Published in final edited form as:

J Magn Reson. 2013 February ; 227: 14–19. doi:10.1016/j.jmr.2012.11.006.

Dissolution DNP-NMR spectroscopy using galvinoxyl as a polarizing agent

Lloyd L. Lumata^a, Matthew E. Merritt^a, Craig R. Malloy^a, A. Dean Sherry^{a,b}, Johan van Tol^c, Likai Song^c, and Zoltan Kovacs^{a,*}

^aAdvanced Imaging Research Center, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75390, United States

^bDepartment of Chemistry, University of Texas at Dallas, 800 West Campbell Road, Richardson, Texas 75080, United States

^cNational High Magnetic Field Laboratory, Florida State University, 1800 East Paul Dirac Drive, Tallahassee, Florida 32310, United States

Abstract

The goal of this work was to test feasibility of using galvinoxyl (2,6-di-tert-butyl- α -(3,5-di-tert-butyl-4-oxo-2,5-cyclohexadien-1-ylidene)-p-tolyloxy) as a polarizing agent for dissolution dynamic nuclear polarization (DNP) NMR spectroscopy. We have found that galvinoxyl is reasonably soluble in ethyl acetate, chloroform, or acetone and the solutions formed good glasses when mixed together or with other solvents such as dimethyl sulfoxide. W-band electron spin resonance (ESR) measurements revealed that galvinoxyl has an ESR linewidth D intermediate between that of carbon-centered free radical trityl OX063 and the nitroxide-based 4-oxo-TEMPO, thus the DNP with galvinoxyl for nuclei with low gyromagnetic ratio γ such as ^{13}C and ^{15}N is expected to proceed predominantly via the thermal mixing process. The optimum radical concentration that would afford the highest ^{13}C nuclear polarization (approximately 6% for $[1-^{13}\text{C}]$ ethyl acetate) at 3.35 T and 1.4 K was found to be around 40 mM. After dissolution, large liquid-state NMR enhancements were achieved for a number of ^{13}C and ^{15}N compounds with long spin-lattice relaxation time T_1 . In addition, the hydrophobic galvinoxyl free radical can be easily filtered out from the dissolution liquid when water is used as the solvent. These results indicate that galvinoxyl can be considered as an easily available free radical polarizing agent for routine dissolution DNP-NMR spectroscopy.

Keywords

dynamic nuclear polarization; NMR; free radical; hyperpolarization; thermal mixing

*corresponding author: Zoltan Kovacs, Ph.D., Tel. 214-645-2750, Fax 214-645-2744, zoltan.kovacs@utsouthwestern.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/xx.xxxx/j.jmr.xxxx.xx.xxx>.

1. INTRODUCTION

Nuclear magnetic resonance (NMR) is one of the most widely used analytical and structural elucidation techniques in chemistry and materials science. It is also the underlying principle of magnetic resonance imaging (MRI), an important clinical imaging modality. NMR, however, is a relatively insensitive technique compared to other spectroscopic methods such as electron spin resonance (ESR), optical, or mass spectroscopy [1]. This inherent insensitivity emanates from the relatively small magnetic moments of the nuclei leading to low nuclear polarization P , a parameter which determines the strength of the NMR signal [2,3].

The problem of low sensitivity in liquid-state NMR spectroscopy has been recently addressed by the invention of the dissolution dynamic nuclear polarization (DNP) method [4]. DNP, a technique used in the production of polarized targets for nuclear and particle physics experiments since the 1960s, amplifies the NMR signal via the microwave-induced transfer of the high electron thermal polarization to the target nuclear spins at low temperature and moderate magnetic field [5–7]. The incorporation of a dissolution device in the DNP polarizer that rapidly dissolves the frozen polarized samples (e.g. compounds containing NMR-active nuclei dissolved in glass formers and doped with paramagnetic electrons) using pressurized superheated water or other solvents has allowed the production of highly polarized solutions at room temperature [4]. As a result, the liquid state NMR signal is enhanced several-thousand-fold thereby facilitating the high sensitivity liquid-state MR spectroscopy and imaging of nuclei with low gyromagnetic ratio γ , in particular, ^{13}C and ^{15}N , in chemistry [8] and biomedical applications [9–11].

One of the crucial parameters to achieve high nuclear polarization levels in DNP is the choice of free radicals used as polarizing agents [12,13]. The ESR properties of the free radical (e.g. the ESR linewidth D) play a central role in the DNP polarization transfer process [5,12,13]. Currently, the commonly used free radical polarizing agents for dissolution DNP-NMR include the carbon-centered trityl OX063 radical as well as the nitroxide-based TEMPO and very recently the carbon-centered BDPA [14] free radical. Other free radicals that have been considered for dissolution DNP-NMR spectroscopy include the perchlorinated trityls, trityl biradicals, and other derivatives [15–17]. In this work, we have investigated the feasibility of a well-known stable free radical, 2,6-di-tert-butyl- α -(3,5-di-tert-butyl-4-oxo-2,5-cyclohexadien-1-ylidene)-p-tolyloxy or galvinoxyl as a polarizing agent for dissolution DNP. Galvinoxyl (see Fig. 1a), first synthesized by Galvin Coppinger in 1957 [18], has been mainly used as scavenging free radical to test the potency of antioxidants in food and pharmaceuticals [19,20]. This free radical has never been tried for dissolution DNP-NMR spectroscopy primarily because it is insoluble in water. However, we have found that galvinoxyl is reasonably soluble in ethyl acetate, chloroform, or acetone and these solvents form a good glassing matrix when mixed together or with other solvents such as dimethyl sulfoxide. The goal of this research was to investigate the utility of galvinoxyl as a polarizing agent for routine dissolution DNP-NMR spectroscopy in chemistry.

