Dynamic nuclear polarization enhancement of protons and vanadium-51 in the presence of pH-dependent vanadyl radicals

Angel J. Perez Linde, Diego Carnevale, Pascal Miéville, Andrzej Sienkiewicz and Geoffrey Bodenhausen

We report applications of dynamic nuclear polarization to enhance proton and vanadium-51 polarization of vanadyl sulfate samples doped with TOTAPOL under magic angle spinning conditions. The electron paramagnetic resonance response stemming from the paramagnetic $^{51}$V species was monitored as a function of pH, which can be adjusted to improve the enhancement of the proton polarization. By means of cross-polarization from the proton bath, $^{51}$V spins could be hyperpolarized. Enhancement factors, build-up times, and longitudinal relaxation times $T_1(1H)$ and $T_1(51V)$ were investigated as a function of pH.

Keywords: NMR; MAS; vanadium-51; DNP; EPR

Introduction

Vanadium possesses two NMR active isotopes, $^{50}$V and $^{51}$V; the latter has a spin $S(51V) = 7/2$, a 99.8% natural abundance, and a favorable gyromagnetic ratio since $\gamma(51V)/\gamma(13O) \approx 1.05$. Vanadium exists in several forms; one of the most stable is the paramagnetic vanadyl ion ($V^{IV}O^{2+}$, with the electronic configuration for the metal $V^{IV}d^{1}$), which can be found in rare minerals[2] and food supplements. The usual source of vanadyl ions is vanadyl sulfate ($VOSO_4$), which has been widely studied for the treatment of diabetes[3–5] and has effects on several mammalian enzymes.[6] The salt is water-soluble and, depending on pH, can form different hydrates.[7] At $2 < pH < 4$, the vanadyl ion is mainly present as a pentahydrate [V$^{IV}O(H_2O)_5$]$^{2+}$. At a pH around 4, the pentahydrate is in equilibrium with the hydroxide [V$^{IV}OOH$]$^{+}$. Both the pentahydrate and the first hydroxide are EPR active and give intense blue solutions. When increasing the pH to ~5, a new equilibrium between the pentahydrate and a second dimeric hydroxide [(V$^{IV}OO$)$_2$]$^{2+}$ is established. Around pH 6, a third insoluble hydroxide V$^{IV}O(OH)_2$ starts to aggregate and precipitate. Both dimeric and aggregate forms are EPR silent. Once the pH reaches ~11, the precipitate is dissolved again to form a gold-brown solution of [VIVO(OH)$_3$] which is known to be EPR active.[7] In the presence of oxygen, vanadyl can be oxidized into vanadate (V$^V$), which may result in a much more complex chemical system.[9]

Solid-state NMR in combination with magic angle spinning (MAS) is widely used to measure inhomogeneous interactions such as anisotropic chemical shifts and quadrupolar and dipolar couplings, all of which yield information about the local environment of quadrupolar nuclei such as $^{51}$V nuclei.[10–14]

Dynamic nuclear polarization (DNP) can be induced in the presence of paramagnetic centers by strong microwave irradiation to improve the polarization of protons by a factor $e_{DNP}(1H)$. In combination with cross polarization CP($1H \rightarrow 51V$), the strength of $^{51}$V NMR signals can be boosted indirectly by a factor $e_{DNP}CP(^{51}V)$, resulting in a reduction of the experimental time proportional to $e_{DNP}CP(^{51}V)$. The combination of MAS, DNP, and CP is becoming a method of choice in high-field solid-state NMR.[15,16] Proton DNP combined with heteronuclear polarization transfer has been successfully applied to various quadrupolar nuclei such as $^{14}$N, $^{13}$C, and $^{27}$Al.[17–21] DNP requires paramagnetic electrons with a suitable longitudinal relaxation time $T_{1e}$. Off-resonance irradiation near the EPR line leads to a transfer of polarization from the electrons to nuclear spins, with a theoretical maximum enhancement of 660 for protons with respect to thermal polarization. For biradicals such as TOTAPOL, the main mechanism for polarization transfer at 9.4 T and 100 K is the cross effect[15,16] involving two...
electron spins and one proton spin. The efficiency of this polarization transfer mechanism can be affected by other EPR transitions.\(^\text{[15,22-30]}\) Careful selection of two radicals with different EPR line shapes and different \(T_{1e}\) can lead to improved DNP enhancements.\(^\text{[25–27,31]}\)

