

Can metal ion complexes be used as polarizing agents for solution DNP? A theoretical discussion

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Abstract Dynamic nuclear polarization (DNP) can be used to dramatically increase the NMR signal intensities in solutions and solids. DNP is usually performed using nitroxide radicals as polarizing agents, characterized by sharp EPR lines, fast rotation, fast diffusion, and favorable distribution of the unpaired electron. These features make the nitroxide radicals ideally suited for solution DNP. Here, we report some theoretical considerations on the different behavior of some inorganic compounds with respect to nitroxide radicals. The relaxation profiles of slow relaxing paramagnetic metal aqua ions [copper(II), manganese(II), gadolinium(III) and oxovanadium(IV)] and complexes have been re-analyzed according to the standard theory for dipolar and contact relaxation, in order to estimate the coupling factor responsible for the maximum DNP enhancement that can be achieved in solution and its dependence on field, temperature and relative importance of outer-sphere versus inner-sphere relaxation.

Keywords Paramagnetic metal ions · Coupling factor · NMRD · Relaxometry · Paramagnetic relaxation

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Introduction

NMR is a powerful and very versatile tool for the study of structure and dynamics of proteins and protein complexes at the atomic level, even in their natural environment through *in-cell* studies. Its major drawback is however represented by the low sensitivity, caused by the small difference in population at equilibrium between nuclei with different spin states in the magnetic fields of the NMR spectrometers (up to around 1,000 MHz of proton Larmor frequency) and temperatures of 20–40 °C. Dynamic nuclear polarization (DNP) is gaining more and more attention as a tool to increase the difference in population between the nuclear spin states, and thus to increase the NMR signal intensity, by transferring polarization from polarized unpaired electrons to nuclei (Hausser and Stehlik 1968). The maximum signal enhancement that can be achieved is provided by the ratio between the free electron γ_S and the nuclear γ_I , which amounts to 658 for the ^1H nucleus. DNP thus requires the use of paramagnetic species, containing the unpaired electron(s), which must be hyperfine coupled to the observed nuclei.

DNP has been successfully applied through a variety of protocols by polarizing samples at very low temperatures, and thus in the solid state. NMR detection can then be either performed with solid state NMR experiments (Hall et al. 1997; Joo et al. 2006; Bajaj et al. 2009; Ravera et al. 2013a), or after sample dissolution by fast increase of temperature (Ardenkjaer-Larsen et al. 2003). Low temperatures make the polarization transfer easier, because solid effect (Corzilius et al. 2011, 2012; Hovav et al. 2010), thermal mixing (Hu et al. 2007; Hovav et al. 2013) and cross effect (Hu et al. 2004; Matsuki et al. 2009; Hovav et al. 2012; Shimon et al. 2012; Thurber and Tycko 2012) are operative, but may not be appropriate for the study of biological

systems, because structure and mobility of biomolecules are likely different than in solution and in living cells, and freezing and quick dissolution can alter or disrupt biomolecular structures. Furthermore, a single time-consuming polarization shot is allowed in the dissolution technique, so that multidimensional experiments are hardly feasible.

DNP was anyway proposed also as a liquid state experiment and practically implemented as such on ammonia solutions of alkali metals (Carver and Slichter 1953). Renaissance of solution DNP experiments has started more recently (Maly et al. 2008; Höfer et al. 2008; Prandolini et al. 2008, 2009; Reese et al. 2008; Denysenkov et al. 2008; Villanueva-Garibay et al. 2010; Reese et al. 2009; Kryukov et al. 2010; Griesinger et al. 2012). NMR acquisition at high magnetic fields is required for a good resolution of the signals of the biomolecules. The active DNP mechanism in the liquid state is the Overhauser effect (Hausser and Stehlik 1968), which originates from the hyperfine interaction coupling electron and nuclear spins. The signal enhancement is in this case provided by the ratio between the free electron γ_S and the nuclear γ_I times the product between the saturation factor, the leakage factor and the coupling factor. The limiting factor among the three is often the coupling factor, which is thus what determines the maximum magnetization enhancement that can be achieved at a fixed magnetic field. Since the coupling factor often decreases with increasing the field, it was also deemed convenient to polarize the electron at low fields (for instance at the EPR X-band, corresponding to 15 MHz ^1H Larmor frequency) and then to shuttle the sample into a higher frequency NMR spectrometer (Höfer et al. 2008; Reese et al. 2008, 2009).

Due to its importance in determining the efficiency of the Overhauser DNP mechanism at a given field, it is thus important to determine the coupling factor of polarizing paramagnetic molecules, to be able to predict the maximum DNP enhancement that such molecules can provide. ^1H relaxometry represents an easy way to estimate the coupling factor as a function of the applied magnetic field. It consists in measuring the relaxation rates of water protons from very low magnetic fields up to 1 T (Luchinat and Parigi 2008; Nilsson et al. 2002; Kowalewski et al. 2005; Bertini et al. 2005b; Luchinat et al. 2013; Ravera et al. 2013b). The relaxation profiles can provide the parameters governing the relaxation rates, from which the coupling factor also depends. The method has been applied to determine the coupling factor of a number of nitroxide radicals (Wind and Ardenkjaer-Larsen 1999; Höfer et al. 2008; Bennati et al. 2010; Turke et al. 2012; Neugebauer et al. 2013).