2. EXPERIMENTAL METHODS

Materials

The galvinoxyl and 4-oxo-TEMPO (4-oxo-2,2,6,6-tetramethylpiperidine-1-oxyl) free radicals, ^{13}C (ethyl acetate, urea, methyl pyruvate, sodium pyruvate, diethyl-1,3-acetonedicarboxylate) and ^{15}N (nitrobenzene, acetonitrile, and pyridine) compounds, glassing agents, and solvents used in this work were obtained from Sigma-Aldrich (St. Louis, Missouri) and were used without further purification. The free radical trityl OX063 (tris{8-

carboxyl-2,2,6,6-benzo(1,2-d:5-d)-bis(1,3)dithiole-4-yl}methyl sodium salt) was obtained from Oxford Instruments (Tubney Woods, U.K.).

ESR measurements

The ESR measurements of galvinoxyl samples at W-band (3.35 T, 5 K base temperature) were done in a commercial Bruker E680 ESR spectrometer (Bruker Biospin, Billerica, Massachusetts) at the National High Magnetic Field Laboratory (NHMFL) in Tallahassee, Florida. The galvinoxyl samples (40 mM in 1:1 (v/v) CHCl₃:DMSO) for ESR were loaded into 0.55 mm OD quartz capillary tubes. For comparison, the ESR spectra of trityl OX063 (15 mM in 1:1 (v/v) glycerol:water) and 4-oxo-TEMPO (15 mM in 1:1 (v/v) glycerol:water) were also measured in the W-band at 100 K (see the Supplementary Information). The W-band ESR spectra at 100 K were measured by echo-detected field sweep by using a two-pulse echo 90°-τ-180°.

Dynamic nuclear polarization of compounds

The samples were polarized in the HyperSense commercial polarizer (Oxford Instruments, Tubney Woods, UK) at 3.35 T and 1.4 K with a 100 mW microwave source as described previously [12]. The ¹³C microwave DNP spectra and polarization buildup curves were done with a built-in automated program in the HyperSense NMR spectrometer.

Hyperpolarized ¹³C compounds

a) [*1-¹³C*]ethyl acetate. A 100 μL solution containing 3.37 mg galvinoxyl in CHCl₃ was prepared and then mixed with an equal volume of [*1-¹³C*]ethyl acetate. The final concentration of galvinoxyl in the sample was 40 mM. 50 μL aliquots were polarized in the HyperSense for 1 hour. 4 mL methanol was used as the dissolution solvent. b) [*1-¹³C*]urea. A 200 μL solution contained 25 mg [*1-¹³C*]urea solution was prepared in 1:1 (v/v) DMSO:CHCl₃ and then doped with 3.4 mg galvinoxyl. 40 μL aliquots were polarized in the HyperSense at 3.35 T and 1.4 K for 2 hours. 4 mL water was used as the dissolution solvent. c) *¹³C-natural abundance methyl pyruvate*. A 200 μL solution containing 6.75 mg galvinoxyl in CHCl₃ was prepared and then mixed with equal volume of methyl pyruvate. 100 μL aliquots were polarized in the HyperSense for 2 hours. 4 mL methanol was used as the dissolution solvent. d) *¹³C-natural abundance diethyl-1,3-acetone-dicarboxylate*. A 200 μL solution containing 6.75 mg galvinoxyl in CHCl₃ was prepared and then mixed with equal volume of diethyl-1,3-acetone-dicarboxylate. 100 μL aliquots were polarized in the HyperSense polarizer for 2 hours. 4 mL methanol was used as the dissolution solvent.

Hyperpolarized ¹⁵N compounds

a) *¹⁵N-enriched nitrobenzene*. A 100 μL solution containing 3.37 mg galvinoxyl in CHCl₃ was prepared and then mixed with an equal volume of ¹⁵N-nitrobenzene. 50 μL aliquots were polarized in the HyperSense at 3.35 T and 1.4 K for 3 hours. 4 mL methanol was used as the dissolution solvent. b) *¹⁵N-natural abundance acetonitrile*. A 200 μL solution containing 6.75 mg galvinoxyl in CHCl₃ was prepared and then mixed with equal volume of acetonitrile. 100 μL aliquots were polarized in the HyperSense at 3.35 T and 1.4 K for 3 hours. c) *¹⁵N-natural abundance pyridine*. A 200 μL solution containing 6.75 mg galvinoxyl in CHCl₃ was prepared and then mixed with equal volume of pyridine. 100 μL aliquots were polarized in the HyperSense polarizer at 3.35 T and 1.4 K for 3 hours. 4 mL water was used as the dissolution solvent.