However, paramagnetic agents with very short longitudinal relaxation times \(T_{1e}\) or with much greater line widths than TOTAPOL may be detrimental for DNP enhancements, especially at high concentrations when they may increase the leakage of polarization.\(^\text{[23,26,28]}\)

DNP with \(^{51}\text{V}\) in solution can also be achieved using the Overhauser effect.\(^\text{[32]}\) In solids, DNP can be achieved using high-spin metal ions\(^\text{[33]}\) or other radicals.\(^\text{[34,35]}\)

In this study, we explore the efficiency of DNP in frozen solutions of vanadyl sulfate, doped with the biradical TOTAPOL as a function of \(pH\). The variation of the proton DNP enhancement as a function of \(pH\) can be related to the simultaneous presence of two distinct paramagnetic agents that may or may not interfere with each other. We demonstrate that direct \(^{51}\text{V}\) DNP can also be achieved on the same samples, albeit with modest enhancement factors. At high \(pH\), the enhanced proton polarization can be best transferred to the quadrupolar \(^{51}\text{V}\) nuclei through cross-polarization CP(\(1\text{H} \rightarrow {^{51}\text{V}}\)).

**Experimental**

The NMR experiments were performed using a Bruker 9.4 T magnet with a field-sweep capability of \(\pm 0.075 \text{ mT} \ (\pm 2.1 \text{ GHz})\), operating at 400 MHz for \(^1\text{H}\) and 263 GHz for EPR, using a gyrotron with a maximum output of 5 W. A triple-channel MAS probe operating at temperatures near 100 K was used with 3.2-mm diameter thin-walled zirconia rotors.\(^\text{[18]}\)

In order to study DNP in the presence of the two distinct paramagnetic agents, \([\text{V}^{2+}(\text{H}_2\text{O})_6]^{2+}\) and TOTAPOL, we prepared two series of solutions, all with 8 mM TOTAPOL, with and without vanadyl sulfate. The \(pH\) was adjusted with 1 M NaOD or DCI using a Hach pH meter, model H160. In the first stage, 40 mM VOSO_4 \cdot 2H_2O was dissolved in D_2O, using NaOD or DCI for \(pH\) adjustment. At a \(pH\) around 12 and above, the color of the solution was yellow. All solutions in D_2O were left to rest for 24 h before freezing, without observing any precipitation. Note that after 24 h, the yellow color had vanished at high \(pH\), although the \(pH\) has not changed during this interval. This suggests a low concentration of \([\text{V}^{2+}\text{(OH)}_3]^+\), which is consistent with the EPR spectrum in Fig. 1(d).

We did not observe any precipitation at neutral \(pH\)\(^\text{[7]}\) but at a concentration of only 40 mM, it is possible that some precipitation was not directly visible to the eye. Glycerol and H_2O with TOTAPOL (with final ratios of glycerol : D_2O : H_2O = 50 : 40 : 10 (v:v:v)) were added in order to obtain a glass-forming mixture with a final concentration of 16 mM vanadyl sulfate. The solutions were left to rest for one more hour before freezing for NMR-MAS experiments. The \(pH\) of the sample containing water and glycerol at room temperature was measured with a pH meter without correcting for the effects of glycerol. EPR spectra of samples containing 16 mM vanadyl and 8 mM TOTAPOL were taken at room temperature for three samples at \(pH\) 3.5, 7.2, and 12.2 (Fig. 1). The solutions were transferred to thin-walled 3.2-mm zirconia rotors suitable for DNP, cooled in the low-temperature MAS probe until the nominal temperature 100 K was reached and spun at \(\nu_R = 10 \text{ kHz}\). Comparison