Nitroxides seem to be the best suited polarizers for DNP for a number of reasons:

1. they are highly stable, compatible with biological samples in water;
2. they have sharp EPR lines that can be easily saturated;
3. they rotate fast and have a relatively large diffusion coefficient, due to their small size;
4. the unpaired electron is partially delocalized on the oxygen nucleus, so that protons of water molecules can approach it very closely.

We here describe the features that could make some inorganic compounds suitable as polarizing agents.

Theoretical background

The overall proton longitudinal relaxation rate of water solutions containing a paramagnetic species, R_1 , is given by

$$R_1 = f_M(T_{1M} + \tau_M)^{-1} + R_{1\text{diff}} + R_{1\text{dia}} \quad (1)$$

where f_M is the mole fraction of protons bound to the paramagnetic molecules and exchanging with bulk water protons, τ_M is their residence time, $T_{1M}^{-1} = R_{1M}$ is the paramagnetic enhancement to the proton longitudinal relaxation rate due to these bound protons, $R_{1\text{diff}}$ is the outer-sphere relaxation from water molecules diffusing around the paramagnetic species, and $R_{1\text{dia}}$ is the relaxation rate in the absence of the paramagnetic species. In the presence of a fast exchange ($\tau_M^{-1} \ll R_{1M}$) of water molecules coordinated to the paramagnetic species with bulk water molecules, the paramagnetic enhancement to the proton longitudinal relaxation rate, $R_{1\text{para}}$, is thus:

$$R_{1\text{para}} = f_M R_{1M} + R_{1\text{diff}} \quad (2)$$

The nuclear relaxation theory for the paramagnetic enhancement of the proton relaxation rate was originally developed by Solomon (Solomon 1955) under a dipole-dipole approximation and extended by Bloembergen (Bloembergen 1957) to include contact contribution. R_{1M} is thus the sum of the $R_{1\text{dip}}$ term, accounting for the dipole-dipole interaction between unpaired electron(s) and the nucleus at a fixed distance r , and $R_{1\text{cont}}$, accounting for the relaxation enhancement due to unpaired electron spin density located on the nucleus:

$$R_{1\text{dip}} = \frac{2}{15} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\gamma_I^2 g_e^2 \mu_B^2 S(S+1)}{r^6} \left(\frac{7\tau_c}{1 + \omega_S^2 \tau_c^2} + \frac{3\tau_c}{1 + \omega_I^2 \tau_c^2} \right) \quad (3)$$

$$R_{1\text{cont}} = \frac{2}{3} S(S+1) \left(\frac{A}{\hbar} \right)^2 \frac{\tau_e}{1 + \omega_S^2 \tau_e^2} \quad (4)$$

where ω_I and ω_S are the nuclear and electron Larmor precession frequency corresponding to the transition frequency between the various spin levels, τ_c is the correlation time for dipolar relaxation, τ_e is the correlation time for

contact relaxation, A/\hbar is the contact coupling constant (in rad s^{-1}), proportional to the electron spin density residing on the nucleus, μ_0 is the permeability of vacuum, μ_B is the electron Bohr magneton, g_e is the electron g factor, and S is the total electron spin.

The correlation time for dipolar relaxation is dominated by the fastest between electron relaxation (τ_s), reorientation (τ_R) and water proton residence time, and is determined by the relationship:

$$\tau_c^{-1} = \tau_s^{-1} + \tau_R^{-1} + \tau_M^{-1}. \tag{5}$$

The correlation time for contact relaxation is instead given by

$$\tau_e^{-1} = \tau_s^{-1} + \tau_M^{-1} \tag{6}$$

because molecular reorientation does not influence the reciprocal orientations of the nuclear magnetic moment and of the fractional electron magnetic moment located at the nucleus site.

According to the modified Solomon-Bloembergen-Morgan (SBM) equations, in $S > 1/2$ systems, electron relaxation is often field dependent because of a modulation of the transient zero field splitting as a result of the collisions with solvent molecules in solution (Bloembergen and Morgan 1961; Rubinstein et al. 1971). The electron relaxation rate is expressed, in this so-called pseudorotational model, through a transient zero-field splitting, Δ_r , and a correlation time for the electron relaxation mechanism τ_v :

$$\tau_s^{-1} = \frac{\Delta_r^2}{25} [4S(S+1) - 3] \left[\frac{\tau_v}{1 + \tau_v^2 \omega_S^2} + \frac{4\tau_v}{1 + 4\tau_v^2 \omega_S^2} \right]. \tag{7}$$

According to the Freed model (Hwang and Freed 1975), outer-sphere relaxation due to water molecules freely diffusing around a paramagnetic moiety is given by

$$R_{1\text{diff}} = k' (7\tilde{J}(\omega_S, \tau_D) + 3\tilde{J}(\omega_I, \tau_D)) \tag{8}$$

with

$$k' = \frac{32000\pi}{405} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{N_A [M] \gamma_I^2 g_e^2 \mu_B^2 S(S+1)}{d(D_{\text{solvent}} + D_{\text{solute}})}$$

$$\tilde{J}(\omega, \tau) = \frac{1 + 5z/8 + z^2/8}{1 + z + z^2/2 + z^3/6 + 4z^4/81 + z^5/81 + z^6/648}$$