NMR signal enhancement ε and spin-lattice T₁ relaxation measurements in the liquid-state

The liquid-state NMR signal enhancement ε and T₁ measurements were all done at 298 K in a 9.4 T Varian VNMRs high resolution spectrometer (Agilent Technologies, Santa Clara,

California). The dissolution liquid from the polarizer was collected directly in a 10-mm NMR tube inside a 9.4 T magnet via a Teflon tube with a dissolution transfer time of 8 s. Thus, the NMR enhancements ϵ were measured 8 s after dissolution of the hyperpolarized ^{13}C or ^{15}N compounds. The liquid-state ϵ was calculated by taking the ratio of the integrated NMR area of the sample in the hyperpolarized state over the thermal NMR signal [12]. In the case of ^{15}N natural abundance compounds, neat ^{15}N natural abundance nitrobenzene was used as the thermal ^{15}N NMR signal for enhancement calculations. The NMR enhancement measurements were done in triplicate ($N=3$) and the averages were calculated along with the standard deviation. For liquid-state T_1 measurements, 1 mL of the 4 mL dissolution liquid collected in the beaker was pipetted into a 10-mm NMR tube. The sample tube was placed higher than normal to make sure that the hyperpolarized liquid is enclosed by the rf coil. The decay of the hyperpolarized NMR signal was monitored by applying a small flip angle θ_{flip} every time interval TR. The T_1 values were calculated using an equation [21,22] which accounts for the loss of nuclear magnetization due to relaxation and rf excitation (see also the Supplementary Information). The analyses of the NMR data were done using the software ACD Labs version 12 (Advanced Chemistry Development Inc., Toronto, Canada) and Igor Pro version 6 (Wavemetrics Inc., Portland, Oregon).

Filtration and UV-Vis spectroscopy

The hydrophobic galvinoxyl free radical naturally precipitated out in the dissolution liquid when water is used as the solvent. In separate experiments, the free radical was rapidly removed from the hyperpolarized solution by simple mechanical filtration using a 0.2-micron syringe filter. The absence of galvinoxyl in the filtered aqueous dissolution liquid was confirmed by UVVis spectroscopy (see the Supplementary Information) using a Shimadzu UV-3600 spectrophotometer (Shimadzu Scientific Instruments, Japan).

3. RESULTS AND DISCUSSION

3.1 ESR measurements

As mentioned previously, the ESR properties of the free radical polarizing agent play an important role in achieving the maximum nuclear polarization of the target nuclei in DNP [5–7, 12, 13]. In this regard, we have performed ESR measurement on the optimum DNP concentration of galvinoxyl in the W-band which operates at approximately the same magnetic field as the HyperSense polarizer (3.35 T). Fig. 1b shows the ESR spectrum of galvinoxyl (40 mM) in 1:1 (v/v) CHCl_3 :DMSO glassing matrix at 100 K in the W-band. The shape of the W-band ESR spectrum at 100 K is expected to be very similar to that at 1.4 K where DNP is performed. The principal values of the g-tensor of galvinoxyl measured at 100 K in the W-band are $g_{xx}=2.00625$, $g_{yy}=2.00445$, and $g_{zz}=2.00237$. As seen from Fig. 1b, it is difficult to determine the ESR linewidth due to the inhomogeneously broadened lineshape, which is dominated by g-anisotropy arising mainly from the spin-orbit interaction with the oxygen atom [23]. In this light, we define the linewidth D in this work as the distance from base to base of the ESR spectrum. Based on this criterion, the ESR D of galvinoxyl (approximately 250 MHz) is estimated to be intermediate between the carbon-centered trityl OX063 [24] (115 MHz; see the Supplementary Information) and the nitroxide-based 4-oxo-TEMPO ESR linewidths [25] ($D=465$ MHz; see the Supplementary Information).

3.2 DNP of galvinoxyl-doped [1- ^{13}C]ethyl acetate in the solid state

Mounting evidence [12, 13, 26, 27] have shown that DNP of low- γ nuclei such as ^{13}C , ^{15}N , and ^{89}Y with trityl OX063 and TEMPO at moderate fields (e.g. 3.35 T) and at temperatures close to 1 K operate predominantly via the thermal mixing mechanism. Since the ESR linewidth of galvinoxyl is significantly larger than the Larmor frequencies of ^{13}C (35.96