**Figure 1.** (a) X-band ESR spectrum of a sample containing 40 mM VOSO_4 in D_2O, \(pH\) 2.9, at room temperature. (b)–(d) X-band spectra of a sample containing 8 mM TOTAPOL and 16 mM VOSO_4 in glycerol : D_2O : H_2O = 50 : 40 : 10 at \(pH\) 3.5, 7.2, and 12.2, respectively, also at room temperature. Twice the number of scans were taken for (c) compared with (b) and (d). The center field was 3345 G, the sweep width 1000 G, and the irradiation power was 0.6335 mW. Data were processed with MATLAB.
of the proton polarization enhancements for two sets of samples, both with 8 mM TOTAPOL but with and without 16 mM VO₃⁻ at different pH, is shown in Fig. 2. In addition, two samples of 16 mM vanadyl sulfate without TOTAPOL were prepared at pH 3.1 and 12.4 to evaluate DNP enhancements stemming only from the paramagnetic vanadyl ions (Table 1).

Cross polarization CP(¹H→⁵¹V) was performed with a 2 µs 90° proton pulse followed by a contact time of 4 ms with ν(⁵¹V) = 100 kHz and ν(¹H) = 80 kHz. The spinning frequency was νₛ = 10 kHz in all cases. The lineshape due to the central transition of the ⁵¹V in the spectra of Fig. 2(b) is nearly isotropic. The Hartmann–Hahn match was optimized experimentally, and ν(¹H) = 80 kHz and ν(⁵¹V) = 100 kHz agree with the simple case of two S = 1/2 spins, for which one expects ν(¹H) = ν(⁵¹V) + nνₛ. In our case, νₛ = 10 kHz and n = 2. Proton spectra were acquired using rotor-synchronized spin echoes with τₕ_{ref} = τₜ_{ref} = 1/νₛ = 100 µs to avoid phase distortions and spurious background signals. Proton build-up times τ_DNP(¹H) and proton longitudinal relaxation times T₁(¹H) were measured by saturation recovery experiments. The enhancements ε_DNP(¹H) were measured by comparing signal intensities observed with rotor-synchronized spin echoes with and without microwave irradiation.

**EPR Spectra**

All EPR spectra were acquired at room temperature with an X-band EPR spectrometer designed by Bruker, Model EleXsys E500. Standard TOPSPIN, and graphs were plotted with MATLAB.

**Figure 2.** (a) Relative enhancements ε_DNP(¹H) of the proton polarization obtained with 5 W microwave irradiation at 263 GHz, with respect to the thermal Boltzmann population obtained without microwave irradiation, as a function of pH: (●) 8 mM TOTAPOL; 16 mM vanadyl and 8 mM TOTAPOL in a glass-forming solvent mixture glycerol:D₂O:H₂O = 50:40:10. The proton spectra were obtained with a spin echo (90°x - s90°y - s90°y - echo), either with or without DNP, accumulating 16 scans each. The lines are merely to guide the eye. The average <ε_DNP(¹H)> obtained from samples doped only with TOTAPOL, averaged over all pH values, was defined to be 100%. (b) ⁵¹V NMR spectra of a solution of 8 mM TOTAPOL and 16 mM vanadyl sulfate in a glass-forming solvent mixture glycerol:D₂O:H₂O = 50:40:10 at pH 12.3. ν₁ = 10 kHz and T = 100 K. The ⁵¹V vanadium spectrum with DNP was obtained with cross polarization (contact time 4 ms, RF field amplitudes of 100 kHz for ¹H and 80 kHz for ⁵¹V, 10 s, 32 scans with recovery delays of 50 s, and a total experimental time of 28 min, while the ⁵¹V spectra obtained in thermal equilibrium without DNP (microwaves off) and with direct DNP (microwaves on) were averaged over 32 scans each with recovery delays of 300 and 120 s, requiring overall experimental times of 165 and 64 min, respectively (τ_DNP(⁵¹V) = 29 s, T₁(⁵¹V) = 70 s). If the curves are assumed to agree with monoexponential functions, these delays allow the recovery of 98% of the polarization. All ⁵¹V spectra were obtained by triggering signal acquisition on top of a rotor-synchronized spin echo (either after CP or after a 90° excitation pulse). The amplitudes of these spectra were normalized with respect to the Boltzmann equilibrium. The equilibrium spectra (and direct DNP spectra for ⁵¹V) are displayed below the hyperpolarized spectra for comparison. For all three spectra, a spectral width of 500 kHz was selected and with a spectral width of 500 kHz was selected and