$z = (2\omega\tau_D + \tau_D/\tau_s)^{0.5}$ (Freed 1978; Bertini et al. 2005b), and $[M]$ representing the molar concentration of the paramagnetic moiety (expressed in mol dm^{-3}). The correlation time τ_D is determined by the distance of closest approach, d , of the water protons to the unpaired electron(s) and by the diffusion coefficient D :

$$\tau_D = \frac{d^2}{D_{\text{solvent}} + D_{\text{solute}}}. \tag{9}$$

The Overhauser DNP enhancement factor is defined as (Hausser and Stehlik 1968)

$$\varepsilon_{OE} = \frac{\langle I_z \rangle - I_0}{I_0} = \frac{\gamma_S}{\gamma_I} \cdot f \cdot s \cdot \xi \tag{10}$$

where $f = R_{1\text{para}}/(R_{1\text{para}} + R_{1\text{dia}})$ represents the leakage factor, s the saturation factor and ξ the coupling factor. The leakage factor is thus a measure of the ratio between the paramagnetic enhancement of the nuclear relaxation rate and the total nuclear relaxation rate, and approaches 1 for large enough concentration of the paramagnetic species. The saturation factor describes how much the electron Zeeman transitions have been saturated, and can vary from zero in the case of electrons at the thermal populations, to 1 for equalized populations of the electron spin states. The coupling factor describes the magnetization transfer from the electron to the nuclear spin when the electron spin is saturated with respect to the capability of the nuclear spin to return to equilibrium, and in the case of fast exchange is provided by (Bennati et al. 2010)

$$\xi = \frac{5}{7} \left[1 - \frac{\frac{2f_M}{15} \left(\frac{\mu_0}{4\pi} \right)^2 \gamma_I^2 g_e^2 \mu_B^2 S(S+1) \frac{3\tau_c}{1+\omega_I^2 \tau_c^2} + 3k'\tilde{J}(\omega_I, \tau_D)}{R_{1\text{para}}} \right] - \frac{12 R_{1\text{cont}}}{7 R_{1\text{para}}}. \tag{11}$$

It ranges from -1 in the case of $R_{1\text{dip}} + R_{1\text{diff}} = 0$ (which implies that the numerator of the first term of Eq. 11 is zero), to 0.5 in the case of $R_{1\text{cont}} = 0$. In the latter case, a coupling factor of 0.5 is obtained for frequencies such that $\omega_S \tau_c \ll 1$ and $\omega_S \tau_D \ll 1$.

Results and discussion

As outlined in the Theoretical background section, all parameters entering the definition of the coupling factor ξ are also governing the paramagnetic nuclear relaxation rate. Obtaining these parameters from the analysis of the relaxation rates can thus permit to calculate the coupling factor and to estimate the maximum DNP enhancement that can be achieved with that paramagnetic molecule. Of course, it is not possible to retrieve the several parameters from which both paramagnetic relaxation rates and coupling factor depend from the measurement of the nuclear relaxation rate at a single field (or in a narrow range of magnetic fields). The availability of relaxation profiles over a large range of magnetic fields, also called nuclear magnetic relaxation dispersion (NMRD) profiles (Ferrante and Sykora 2005; Bertini et al. 2005a, b), can however provide the values of all relevant parameters. We have thus re-analyzed the NMRD profiles of slow relaxing paramagnetic

metal aqua ions and complexes to determine possible advantages in their use as polarizing agents for DNP. We have compared the maximum achievable enhancements of these metal compounds with respect to nitroxides, under the assumption of complete saturation of the electron transitions, which is a goal of all ongoing improvements of the instrumental setups. Efforts are actually devoted to improve the microwave resonators (Villanueva-Garibay et al. 2010). Increasing saturation levels are achieved at high fields using extended interaction klystron amplifiers (Kryukov et al. 2010) and high-power gyrotron microwave sources (Denysenkov et al. 2010; Bajaj et al. 2007; Griesinger et al. 2012).

From Eq. 11 it is clear that, in the absence of contact contributions to relaxation, the coupling factor increases with decreasing the correlation time of the coordinated water protons dipole–dipole coupled to the unpaired electron(s) and/or the diffusion correlation time of outer-sphere water molecules. In fact, $R_{1\text{para}}$, at the denominator in the first term of Eq. 11, is 10/3 of the numerator at low fields, and decreases with increasing field. The smaller the correlation time, the higher the frequency at which $R_{1\text{para}}$ starts decreasing. A decrease of a factor 2 in the correlation time for inner-sphere or outer-sphere relaxation can actually have a large effect on the coupling factor at high fields (see Fig. 1).

Since most of the favorable features of nitroxides are determined by their small size, before examining the case of paramagnetic metals, it is straightforward to think of the simplest nitroxide, nitrogen(II) oxide (NO), as polarizing agent, even if its EPR lines are much broader than those of nitroxide radicals (Hogg 2010). Accurate calculations and fitting of EPR data indicate that 65 % of the unpaired electron density resides on the oxygen atom. Molecular dynamics simulations suggest that the distance of closest approach of water protons is about 2 Å (Zhou et al. 2005; Yazyev and Helm 2007). From the diffusion coefficients of water and NO at 25 °C of 2.3×10^{-9} and 3×10^{-9} m²/s (Zhou et al. 2005), respectively, the diffusion correlation

time, obtained from Eq. 9, results of 7.5 ps, i.e., about 3.5 times smaller than that of the TEMPONE nitroxide radical (Bennati et al. 2010). This quite low τ_D value provides coupling factors of 0.43, 0.22, 0.09 at 15, 150 and 400 MHz, respectively, corresponding to maximum DNP enhancements of 285, 145 and 57 (calculated from Eq. 10, with $s = f = 1$). These values are much larger than those foreseen (and experimentally verified at the two lowest fields) for TEMPONE (Bennati et al. 2010), the coupling factor of which being 0.35, 0.05, 0.01 at 15, 150 and 400 MHz, respectively. Molecular dynamics simulations have also been performed for TEMPOL solutions, providing coupling factors very similar to what calculated for TEMPONE (Sezer et al. 2009).