MHz) or ^{15}N (14.45 MHz) in the HyperSense polarizer, the expected DNP mechanism for such low- γ nuclei is the thermal mixing process [5–7, 12, 13]. As described by the spin temperature or the Borghini model of DNP [28], off-center microwave irradiation by a certain optimum offset frequency Δ (upfield or downfield from the center of the ESR lineshape) will lead to minimum spin temperature of the electron dipolar system, which consequently cools the nuclear Zeeman system [5–7, 12, 13]. Fig. 2a shows the location of the optimum polarization peaks in the ^{13}C microwave DNP spectrum (plot of ^{13}C NMR signal enhancements as a function of microwave irradiation frequency) of 1:1 (v/v) $[1-^{13}\text{C}]$ ethyl acetate: CHCl_3 sample doped with 40 mM galvinoxyl. The separation between the positive P(+) and negative P(-) polarization peaks is about 150 MHz. In comparison, the ^{13}C microwave DNP spectrum of a $[1-^{13}\text{C}]$ pyruvate sample doped with the carbon-centered trityl OX063 free radical also shown in Fig. 2a has a separation $|P(+)-P(-)|$ that is roughly half that of galvinoxyl. These values reflect the size of their ESR linewidths. A ^{13}C polarization close to 10 % was obtained for 1.4 M $[1-^{13}\text{C}]$ sodium pyruvate sample in 1:1 (v/v) glycerol:water glassing matrix doped with 15 mM trityl OX063 at 3.35 T and 1.4 K. On the other hand, galvinoxyl ($D=250$ MHz) has a narrower ESR spectrum than 4-oxo TEMPO ($D=465$ MHz; see W band ESR data in the Supplementary Information). ^{13}C polarization values in the range of 5–10% were reported elsewhere [25, 26, 29–31] for ^{13}C samples doped with TEMPO radicals, whereas under similar DNP conditions we have obtained $5.9 \pm 0.3\%$ ^{13}C polarization for $[1-^{13}\text{C}]$ ethyl acetate sample doped with galvinoxyl as shown in Fig. 2. It should be noted that parameters such as the type of glassing matrix, lattice temperature, microwave power, and other factors that influence DNP performance [12, 13, 25] may be different in some polarizers, which may preclude direct comparison of polarization values obtained in different instruments. However, DNP of ^{13}C sodium pyruvate sample doped with 4-oxo-TEMPO performed in the same polarizer yielded $\sim 5.5\%$ [30], thus, we can say that galvinoxyl DNP performance is at least comparable with the results obtained with the nitroxide-based 4-oxo-TEMPO radical.

Another note is that although trityl OX063 is expected to be a better polarizing agent in DNP of low- γ nuclei such as ^{13}C via thermal mixing, it provides low DNP enhancements for high- γ nuclei such as protons using the same polarizer setup as shown in a previous work [32]. Thus, there are some cases in which free radicals with large D are advantageous. For instance, TEMPO was used to simultaneously polarize ^1H and ^{13}C spins in ^1H - ^{13}C DNP cross polarization (CP) transfer experiments [31, 33, 34] where ^1H , owing to its larger γ , yielded higher polarization and consequently led to significant improvements of ^{13}C DNP polarization after transfer at shorter microwave irradiation times. Our present DNP hardware is currently not equipped for such experiments, but it should be noted that the addition of galvinoxyl to the list of polarizing agents for dissolution DNP could offer such potential advantages.

Fig. 2b displays the polarization buildup time constants τ_{bu} across the ^{13}C microwave DNP spectrum. The τ_{bu} values were extracted by fitting the corresponding polarization buildup curves at each microwave frequency with a single exponential buildup equation $P(t) = P_0[1 - \exp(-t/\tau_{\text{bu}})]$ where P_0 is the maximum polarization achieved and t is the microwave irradiation time. It is interesting to note that τ_{bu} remains roughly constant in between the polarization peaks P(+) and P(-), while it increases as the microwave irradiation frequency is set outside this range. The large values of τ_{bu} further out in the DNP spectrum was also previously reported in TEMPO-doped ^{13}C samples at 94 GHz and 140 GHz [25] where the experimental plot of DNP spectrum is narrower than the ones predicted by the Borghini model. The increase in τ_{bu} 's on the wings of the DNP spectrum is attributed to the partial saturation of the ESR lineshape as the irradiation frequency is set further away from the center of the ESR lineshape [35]. Fig. 2c shows the polarization buildup curves of 1:1 (v/v) $[1-^{13}\text{C}]$ ethyl acetate: CHCl_3 doped with different concentrations of galvinoxyl free radical.

The maximum ^{13}C polarization approaching 6% is reached at a concentration of 40 mM. The faster ^{13}C polarization buildup (shorter τ_{bu}) with increasing galvinoxyl concentration shown in Fig. 2d is attributed to more paramagnetic centers polarizing the ^{13}C nuclear spins.