**Table 1.** Proton and ⁵¹V DNP build-up time constants τ_DNP(¹H) and τ_DNP(⁵¹V) and relaxation times T₁(¹H) and T₁(⁵¹V) for 16 mM vanadyl sulfate samples with and without TOTAPOL at different pH, in a glassy matrix of glycerol:D₂O:H₂O = 50:40:10 (v:v:v) at 100 K and a spinning frequency νₛ = 10 kHz

<table>
<thead>
<tr>
<th>pH</th>
<th>[TOTAPOL] (mw)</th>
<th>[VO₃⁻] (mw)</th>
<th>τ_DNP(¹H) (s)</th>
<th>T₁(¹H) (s)</th>
<th>τ_DNP(⁵¹V) (s)</th>
<th>T₁(⁵¹V) (s)</th>
<th>ε_DNP(¹H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.4</td>
<td>0</td>
<td>16</td>
<td>94 ± 5</td>
<td>130 ± 10</td>
<td>62 ± 2</td>
<td>110 ± 0</td>
<td>1</td>
</tr>
<tr>
<td>12.3</td>
<td>8</td>
<td>16</td>
<td>10.7 ± 1.0</td>
<td>18.2 ± 0.3</td>
<td>29 ± 2</td>
<td>70 ± 5</td>
<td>17.8</td>
</tr>
<tr>
<td>12.2</td>
<td>8</td>
<td>0</td>
<td>9.8 ± 0.6</td>
<td>15.5 ± 0.1</td>
<td>65 ± 0.1</td>
<td>11.5 ± 1</td>
<td>2</td>
</tr>
<tr>
<td>11.8</td>
<td>8</td>
<td>16</td>
<td>8.0 ± 1.0</td>
<td>15.5 ± 0.5</td>
<td>62 ± 2</td>
<td>11.5 ± 1</td>
<td>16</td>
</tr>
<tr>
<td>8.6</td>
<td>8</td>
<td>0</td>
<td>6.5 ± 0.5</td>
<td>11.2 ± 0.1</td>
<td>65 ± 0.5</td>
<td>11.5 ± 1</td>
<td>13</td>
</tr>
<tr>
<td>7.6</td>
<td>8</td>
<td>16</td>
<td>7.0 ± 0.7</td>
<td>10.8 ± 0.5</td>
<td>65 ± 0.5</td>
<td>11.5 ± 1</td>
<td>13.2</td>
</tr>
<tr>
<td>7.3</td>
<td>8</td>
<td>0</td>
<td>6.6 ± 0.5</td>
<td>10.2 ± 0.2</td>
<td>65 ± 0.5</td>
<td>11.5 ± 1</td>
<td>14.8</td>
</tr>
<tr>
<td>6.4</td>
<td>8</td>
<td>0</td>
<td>6.0 ± 0.7</td>
<td>10.9 ± 0.1</td>
<td>65 ± 0.5</td>
<td>11.5 ± 1</td>
<td>12.9</td>
</tr>
<tr>
<td>3.2</td>
<td>8</td>
<td>16</td>
<td>9.8 ± 0.5</td>
<td>13.5 ± 0.5</td>
<td>65 ± 0.5</td>
<td>11.5 ± 1</td>
<td>5.1</td>
</tr>
<tr>
<td>3.1</td>
<td>0</td>
<td>16</td>
<td>15 ± 2</td>
<td>15 ± 1</td>
<td>65 ± 0.5</td>
<td>11.5 ± 1</td>
<td>1</td>
</tr>
<tr>
<td>2.6</td>
<td>8</td>
<td>0</td>
<td>6.3 ± 0.5</td>
<td>10.7 ± 0.1</td>
<td>65 ± 0.5</td>
<td>11.5 ± 1</td>
<td>13</td>
</tr>
</tbody>
</table>