Aqua ions, and metal complexes in general, have longer reorientation correlation times than nitroxides; they are thus expected to yield worse enhancements at high fields. Anyway, as it is clear from Fig. 1, it is important to notice that the outer-sphere contribution to relaxation might be quite important.

The NMRD profiles for copper(II), manganese(II) and gadolinium(III) aqua ions were collected at 25 and 40 °C (see Supplementary Material); those of oxovanadium(IV) have been taken from (Bertini et al. 1992). All profiles have been analyzed using standard SBM theory for inner-sphere relaxation and the Freed model for outer-sphere relaxation. Copper(II), manganese(II) and gadolinium(III) are known to have coordinated water molecules in fast exchange (Powell et al. 1991; Pasquarello et al. 2001), so that τ_M can be neglected in Eq. 1.

Copper(II) has an $S = 1/2$ spin, and therefore it has no zero-field splitting. Since the first excited state is far from the ground state, electron relaxation can be related to modulation of hyperfine coupling with the metal nucleus and of the g tensor, to Raman type processes (Bertini et al. 1989), and/or to Jahn–Teller dynamics (Powell et al. 1991; Freed and Kooser 1968; Kooser et al. 1969), and is relatively long among transition metal ions, although much shorter than for nitroxide radicals. No field dependence in

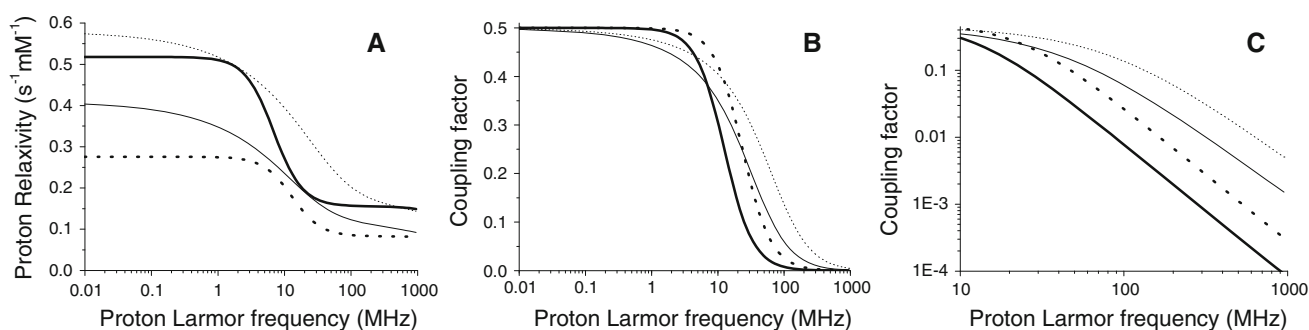


Fig. 1 The effect of decreasing the correlation time for dipolar relaxation or the diffusion correlation time on proton relaxivity (a) and coupling factor (b and c). The *solid* and *dotted* lines are

calculated for $\tau_c = 40$ and 20 ps (*thick lines*), respectively, or $\tau_D = 40$ ps and 20 ps (*thin lines*), respectively. The parameter τ_D has been changed by varying the distance of closest approach

electron relaxation has been reported up to now. According to the available estimates of the electron relaxation time, of the order of the nanoseconds, a lower limit of 0.3 ns was fixed for τ_s (Banci et al. 1985).

As shown in Fig. S1, the water proton NMRD profiles of the copper(II) aqua ion have only one dispersion. The fit performed for the two parameters r and τ_c indicates that the distance between 12 water protons ($q = 6$ coordinated water molecules) and the paramagnetic center, r , is equal to 2.6 Å, and that the correlation time τ_c is equal to 3×10^{-11} and 2×10^{-11} s at 25 and 40 °C, respectively (see Table 1). These values are of the order of what is expected for an aqua ion from the Stokes–Einstein equation for the reorientation time, being orders of magnitude smaller than the electron relaxation time. From the analysis of this profile we can also exclude possible contact contributions to relaxation, because otherwise the corresponding $\omega_S \tau_s$ dispersion would have been detected. Inclusion of outer-sphere relaxation slightly increases the quality of the fit (solid lines in Fig. S1) and the distance r to 2.7 Å. The diffusion coefficient (for all the aqua ions) was fixed to 3×10^{-9} m²/s at 25 °C, being the diffusion coefficient of metal ions equal to 7×10^{-10} m²/s. We note that an optimal fit could be obtained by including a field dependent electron relaxation time of the order of the nanosecond at fields corresponding to 40 MHz proton Larmor frequency and of 150 ps at low fields. However, the coupling factor does not change significantly whether calculated using a field dependent or independent electron relaxation time (see below).

A fascinating debate is present in the literature on the coordination number of copper(II) aqua ion: it has been proposed (Pasquarello et al. 2001) that the ion, rather than six-coordinated, as widely believed, is mostly five coordinated, rapidly cycling between square pyramidal and trigonal bipyramidal geometries. Recently, a unified model has been proposed on the basis of a sixfold coordination undergoing Jahn–Teller dynamics, implying shortening of

one of the two long copper–oxygen bonds and elongation of the opposite bond (Gómez-Salces et al. 2012). A smaller coordination number however only slightly decreases the best fit value of r , with a negligible effect on the calculated coupling factor.