3.3 Hyperpolarized ^{13}C compounds in the liquid-state

Dissolution DNP has been very successful in amplifying the NMR signals of ^{13}C (spin-1/2, $\gamma=10.705$ MHz/T) spins for NMR spectroscopy and imaging where several thousand-fold signal enhancements are routinely achieved using trityl OX063 as the polarizing agent [4, 9–11]. Here we have demonstrated that large ^{13}C NMR signal enhancements could also be obtained with galvinoxyl free radical. Fig. 3 shows the hyperpolarized (HP) and thermal (TH) ^{13}C NMR signals of four model compounds that contain at least one ^{13}C located in carbonyl or carboxyl functionality. Such carbon nuclei are termed as “long T_1 carbons” since their spin-lattice relaxation time is usually very long (over 30 s) due to the absence of directly-bonded H. Although long T_1 values are considered a nuisance in conventional NMR, they are very useful in experiments with hyperpolarized compounds since the hyperpolarized state decays with T_1 relaxation. The HP spectrum of ^{13}C enriched $[1-^{13}\text{C}]$ ethyl acetate was measured 8 s after the dissolution and transfer of the frozen polarized sample (50 μL 1:1 $[1-^{13}\text{C}]$ ethyl acetate: CHCl_3 doped with 40 mM galvinoxyl) with 4 mL methanol. Owing to the relatively long T_1 (45 s) of ^{13}C in the C1 position of ethyl acetate, a substantial NMR signal enhancement ≈ 5500 -fold in the liquid state was obtained relative to the ^{13}C TH NMR signal. Fig. 3b shows that the hydrophobic galvinoxyl free radical could also be used in the dissolution DNP of water-soluble compounds such as $[^{13}\text{C}]$ urea where the ^{13}C NMR signal was enhanced 2800-fold in the liquid state. In this case, water was used as the dissolution solvent, and the water-insoluble galvinoxyl free radical naturally precipitated out from the dissolution liquid and could easily be removed by a simple filtration using a 0.2-micron syringe filter. UV-Vis spectroscopy confirmed the absence of the free radical in the solution after filtration (see results in the Supplementary Information). Fig. 3c and Fig. 3d illustrate that hyperpolarization with galvinoxyl is not limited to ^{13}C -labeled compounds but could facilitate the structural characterization of compounds containing ^{13}C at natural abundance (1.11 %). The HP and TH NMR spectra of unlabeled methyl pyruvate and diethyl-1,3-acetone-dicarboxylate are shown in Fig. 4b and Fig. 4c, respectively, where the ^{13}C spins present in the carbonyl and carboxyl groups were enhanced about 3000–4000-fold. Galvinoxyl, however, was not compatible with $[1-^{13}\text{C}]$ pyruvic acid; it appears to react with pyruvic acid as indicated by a color change from green to red.

3.4 Hyperpolarized ^{15}N compounds in the liquid-state

In addition to ^{13}C compounds, we have also polarized a number of ^{15}N (spin-1/2, $\gamma=4.3143$ MHz/T) substrates using galvinoxyl as the DNP polarizing agent. It is well established in previous works [12, 13, 27, 36] that in the thermal mixing regime, the locations of the optimum polarization peaks should be approximately the same for all low- γ nuclei because the shape of the microwave DNP spectrum depends mainly on the properties of the free radical polarizing agent. Thus, the ^{15}N compounds were polarized at the optimum positive polarization peak (94.10 GHz) determined for ^{13}C compounds. The ^{15}N NMR spectra in the liquid-state were referenced to nitrobenzene (^{15}N $\delta=372$ ppm with respect to anhydrous ammonia) [37]. Fig. 4 shows the HP and TH ^{15}N NMR signals of a) ^{15}N -enriched nitrobenzene b) ^{15}N natural abundance acetonitrile, and c) ^{15}N natural abundance pyridine measured at 298 K in a 9.4 T high resolution magnet. The T_1 of ^{15}N is generally longer than that of ^{13}C owing to its lower gyromagnetic ratio. Despite the low number of naturally abundant ^{15}N spins (natural abundance is only 0.36 %), we were able to detect the ^{15}N NMR spectra of acetonitrile and pyridine with 1 scan as shown in Fig. 4b and Fig. 4c. The thermal equilibrium NMR signal of neat ^{15}N natural abundance nitrobenzene was used for

the liquid-state NMR enhancement calculations of ^{15}N natural abundance compounds. The average ^{15}N NMR signal enhancement ϵ of acetonitrile was 4200-fold with a liquid-state T_1 of 110 s while the natural abundance pyridine sample yielded an average enhancement of 3300-fold. The T_1 of ^{15}N pyridine was slightly shorter (58 s) due to the proximity of ^{15}N to ^1H nuclei in the molecule.

4. CONCLUSION

In summary, we have demonstrated that the commercially available galvinoxyl free radical can be used as a polarizing agent for dissolution DNP-NMR characterization of a variety of compounds. The optimum concentration of galvinoxyl for DNP at 3.35 T and 1.4 K was found to be around 40 mM. Several-thousand-fold NMR signal enhancements were achieved in the liquid-state for ^{13}C - and ^{15}N -enriched compounds as well as for naturally abundant samples. Dissolution DNP with galvinoxyl is currently restricted to the glassing solvents in which this radical is soluble (e.g. CHCl_3 , ethyl acetate, acetone, or DMSO). The water-insoluble galvinoxyl can readily be filtered off from the dissolution liquid by mechanical filtration when water is used as the dissolution solvent. Certain optimization steps such as Gd^{3+} doping [12,30], solvent and sample deuteration [26,38], and lower operating temperatures of the polarizer [39] may be explored in the future to further increase the NMR signal enhancements of DNP samples doped with galvinoxyl.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work is supported by the National Institutes of Health (NIH) grant numbers R21EB009147 and NIBIB RR02584. The ESR measurements were done at the National High Magnetic Field Laboratory (NHMFL) which is supported by the National Science Foundation (NSF) through the Cooperative Agreement No. DMR-0654118, the State of Florida, and the U.S. Department of Energy (DOE). L.S. also acknowledges the support of NHMFL UCGP grant No. 5080.