Within the the marging of error, no proton enhancement was obtained in samples containing vanadyl sulfate without TOTAPOL.
first derivative of absorption spectra was recorded using 100 kHz magnetic field modulation with a peak-to-peak amplitude of 5 G. Figure 1 shows EPR spectra of 40 mM VOSO4 in D2O (Fig. 1(a)) and samples containing 8 mM TOTAPOL and 16 mM vanadyl in glycerol: D2O:H2O = 50:40:10 (Fig. 1(b)-(d)).

In the EPR spectrum, the characteristic narrow signal of TOTAPOL dominates over the broad features of vanadyl, especially in solutions with neutral to high pH, as can be seen in Fig. 1(b)-(d). At low pH, 40 mM vanadyl in D2O shows a clear response (Fig. 1(a)), with a width extending over 2 GHz. Still at low pH, in a sample containing 16 mM vanadyl and 8 mM TOTAPOL, the wide vanadyl signals overlap with the narrow signal of the nitroxide biradical (Fig. 1(b)).

**Proton Polarization**

Figure 2(a) shows the proton polarization enhancements $\epsilon_{\text{DNP}}(1H)$ for samples containing 8 mM TOTAPOL with and without 16 mM vanadyl sulfate as a function of pH. The enhancements obtained for $^{51}$V with CP($^{1H} \to ^{51}$V) at high pH are shown in Fig. 2(b).

Samples doped only with TOTAPOL show proton DNP enhancements $\epsilon_{\text{DNP}}(1H) = 12.3 \pm 1.2$. These enhancements may have suffered from the use of deuterated glycerol and opaque zirconia instead of transparent sapphire rotors. The enhancements were also hampered by the use of low (8 mM) concentrations of TOTAPOL and high spinning frequencies. In the samples containing both $^{51}$Vo$^{2+}$ and TOTAPOL, the enhancements $\epsilon_{\text{DNP}}(1H)$ clearly improve with increasing pH. For samples containing both $^{51}$Vo$^{2+}$ and TOTAPOL at pH < 6, the enhancements were much smaller than for samples containing only TOTAPOL. This is consistent with vanadyl ions acting as a sink of polarization leakage. Near neutral pH, the enhancements approach those obtained with only TOTAPOL. This observation is consistent with the disappearance of the EPR response because of the aggregation and precipitation of vanadyl hydroxides. For higher pH (Table 1), the enhancements have either similar or greater. Build-up times $\tau_{\text{DNP}}(1H) = 8 \pm 2$ s and relaxation times $T_1(1H) = 15 \pm 5$ s were found for samples containing both TOTAPOL and vanadyl while $\tau_{\text{DNP}}(1H) = 8 \pm 2$ s and $T_1(1H) = 12.5 \pm 3$ s for samples doped only with TOTAPOL (Table 1). The time constants $\tau_{\text{DNP}}(1H)$ and $T_1(1H)$ for two samples containing vanadyl but no TOTAPOL are also shown in Table 1.

At neutral pH, according to Francavilla and Chasteen, and as confirmed in Fig. 1(c), the vanadyl EPR response vanishes. This observation pleads in favor of an EPR response stemming only from TOTAPOL in this pH range and therefore explains that the enhancements are similar to those acquired with TOTAPOL as unique paramagnetic agent.

Table 1 shows that, in the absence of TOTAPOL, vanadyl complexes only contribute weakly to the proton relaxation rates at pH = 12.4. Indeed, no EPR signals of vanadyl ions can be observed in Fig. 1(d), using the same acquisition parameters as in Fig. 1(b). Nevertheless, in solutions at high pH, one expects another EPR signal coming from vanadyl ions. A small paramagnetic contribution of vanadyl ions in samples with only VOSO4 at pH = 12.4 is coherent with the significant difference between the proton DNP build-up time $\tau_{\text{DNP}}(1H) = 94$ s and the proton longitudinal relaxation time $T_1(1H) = 130$ s. A small concentration of another paramagnetic agent in addition to the one being used for DNP, such as demonstrated with gadolinium in other studies, could instead change or improve the DNP enhancement.