Figure 2 shows the field dependence of the coupling factor, calculated from the best fit parameters reported in Table 1, with and without inclusion of outer-sphere relaxation in the fit of the NMRD profiles. As expected the coupling factor is 0.5 at low fields, and decreases down to zero for increasing fields, in line with the $\omega_S \tau_c$ dispersion. At 15 MHz the coupling factor is 0.29 at 25 °C and 0.36–0.37 at 40 °C, i.e., it is only somewhat smaller than for nitroxide radicals, for which values of about 0.35 and 0.4 at the two temperatures have been obtained (Höfer et al. 2008; Bennati et al. 2010; Turke et al. 2012). As expected, the inclusion of outer-sphere relaxation, although not

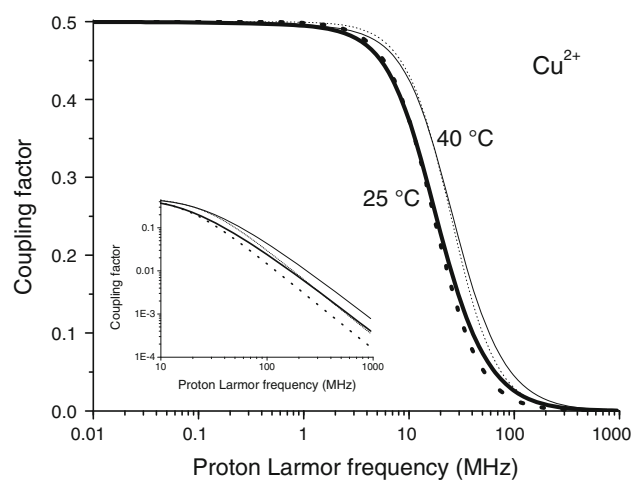


Fig. 2 Coupling factor at 25 °C (thick lines) and at 40 °C (thin lines) calculated for a water solution with copper(II) aqua ions. Dotted lines and solid lines show the values obtained without and with including outer-sphere relaxation in the fit of the NMRD profiles, respectively. In the inset, the coupling factor values at high fields can be better appreciated

Table 1 Values of the parameters providing the NMRD profiles of the aqua ions reported in the Figures S1–S3

	Cu ²⁺		Mn ²⁺				Gd ³⁺					
T (°C)	25	40	25	40	25	40	25	40	25	40	25	40
S	0.5				2.5				3.5			
q	6				6				8			
r (Å) (*)	2.59	2.59	2.66	2.66	2.85	2.85	3.00	3.00	3.00	3.00	3.07	3.07
τ_R (ps) (*)	28	19	28	19	27	18	29	19	38	27	36	26
A/h (MHz) (*)					0.75	0.75	0.74	0.74				
Δ_r (cm ⁻¹) (*)					0.014	0.014	0.015	0.015	0.036	0.036	0.030	0.030
τ_v (ps) (*)					7.8	7.5	7.1	6.4	16	14	18	15
d (Å) (*)			2.9	2.9			3.0	3.0			3.6	3.6
D (10 ⁻⁹ m ² /s)			3.0	3.9			3.0	3.9			3.0	3.9

Best fit values are indicated with (*)

important in determining the value of ξ at relatively low fields, is responsible of a very large increase in percent of the coupling factor at high fields.

In the applied model, the contribution to relaxation of water molecules outside the first coordination sphere of the metal are all included in the outer-sphere term. In reality, water molecules should be distinguished in hydrogen-bonded second-sphere water molecules and freely diffusing water molecules (Botta 2000). Therefore, the distance of closest approach calculated without considering the second-sphere contribution is a fit parameter without a strict physical meaning. Second-sphere water molecules are expected to be dipole–dipole coupled to the metal with correlation time determined by the reorientation time of the aqua ion and their lifetime. This correlation time is expected to be in the range from several picoseconds up to few tens of picoseconds (Bertini et al. 2001; Chen et al. 1998). The inclusion of second-sphere water molecules in the calculation of the coupling factor thus causes a minor effect, providing ξ values close or slightly smaller than those calculated with the fit performed by considering only inner-sphere and outer-sphere relaxation.

Manganese(II) is an $S = 5/2$ ion, with a field dependent electron relaxation determined by modulation of transient zero field splitting. The ^1H NMRD profiles show two dispersions (Fig. S2): the first, at 0.1 MHz, is due to the contact relaxation, and the second, at about 10 MHz, is due to the dipolar relaxation (Bertini et al. 1993). The fit of the profiles provides the contact coupling constant and the parameters determining the electron relaxation times (Δ_r and τ_v), which reproduce the first dispersion, as well as r and the correlation time for dipolar relaxation, which is again the reorientation time, responsible for the second dispersion. The best fit parameters are in reasonable agreement with previous analyses (Bertini et al. 1993, 2005b). Again, the fit improves slightly if the effect of outer-sphere contributions is considered.

Figure 3 and Table 2 report the coupling factors calculated from the best fit values of the NMRD profiles of manganese(II) aqua ion. The coupling factor at low field is large and negative (around -0.5 at 25°C), as expected from the dominant contribution to relaxation of the contact interaction. It becomes close to 0.5 with increasing the magnetic field up to the point that contact relaxation is basically dispersed and dipolar relaxation is not. Further increase of the magnetic field causes a fast decrease of the coupling factor as a consequence of the occurrence of the dipolar dispersion, similarly to the copper(II) case.