REFERENCES

1. Webb A. Increasing the sensitivity of magnetic resonance spectroscopy and imaging. *Anal. Chem.* 2012; 84:9–16. [PubMed: 21978101]
2. Slichter, CP. *Principles of Magnetic Resonance*. New York: Springer; 1989.
3. Abragam, A. *Principles of Nuclear Magnetism*. England: Oxford University Press; 1961.
4. Ardenkjær-Larsen JH, Fridlund B, Gram A, Hansson G, Hansson L, Lerche MH, Servin R, Thaning M, Golman K. Increase in signal-to-noise ratio of >10,000 times in liquid-state NMR. *Proc. Natl. Acad. Sci. U.S.A.* 2003; 100:10158–10163. [PubMed: 12930897]
5. Abragam A, Goldman M. Principles of dynamic nuclear polarization. *Rep. Prog. Phys.* 1978; 41:395–467.
6. de Boer W. Dynamic orientation of nuclei at low temperatures. *J. Low Temp. Phys.* 1976; 22:185–212.
7. Crabb DG, Meyer W. Solid polarized targets for nuclear and particle physics experiments. *Ann. Rev. Nucl. Part. Sci.* 1997; 47:67–109.
8. Gunther, U. *Top. Curr. Chem.* Berlin/Heidelberg: Springer-Verlag ; 2012. *Dynamic Nuclear Hyperpolarization in Liquids*; p. 1-47.
9. Gallagher FA, Kettunen MI, Brindle KM. Biomedical applications of hyperpolarized ^{13}C magnetic resonance imaging. *Prog. Nucl. Magn. Reson. Spectrosc.* 2009; 55:285–295.
10. Kurhanewicz J, Vigneron DB, Brindle K, Chekmenev EY, Comment A, Cunningham CH, DeBerardinis RJ, Green GG, Leach MO, Rajan SS, Rizi RR, Ross BD, Warren WS, Malloy CR.

Analysis of cancer metabolism by imaging hyperpolarized nuclei: prospects for translation to clinical research. *Neoplasia*. 2011; 13:81–97. [PubMed: 21403835]

