeneity (improve shimming for reducing R_2^{inh}); in addition, deuterated solvents (for field locking) not containing oxygen are preferable.

- The sample should be enriched in the water ^{17}O isotope. An enrichment of ca. 0.2 % in the sample under observation is enough for acquiring the entire profile in reasonable times.

- The minimum concentration of Gd(III) complex in the sample depends on many factors, among them magnetic field strength, k_{ex} and q values are the most relevant.
  The concentration should be sufficient to detect a significant paramagnetic contribution.
  Usually, concentrations around 10 mM are optimal for any conditions.

NMRD and ^{17}O-R^{2p} parameters

hydration numer q

reorientational correlation time τ_R

exchange lifetime τ_M

Gd-H_w distance r

Gd-Ow distance r'

electronic relaxation times (Δ^2 ; τ_V)

a and D (outer sphere)

The reliability of a multi-parameter fitting is
sensibly improved if the highest number
of parameter as possible is determined by
independent measurements

the hydration number q

luminescence lifetime: Tb(III) and Eu(III) complexes luminescence is better quenched in H_2O than in D_2O because of a better energy transfer to the OH vibrational streching than to the OD ones. The difference in the luminescence lifetimes in the two solvents is proportional to the hydration number.

Horroks and Sudnik

$$q = A\left(k_{H_2O} - k_{D_2O}\right)$$

A = 4.20 ms^{-1} for Tb(III)
     1.05 ms^{-1} for Eu(III)

Parker et al. ⟹ Correction which allows for the contribution of other exchangeable X-H oscillators (N-H and C-H) and of the unbound water molecules

$$q_{Eu} = 1.2\left[\left(k_{H_2O} - k_{D_2O}\right) - 0.25\right] \quad q_{Tb} = 5\left[\left(k_{H_2O} - k_{D_2O}\right) - 0.06\right]$$

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the hydration number q

Dy-induced ^{17}O shift : for the Dy(III) cation the hyperfine shift is largely dominated by the contact term; the contact ^{17}O shifts of the bound water molecules do not depend on the structure of the complex and thus they are directly proportional to the hydration number. The method consists in measurement of the ^{17}O shift of the water signal as a function of the concentration of the paramagnetic complex.
A straight line is obtained whose slope is proportional to the q value of the Dy(III) complex. The actual value is then obtained by comparison with the slope of the line obtained with a reference sample, normally the aquoion $[Dy(H_2O)_8]^{3+}$.
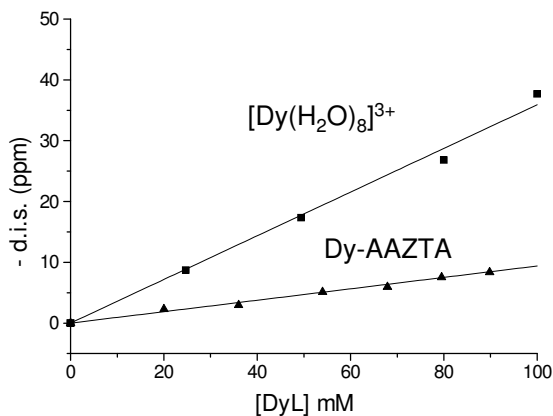
d.i.s. = (qΔ/[H$_2$O])×([Dy-L(H$_2$O)$_q$])
q = inner sphere water molecules
Δ= ^{17}O shift of the water signal

qΔ/[H2O]= slope of the line

Slope(Dy(H$_2$O)$_8$): 8 = Slope(Dy-L):q

Data Analysis

1) Only the paramagnetic contribution to the observed NMRD and ^{17}O-R_{2p} vs. T profiles
   must be analyzed.

$$^1H\text{-NMRD} \longrightarrow R_{1p} = R_{1obs} - R_{1d} \qquad ^{17}O\text{-}R_{2p} \longrightarrow R_{2p}^O = R_{2obs}^O - R_{2d}^O$$

 - An accurate determination of the diamagnetic contribution is very important,
   especially when the concentration of the paramagnetic complex is low.

 - In principle, the diamagnetic terms should be measured in a solution containing a
   diamagnetic Ln(III) complex (e.g. La(III), Lu(III) or Y(III)). In practice, it is
   sufficient to investigate the behaviour of the solution without the Gd(III) complex.

 - Therefore, in a simple aqueous solution of a Gd(III) complex, the diamagnetic term
   is measured on pure water.
   The contribution to the NMRD profile
   is rather small (ca. 0.4 s-1 at 25°C at
   any field), whereas for water ^{17}O-R_2
   is rather large (^{17}O is a quadrupolar
   nucleous) but again field independent
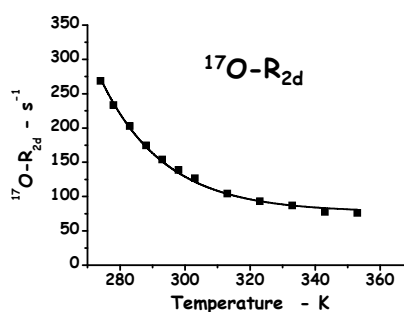
Data Analysis

2) In order to compare profiles (NMRD and ^{17}O-R_{2p}) measured in different conditions,
   it is convenient to normalize the paramagentic contribution to a given Gd(III)
   concentration, e.g 1 mM for NMRD profiles (in this condition R_{1p} correspond to
   the relaxivity of the complex). The normalization is simply done dividing the R_{ip}
   values by the reference concentration.

3) Then, a proper fitting model for the sample to be analyzed has to be selected:
   - for NMRD profiles the following models may be considered:
     - classical inner sphere + outer sphere (total number of parameters = 8)
     - classical inner sphere + outer sphere + second sphere (treated as modified inner
       sphere) (number of parameters ≥ 11)
     - Lipari-Szabo inner sphere (used when the complex experiences a fast and
       a slow tumbling motion) + outer sphere (number of parameters = 10)
     - Lipari-Szabo inner sphere + outer sphere + second sphere
       (number of parameters ≥ 13)
   - for ^{17}O-R_{2p} vs. T profiles the total number of parameters involved is 10.

Data Analysis

4) Since most of the parameters controlling ^{1}H-NMRD and water ^{17}O-R_{2p} vs. T profiles are the same, two fitting strategies can be adopted:

- each dataset (NMRD and ^{17}O-R_{2p} vs. T) is fitted independently and the obtained results are compared and successively averaged.

- all datasets (eventually including NMRD acquired at different temperatures and/or ^{17}O-R_{2p} vs. T profiles acquired at different fields) are analysed simultaneously leading to a unique set of fitted parameters.

In the former approach each dataset is fitted as better as possible, whereas in the simultaneous approach the fitting quality of all the datasets are invariantly worse.