Gadolinium(II) is an $S = 7/2$ ion, with a field dependent electron relaxation, which, analogously to manganese(II), is determined by modulation of transient zero field splitting. Fig. S3 shows the NMRD profiles acquired at 25 and 40°C . One dispersion only is observed with a correlation

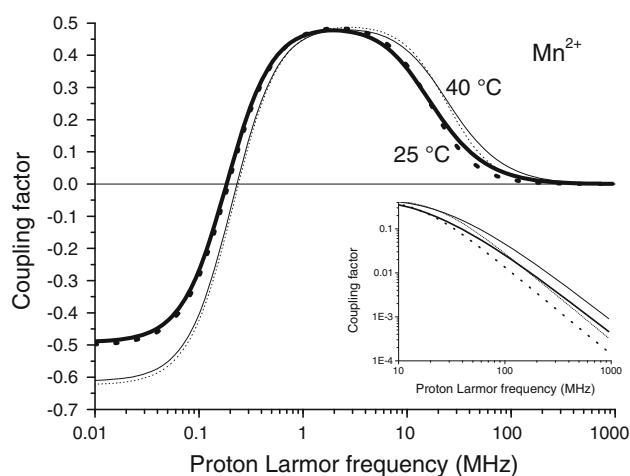


Fig. 3 Coupling factor at 25°C (thick lines) and at 40°C (thin lines) calculated for a water solution with manganese(II) aqua ions. Dotted lines and solid lines show the values obtained without and with including outer-sphere relaxation in the fit of the NMRD profiles, respectively. In the inset, the coupling factor values at high fields can be better appreciated

Table 2 Coupling factor at 15, 150 and 400 MHz, obtained from the parameters in best agreement with the NMRD profiles

	T ($^\circ\text{C}$)	ξ (15 MHz)	ξ (150 MHz)	ξ (400 MHz)
Cu^{2+}	25	0.29	0.007–0.013	0.0009–0.002
	40	0.37	0.014–0.024	0.002–0.004
Mn^{2+}	25	0.27–0.28	0.006–0.015	0.0009–0.003
	40	0.35–0.36	0.013–0.026	0.002–0.005
Gd^{3+}	25	0.21–0.23	0.003–0.007	0.0004–0.001
	40	0.29–0.30	0.006–0.01	0.0008–0.002
VO^{2+}	25	0.17–0.20	0.003–0.009	0.0004–0.002
	35	0.23–0.25	0.004–0.01	0.0006–0.003
NO^a	25	0.43	0.22	0.09
TEMPONE^b	25	0.35	0.05	0.01
Mn-DTPA^c	25	0.30	0.03	0.006
VO-EDTA^d	25	0.41	0.07	0.01

The indicated ranges correspond to the fit performed with and without including outer-sphere relaxation in the fit of the NMRD data

^a Calculated from the parameters estimated through MD calculations in (Zhou et al. 2005)

^b Calculated from the best fit parameters reported in (Bennati et al. 2010)

^c Calculated from the best fit parameters reported in (Wagon and Jackels 1989)

^d Calculated from the best fit parameters reported in (Chen et al. 1998)

time of about $3\text{--}4 \times 10^{-11}$ s, which can be ascribed to τ_R . However a good fit of the profile at high fields cannot be obtained without including in the correlation time a contribution from a field dependent τ_s , which accounts for a reduced ratio between the high field and the low field

relaxation rate with respect to the 3/10 ratio, expected from the Solomon equation in the presence of a field independent correlation time. The reorientation correlation time and the effective electron relaxation time must thus be of the same order at fields around 10 MHz. The resulting best fit data, reported in Table 1, are in good agreement with previous results obtained using the SBM theory (Banci et al. 1985; Borel et al. 2002). Different models for electron relaxation and molecular dynamics calculations were also employed to analyze the profile, providing quite different values for the electron relaxation parameters and its dependence on the static zero field splitting (Banci et al. 1985; Borel et al. 2002; Lindgren et al. 2009). It must be also pointed out that there is actually a large covariance in the Δ_r and τ_r values; however, the resulting correlation time, from which both the relaxation profile and the coupling factor depend, is well determined and consistent. Furthermore, a possible, proposed, $q = 9$ coordination basically affects only the r parameter, without significant changes in the derived coupling factor.

The coupling factors calculated from the best fit parameters with and without outer-sphere relaxation are shown in Fig. 4 and reported in Table 2. Slightly smaller values are calculated at high fields with respect to the other metal ions because of the larger correlation time, which causes the dipolar dispersion to occur at lower fields.

Oxovanadium(IV) has $S = \frac{1}{2}$ and, since in the aqua ion the first excited state is expected to be high in energy from the ground state (Bencini and Gatteschi 1982), electron relaxation is relatively slow, analogously to copper(II). The NMRD profiles, available from Bertini et al. (1992) and reported in Figure S4, show two dispersions, the first