11. Brindle KM, Bohndiek SE, Gallagher FA, Kettunen MI. Tumor imaging using hyperpolarized ^{13}C magnetic resonance spectroscopy. *Magn. Reson. Med*. 2011; 66:505–519. [PubMed: 21661043]
12. Lumata L, Jindal AK, Merritt ME, Malloy CR, Sherry AD, Kovacs Z. DNP by thermal mixing under optimized conditions yields >60000-fold enhancement of ^{89}Y NMR signal. *J. Am. Chem. Soc.* 2011; 133:8673–8680. [PubMed: 21539398]
13. Heckmann J, Meyer W, Radtke E, Reicherz G, Goertz S. Electron spin resonance and its implication on the maximum nuclear polarization of deuterated solid target materials. *Phys. Rev. B*. 2006; 74:134418.
14. Lumata L, Ratnakar SJ, Jindal A, Merritt M, Malloy C, Sherry AD, Kovacs Z. BDPA: an efficient polarizing agent for fast dissolution dynamic nuclear polarization NMR spectroscopy. *Chem. Eur. J.* 2011; 17:10825–10827. [PubMed: 21919088]
15. Gabellieri C, Mugnaini V, Paniagua JC, Roques N, Oliveros M, Feliz M, Veciana J, Pons M. Dynamic nuclear polarization with polychlorotriphenylmethyl radicals: supramolecular polarization-transfer effects. *Angew. Chem. Int. Ed.* 2010; 49:3360–3362.
16. Paniagua JC, Mugnaini V, Gabellieri C, Feliz M, Roques N, Veciana J, Pons M. Polychlorinated trityl radicals for dynamic nuclear polarization: the role of chlorine nuclei. *Phys. Chem. Chem. Phys.* 2010; 12:5824–5829. [PubMed: 20454741]
17. Macholl S, Johannesson H, Ardenkjaer-Larsen JH. Trityl biradicals and ^{13}C dynamic nuclear polarization. *Phys. Chem. Chem. Phys.* 2010; 12:5804–5817. [PubMed: 20458385]
18. Coppinger GM. A stable phenoxy radical inert to oxygen. *J. Am. Chem. Soc.* 1957; 79:501–502.
19. Barzegar A, Moosavi-Movahedi AA. Intracellular ROS protection efficiency and free radical-scavenging activity of curcumin. *PLoS ONE*. 2011; 6:e26012. [PubMed: 22016801]
20. Guo Q, Rimbach G, Moini H, Weber S, Packer L. ESR and cell culture studies on free radical-scavenging and antioxidant activities of isoflavonoids. *Toxicology*. 2002; 179:171–180. [PubMed: 12204553]
21. Kaptein R, Dijkstra K, Tarr CE. A. single-scan Fourier transform method for measuring spinlattice relaxation times. *J. Magn. Reson.* 1976; 24:295–300.
22. Patyal BR, Gao JH, Williams RF, Roby J, Saam B, Rockwell BA, Thomas RJ, Stolarski DJ, Fox PT. Longitudinal relaxation and diffusion measurements using magnetic resonance signals from laser-hyperpolarized ^{129}Xe nuclei. *J. Magn. Reson.* 1997; 26:58–65. [PubMed: 9177796]
23. Bresgunov AY, Dubinsky AA, Poluektov OG, Lebedev YS, Prokofev AI. The structure of phenoxyl radicals as studied by 2 mm band ESR. *Mol. Phys.* 1992; 75:1123–1131.
24. Ardenkjaer-Larsen JH, Macholl S, Johannesson H. Dynamic nuclear polarization with trityls at 1.2 K. *Appl. Magn. Reson.* 2008; 34:509–522.
25. Jannin S, Comment A, Kurdzesau F, Konter JA, Hautle P, Van der Brandt B, van der Klink JJ. A 140 GHz prepolarizer for dissolution dynamic nuclear polarization. *J. Chem. Phys.* 2008; 128:241102. [PubMed: 18601309]
26. Kurdzesau F, van den Brandt B, Comment A, Hautle P, Jannin S, van der Klink JJ, Konter JA. Dynamic nuclear polarization of small labelled molecules in frozen water–alcohol Solutions. *J. Phys. D: Appl. Phys.* 2008; 41:155506.
27. Lumata L, Merritt M, Malloy C, Sherry AD, Kovacs Z. Fast dissolution dynamic nuclear polarization NMR of ^{13}C -enriched ^{89}Y -DOTA complex: experimental and theoretical considerations. *Appl. Magn. Reson.* 2012; 43:69–79.
28. Borghini M. Spin-temperature model of nuclear dynamic polarization using free radicals. *Phys. Rev. Lett.* 1968; 20:419–421.
29. Comment A, van den Brandt B, Uffmann K, Kurdzesau F, Jannin S, Konter JA, Hautle P, Wenckebach WT, Gruetter R, van der Klink JJ. Design and performance of a DNP prepolarizer coupled to a rodent MRI scanner. *Concepts Magn. Reson., Part B*. 2007; 31:255–269.
30. Lumata L, Merritt ME, Malloy CR, Sherry AD, Kovacs Z. Impact of Gd^{3+} on DNP of $1\text{-}^{13}\text{C}$.pyruvate doped with trityl OX063, BDPA, or 4-oxo-TEMPO. *J. Phys. Chem.* 2012; A 116:5129–5138.

31. Jannin S, Bornet A, Colombo S, Bodenhausen G. Low-temperature cross polarization in view of enhancing dissolution Dynamic Nuclear Polarization in NMR. *Chem. Phys. Lett.* 2011; 517:234–236.
32. Wolber J, Ellner F, Fridlund B, Gram A, Johannesson H, Hansson G, Hansson LH, Lerche MH, Mansson S, Servin R, Thaning M, Golman K, Ardenkjaer-Larsen JH. Generating highly polarized nuclear spins in solution using dynamic nuclear polarization. *Nucl. Instrum. Methods Phys. Res. A.* 2004; 526:173–181.
33. Bornet A, Melzi R, Jannin S, Bodenhausen G. Cross polarization for dissolution dynamic nuclear polarization experiments at readily accessible temperatures $1.2 < T < 4.2$ K. *Appl. Magn. Reson.* 2012; 43:107–117.
34. Jannin S, Bornet A, Melzi R, Bodenhausen G. High field dynamic nuclear polarization at 6.7 T: carbon-13 polarization above 70% within 20 min. *Chem. Phys. Lett.* 2012; 549:99–102.
35. Jannin S, Comment A, van der Klink JJ. Dynamic nuclear polarization by thermal mixing under partial saturation. *Appl. Magn. Reson.* 2012; 43:59–68.
36. Reynolds S, Patel H. Monitoring the solid-state polarization of ^{13}C , ^{15}N , ^2H , ^{29}Si , and ^{31}P . *Appl. Magn. Reson.* 2008; 34:495–508.
37. Lambert JB, Binsch G, Roberts JD. Nitrogen-15 magnetic resonance spectroscopy, I. chemical shifts. *Proc. Natl. Acad. Sci. U.S.A.* 1964; 51:735–737. [PubMed: 14172986]
38. Ludwig C, Marin-Montesinos I, Saunders MG, Gunther UL. Optimizing the polarization matrix for ex situ dynamic nuclear polarization. *J. Am. Chem. Soc.* 2010; 132:2508–2509. [PubMed: 20131776]
39. Meyer W, Heckmann J, Hess C, Radtke E, Reicherz G, Triebwasser L, Wang L. Dynamic polarization of ^{13}C nuclei in solid ^{13}C labeled pyruvic acid. *Nucl. Instrum. Methods Phys. Res. A.* 2011; 631:1–5.