**Vanadium-51 NMR**

In this study, $^{51}$V NMR spectra could only be obtained for samples with a pH around 11 and above, either by direct excitation or by using CP. A sample containing 16 mM vanadyl sulfate and 8 mM TOTAPOL at pH 12.3 was found to have $T_{\text{CP}}(^{51}$V) = 29 ± 2 s (microwaves on) and $T_1(^{51}$V) = 70 ± 5 s (microwaves off), determined by saturation recovery of $^{51}$V. With CP, the final enhancement on $^{51}$V was measured to be $\epsilon_{\text{DNP-CP}}(^{51}$V) = 9 ± 1 with respect to thermal equilibrium. This proves the efficiency of DNP and CP even for CP from protons to quadrupolar spins. Direct DNP enhancement of $^{51}$V gave only $\epsilon_{\text{DNP}}(^{51}$V) = 1.4 at pH 12.3 (Fig. 2(b)). A fit with SOLA (Topsin) of the central transition of the $^{51}$V spectrum yielded a quadrupolar coupling constant $C_Q = 703$ kHz and an asymmetry parameter $\eta = 1.0$.

**Conclusions**

It has been possible to hyperpolarize protons in frozen solutions containing 16 mM vanadyl ion $^{51}$Vo$^{2+}$ and 8 mM TOTAPOL at various pH, at temperatures near $T = 100$ K and spinning frequencies $\nu_{\text{sp}} = 10$ kHz. The enhancement $\epsilon_{\text{DNP}}(1H)$ of the proton polarization increases with pH, which can be correlated with the effects of pH on the EPR response of the vanadyl radical. The pH can be adjusted to ‘silence’ the EPR response of the paramagnetic vanadyl species so that the EPR response of TOTAPOL becomes dominant. At pH > 11, we obtained similar or better enhancements $\epsilon_{\text{DNP}}(1H)$ in samples containing both TOTAPOL and VOSO4 than in samples containing only TOTAPOL. These observations are reminiscent of effects previously observed when doping DNP samples with small amounts of gadolinium.

At high pH, the EPR response of vanadyl was not visible using the same EPR acquisition parameters as those used for samples at low pH. However, it is possible to deduce the presence of a small amount of a paramagnetic agent from the difference between $\tau_{\text{DNP}}(1H) = 94$ s and $T_1(1H) = 130$ s at pH 12.4, as stated in the literature. In combination with TOTAPOL, this paramagnetic agent at low concentrations could increase the proton enhancement. The proton polarization can be enhanced by DNP by a factor $\epsilon_{\text{DNP}}(1H) = 10$, with a proton build-up time $\tau_{\text{DNP}}(1H) = 10$ s, which is seven times shorter than $T_1(^{1H}) \approx 70$ s in a sample at pH 12.3 containing TOTAPOL and vanadyl. Direct DNP of vanadium-51 with $\epsilon_{\text{DNP}}(^{51}$V) = 1.4 with respect to thermal equilibrium is possible in this sample, but the indirect enhancement using proton DNP and CP($^{1H} \to ^{51}$V) is to be preferred because it yields $\epsilon_{\text{DNP-CP}}(^{51}$V) = 9. In $^{51}$V complexes aimed at mimicking the effects of insulin, the enhanced proton polarization could be transferred by CP not only to $^{51}$V but also to $^{13}$C or $^{31}$P.

**Acknowledgements**

The authors would like to thank Martial Rey, Anto Basiric, Dr Marc Caporini and Dr Christian Reiter for their technical assistance. This work was supported by the Swiss National Science Foundation (SNSF), the Ecole Polytechnique Fédérale de Lausanne (EPFL), the Swiss Commission for Technology and Innovation (CTI), the French CNRS, and the European Research Council (ERC) advanced grant 339754 ‘Dilute Para-Water’.

**References**