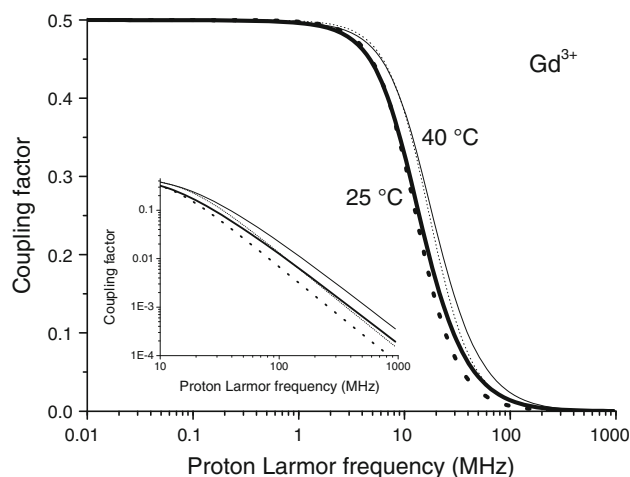


Fig. 4 Coupling factor at 25 °C (*thick lines*) and at 40 °C (*thin lines*) calculated for a water solution with gadolinium(III) aqua ions. *Dotted lines* and *solid lines* show the values obtained without and with including *outer-sphere* relaxation in the fit of the NMRD profiles, respectively. In the *inset*, the coupling factor values at high fields can be better appreciated

ascribed to the contact relaxation, and the second to the dipolar relaxation. From the position of the first dispersion it was possible to estimate τ_s around 5×10^{-10} s. The profiles were fitted by considering four slowly exchanging water molecules in the equatorial plane and a fifth fast exchanging axial water molecule (Bertini et al. 1992). The contact interaction was ascribed to the equatorial water molecules. The profiles have now been fitted again by including an additional contribution from outer sphere relaxation, and the resulting best fit parameters are reported in Table 3. The coupling factors calculated from these parameters with and without outer-sphere relaxation are shown in Fig. 5 and reported in Table 2. In order to take into account the presence of slow exchanging water molecules, the coupling factor has been reduced by a factor

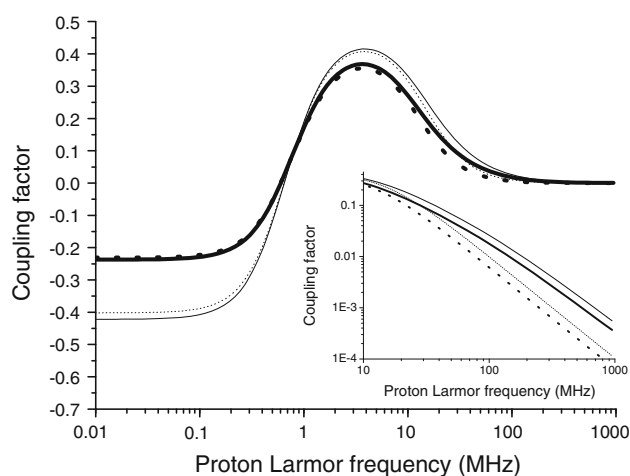
$$\frac{\left[f_{slow}(T_{1Mslow} + \tau_M)^{-1} + f_{fast}T_{1Mfast}^{-1} + R_{1diff} + R_{1dia} \right]}{\left[f_{slow}T_{1Mslow}^{-1} + f_{fast}T_{1Mfast}^{-1} + R_{1diff} + R_{1dia} \right]}$$

In conclusion, metal ions like copper(II), manganese(II) and, to a smaller extent, gadolinium(III) and VO^{2+} are expected to have coupling factors similar to those of nitroxide radicals at magnetic fields of 1–15 MHz. Therefore, they can provide signal enhancements of about 2 orders of magnitude at 15 MHz. As a result, metal aqua ions are almost equally effective than nitroxides if shuttling is performed from a polarizing field of 15 MHz proton Larmor frequency or below.

At fields larger than 15 MHz the coupling factor is sizably smaller than what calculated for nitroxide radicals because both the reorientation correlation time and the diffusional time increase, as a consequence of the larger size of the compound and of the larger distance of closest approach with respect to nitroxide radicals like TEM-PONE. Furthermore, an outer-sphere contribution to relaxation smaller than that of regularly coordinated water molecules at high fields causes the paramagnetic relaxation to decrease more quickly with increasing the magnetic field, so that also the coupling factor decreases more quickly than for nitroxides. In this respect Gd-complexes with one coordinated water molecule are expected to have sizably larger coupling factors in percent at large fields than systems with more coordinated water molecules and the same τ_D and τ_c values. Even better is the case of a complex without any coordinated water molecule, so that water relaxation has a paramagnetic enhancement only due to diffusion around the paramagnetic molecule. In this case, assuming a distance of closest approach of 3.6 Å from the gadolinium ion, as usually estimated for many Gd-complexes, coupling factors of 0.27, 0.02, 0.004 can be

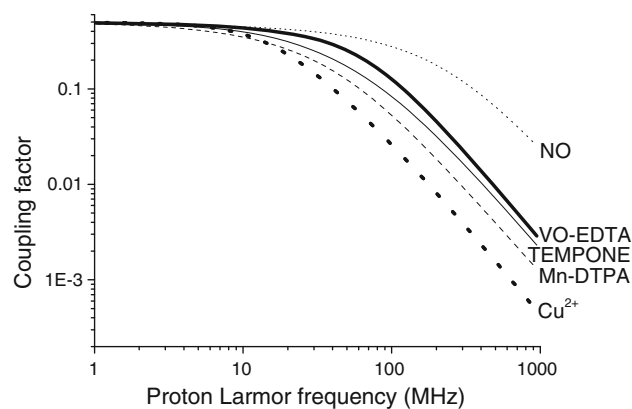
Table 3 Values of the parameters providing the NMRD profiles of VO²⁺ aqua ion reported in Figure S4