Highlights

- Galvinoxyl was tested in ^{13}C and ^{15}N dissolution dynamic nuclear polarization (DNP) NMR.
- Several-thousand-fold ^{13}C and ^{15}N NMR signal enhancements were achieved.
- The water-insoluble galvinoxyl can be filtered off from aqueous dissolution liquids.

Fig. 1.
(a) Structure of galvinoxyl free radical. (b) Field-swept ESR spectrum of 40 mM galvinoxyl in 1:1 (v/v) CHCl_3 :DMSO glassing matrix measured at 100 K in a W-band ESR spectrometer.

Fig. 2.

(a) ^{13}C microwave DNP spectrum of 1:1 (v/v) $[1-^{13}\text{C}]$ ethyl acetate: CHCl_3 doped with 40 mM galvinoxyl (solid circles) at 3.35 T and 1.4 K. The up $[P(+)=94.10 \text{ GHz}]$ and down $[P(-)=94.255 \text{ GHz}]$ arrows represent the positive and negative polarization peaks, respectively. The ^{13}C microwave DNP spectrum of 1.4 M $[1-^{13}\text{C}]$ sodium pyruvate in 1:1 (v/v) glycerol:water doped with 15 mM trityl OX063 (open squares) is also shown for comparison. (b) Polarization buildup time constants τ_{bu} taken at different microwave irradiation frequencies. The error bars are due to fits with a mono-exponential buildup equation. (c) ^{13}C polarization buildup curves of 1:1 (v/v) $[1-^{13}\text{C}]$ ethyl acetate: CHCl_3 doped with different concentrations of galvinoxyl. These data were taken at 3.35 T and 1.4 K using a 100 mW microwave source. The dashed lines are fits to a mono-exponential buildup equation. (d) The corresponding polarization buildup time constants extracted by fitting the data in (c). The error bars are due to fits with a mono-exponential buildup equation.

Fig. 3.

(a) Hyperpolarized (HP; $\theta_{\text{flip}}=0.5^\circ$) and thermal (TH; $\theta_{\text{flip}}=90^\circ$, 1 scan) ^{13}C NMR spectra of $[1\text{-}^{13}\text{C}]$ ethyl acetate (64 mM in MeOH at 298 K and 9.4 T). The average ($N=3$) ^{13}C NMR signal enhancement ϵ is 5500 ± 800 and the liquid-state T_1 is 45 s. (b) HP ($\theta_{\text{flip}}=2^\circ$) and TH ($\theta_{\text{flip}}=90^\circ$, 1 scan) NMR spectra of $[^{13}\text{C}]$ urea (23 mM in H_2O at 298 K and 9.4 T); average $\epsilon=2800 \pm 450$ ($T_1=45$ s). (c) HP ($\theta_{\text{flip}}=90^\circ$) and TH ($\theta_{\text{flip}}=90^\circ$, 16 scans) NMR spectra of ^{13}C natural abundance methyl pyruvate (138 mM in MeOH at 298 K and 9.4 T); average C1 $\epsilon=4300 \pm 930$ ($T_1=42$ s), C3 $\epsilon=4100 \pm 710$ ($T_1=44$ s). (d) HP ($\theta_{\text{flip}}=90^\circ$) and TH ($\theta_{\text{flip}}=90^\circ$, 16 scans) NMR spectra of ^{13}C natural abundance diethyl-1,3-acetone-dicarboxylate (68 mM in MeOH at 298 K and 9.4 T); average C1 $\epsilon=3400 \pm 820$ ($T_1=32$ s), C3 $\epsilon=3700 \pm 760$ ($T_1=34$ s).

Fig. 4.

(a) Hyperpolarized (HP; $\theta_{\text{flip}}=5^\circ$) and thermal (TH; $\theta_{\text{flip}}=90^\circ$, 1 scan) NMR signals of ^{15}N -enriched nitrobenzene (61 mM in MeOH) at 298 K and 9.4 T. The average ^{15}N NMR signal enhancement ($N=3$) is 4800 ± 540 and the liquid-state T_1 is 135 s. (b) HP ($\theta_{\text{flip}}=90^\circ$) and TH ($\theta_{\text{flip}}=90^\circ$, 1 scan) NMR spectra of ^{15}N natural abundance acetonitrile (239 mM in MeOH) at 298 K and 9.4 T.; average $\epsilon=4200 \pm 730$ ($T_1=110$ s). (c) HP ($\theta_{\text{flip}}=90^\circ$) and TH ($\theta_{\text{flip}}=90^\circ$, 1 scan) NMR spectra of ^{15}N natural abundance pyridine (155 mM in H_2O) at 298 K and 9.4 T.; average $\epsilon=3300 \pm 770$ ($T_1=58$ s). The coupling of ^{15}N to protons is also visible [$J(^1\text{H}-^{15}\text{N})=9.7$ Hz]. The ^{15}N NMR spectra were referenced to nitrobenzene (^{15}N $\delta=372$ ppm with respect to anhydrous ammonia).