	VO ²⁺			
	25	35	25	35
<i>T</i> (°C)	25	35	25	35
<i>S</i>	0.5			
<i>q</i>	4 equatorial (slow) + 1 axial (fast)			
τ_M (slow water molecules) (μ s)	8.8	4.2	9.4	4.0
<i>r</i> (Å)	2.62 (equatorial), 2.8 (axial)		2.72 (equatorial), 2.8 (axial)	
τ_R (ps)	41	32	42	32
<i>A/h</i> (MHz)	2.10	2.10	2.05	2.05
τ_s (ps)	540	700	530	700
<i>d</i> (Å)			2.9	2.9
<i>D</i> (10^{-9} m ² /s)			3.0	3.6

**Fig. 5** Coupling factor at 25 °C (*thick lines*) and at 35 °C (*thin lines*) calculated for a water solution with VO²⁺ aqua ions. *Dotted lines* and *solid lines* show the values obtained without and with including *outer-sphere* relaxation in the fit of the NMRD profiles, respectively. In the *inset*, the coupling factor values at high fields can be better appreciated

calculated at 15, 150 and 400 MHz, respectively, at 25 °C, corresponding to maximum DNP enhancements of 175, 13 and 2.5. Similarly, manganese(II) complexes, like Mn-DTPA (Wagnon and Jackels 1989) or Mn-EDTA(BOM)₃ (Aime et al. 2002) are expected to have similar features, the distance of closest approach being around 3.5 Å.

In order to further increase the coupling factor it would be necessary to decrease the distance of closest approach and possibly increase the diffusion coefficient. To a smaller extent, the coupling factor can be increased if coordinated water molecules have a very short correlation time. The latter cannot be determined by the electron relaxation time (because it must be long, since otherwise saturation of the electron transitions would not be possible), or by the

**Fig. 6** Coupling factor at 25 °C calculated for NO, VO-EDTA, Mn-DTPA and copper(II) aqua ions, compared to the coupling factor of TEMPONE

reorientation time, which in metal complexes will be larger than for aqua ions. Therefore, the correlation time should be the lifetime of coordinated water protons. This is possible if second-sphere coordination is present. Vanadyl complexes with EDTA or DTPA, without any coordinated water molecules in the first coordination sphere, have been shown to have a relaxivity ascribed to diffusive water protons and second sphere water molecules (Chen et al. 1998). The analysis of the NMRD profiles of these complexes provided a correlation time of 5–6 ps for the dipolar relaxation of second-sphere water molecules (if the same profiles were fitted using outer-sphere relaxation only, effective (unreasonably small) *d* values around 2.0 Å were calculated). Using the best fit parameters reported in (Chen et al. 1998), Eq. 11 predicts a coupling factor for VO-EDTA of 0.41, 0.07, 0.01 at 15, 150 and 400 MHz, respectively, at 25 °C, corresponding to maximum DNP enhancements of 268, 47 and 9. As shown in Fig. 6, these features are somewhat better than those of TEMPONE, although worse than those expected for NO.

From the above discussion on the coupling factors, it appears that some metal ion complexes can compete with nitroxide radicals at all fields. In order for these coupling factors to translate into enhancements, one has to assume that 100 % saturation of the EPR lines can be achieved even for metal ions, which may not be the case with the current technology. The maximum DNP enhancements calculated for *s* = 1 and *f* = 1 at 15, 150 and 400 MHz and 25 °C are summarized in Table 4.

The table clearly points out that an ideal polarizing agent for solution DNP must be a long relaxing, small molecule, with a relaxation rate dominated by outer-sphere contributions (or contributions from second-sphere water protons exchanging on the picoseconds time scale): for this reason, NO would in principle determine an enhancement much larger than all other molecules. Interestingly, VO-EDTA

Table 4 Maximum DNP enhancement (calculated for $s = 1$ and $f = 1$) at 15, 150 and 400 MHz and 25 °C obtained from the coupling factors reported in Table 2, corresponding to the fit of the NMRD profiles performed with including outer-sphere relaxation

	ϵ_{\max} (15 MHz)	ϵ_{\max} (150 MHz)	ϵ_{\max} (400 MHz)
Gd ³⁺	152	4.5	0.8
VO ²⁺	130	6.2	1.2
Cu ²⁺	193	8.7	1.5
Mn ²⁺	183	9.7	1.8
Mn-DTPA ^a	197	19	3.8
TEMPONE ^b	231	32	6.8
VO-EDTA ^c	268	47	9.1
NO ^d	285	145	57

The polarizing agents are listed in order of increasing theoretical ϵ_{\max} at 400 MHz

^a Calculated from the best fit parameters reported in (Wagnon and Jackels 1989)

^b Calculated from the best fit parameters reported in (Bennati et al. 2010)

^c Calculated from the best fit parameters reported in (Chen et al. 1998)

^d Calculated from the parameters estimated through MD calculations in (Zhou et al. 2005)

appears to be the second best candidate polarizer among those analyzed here. Other oxovanadium(IV) complexes or complexes containing different paramagnetic metal ions without first-sphere water molecules may behave equally well. In general, metal aqua ions are not preferable at high fields: in fact, with respect to the values of 32 and 7 calculated for TEMPONE, the highest enhancement factor determined by aqua ions is only around 10 at 150 MHz and around unity at 400 MHz. To be noted that some paramagnetic metals (like Mn²⁺, Gd³⁺ and VO²⁺) have a field dependent electron relaxation: the longitudinal (not the transverse) electron relaxation time increases at fields corresponding to proton Larmor frequencies of hundreds MHz up to values of the order of 10⁻⁶–10⁻⁷ s, so that conditions for electron saturation can be more easily achieved.

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