Journal of Magnetic Resonance 213 (2011) 410–412

Contents lists available at SciVerse ScienceDirect

Journal of Magnetic Resonance

journal homepage: www.elsevier.com/locate/jmr

Historical Perspective

Dynamic nuclear polarization at 9T using a novel 250 Gyrotron microwave source

Robert G. Griffin *

Massachusetts Institute of Technology, Department of Chemistry and Francis Bitter Magnet Laboratory NW14-3220, MIT, 170 Albany Street, Cambridge, MA 02139, United States

ARTICLE INFO

Keywords:
Dynamic nuclear polarization
Magic angle spinning
Gyrotron
Microwaves

ABSTRACT

In the 1990's we initiated development of high frequency gyrotron microwave sources with the goal of performing dynamic nuclear polarization at magnetic fields (~5–23 T) used in contemporary NMR experiments. This article describes the motivation for these efforts and the developments that led to the operation of a gyrotron source for DNP operating at 250 GHz. We also mention results obtained with this instrument that would have been otherwise impossible absent the increased sensitivity. Finally, we describe recent efforts that have extended DNP to 460 GHz and 700 MHz ^{1}H frequencies.

Interview with the author(s).

A video interview with the author(s) associated with this Historical Perspective and the original article can be found in the online version, at doi:10.1016/j.jmr.2011.08.015.

In the early 1980s the combination of cross polarization (CP) [1] and magic angle spinning (MAS) [2] began to be used to examine biological systems, for example membranes and membrane proteins [3,4]. Later, in the 1990s, the first studies of amyloid appeared [5,6]. In addition, two-dimensional MAS experiments were developed [7–9], and we began to appreciate the fact that it was possible to reintroduce dipolar couplings into the spectra in a manner consistent with the goal of high resolution [10,11]. These developments subsequently lead to rotational resonance (R^2) [12], rotary resonance (R^3) [13], REDOR [14] and a plethora of approaches to measure ^{13}C—^{13}C and ^{13}C—^{15}N distances and torsion angles, and what today is known as dipolar recoupling and to structures of peptides [15] and proteins [16]. However, from the beginning of these experiments, which were designed to elucidate the structure of biological molecules, it was clear that the signal-to-noise was lower than those performed in solution. The reason for this was well understood and is because in most MAS experiments we detect ^{13}C, ^{15}N, etc. directly rather than observing these nuclei through ^{1}H [17]. For this reason we began to consider approaches to increase the sensitivity of MAS experiments. At the time our thought was that if ^{1}H could be incorporated into the experiment for indirect detection, then so much the better. Accordingly, we initiated experiments using ^{2}H labeled samples with ~1% ^{1}H and showed that even at 10 kHz we were able to obtain reasonably narrow lines, and study exchange processes in solids [18]. These experiments have continued in labs of our colleagues [19,20], and today the availability of 40–70 kHz MAS rotors and perdeuterated proteins, together with ^{1}H back exchange of amide ^{1}Hs is making HSQC type experiments a reality. Nevertheless, there remains a considerable loss in sensitivity when compared to ^{1}H detected solution experiments.

Because of my own Ph.D. research in EPR, I have long been aware of the fact that it is in principle possible to introduce stable free radicals into solutions, to irradiate the EPR spectrum with microwaves, and therefore to transfer the much higher electron spin polarization to nuclei. This experiment, first predicted by Overhauser [21] and demonstrated by Carver and Slichter [22], is known as dynamic nuclear polarization (DNP) and we started to consider the possibility of integrating DNP into MAS NMR experiments in the late 1970s and early 1980s. In addition, during this period MAS and other NMR experiments were rapidly transitioning from electromagnets to superconducting systems at ^{1}H operating frequencies of 200–600 MHz. Today, contemporary NMR experiments are performed at these or the higher frequencies currently emerging –700–1000 + MHz. To perform DNP with g = 2 electrons at these fields/^{1}H frequencies requires microwaves in the millimeter wave regime – roughly 2.3–0.75 mm – or 130–400 GHz. Thus, while it was very intriguing to think about achieving large signal enhancements with DNP ($\varepsilon \geqslant 100$), it was also clear that the barrier to wide applicability of these experiments was microwave sources operating stably in the millimeter wave regime. If these were available and we were able to perform signal enhancements, the MAS dipolar recoupling experiments would be widely applica-

* Fax: +1 617 253 5405.
E-mail address: rgg@mit.edu

1090-7807/$ - see front matter © 2011 Published by Elsevier Inc.
doi:10.1016/j.jmr.2011.08.015

ble to structural studies of proteins, nucleic acids and many other problems including imaging.

Accordingly, in the late 1970s we began to investigate the available microwave sources and found that there were diodes (Gunn and Impatt diodes), devices based on slow wave structures (extended interaction oscillators and amplifiers, EIO's and EIA's), and a variety of other devices all with names ending in "tron" – klystrons, oratrons, etc., and gyrotrons [23]. With some exceptions they operated at $\leqslant$100 GHz. In addition, it was also clear that we would need a substantial amount of microwave power (10–100 W) since the experiment we clearly wanted to perform was DNP/MAS and that meant that the sample would consist of a rotor with an rf coil wrapped around it. Thus, the prospects of achieving a high-Q in the microwave circuit were small. It is for these and similar reasons, that the first efforts at performing DNP/MAS were limited to 60 MHz and used 10 W, 40 GHz klystrons as the microwave source and a horn and reflector type arrangement [24–27]. However, it was not possible to move these experiments to the fields used in contemporary NMR experiments because of the paucity of higher frequency microwave sources. Thus, during the 1980s DNP relaxed into a position of an interesting intellectual exercise, but one whose potential had yet to be realized.

In the mid 1980s the chronic need for increase signal intensities for multidimensional experiments stimulated us to think seriously about possible microwave sources for DNP. We found that not much had changed – for example, there were Gunn and IMPATT diodes available, and we could purchase EIO's that operated at 220 GHz from Varian-Canada that would deliver 1 W CW and was guaranteed to operate for 500 h (a rather modest period considering that we would want this to perform NMR experiments on a 24/7/52 basis). We also discovered that our colleagues in the lab across the street in the MIT Plasma Science and Fusion Center were working with gyrotrons and they were functioning in what was termed CW mode at 140 GHz. In fact, Paul Woskow and Richard Temkin has just completed a plasma physics experiment and had a 140 GHz tube that was not being used. We also learned that gyrotrons could be operated at higher frequencies, although not much work had been done above about 100 GHz, and furthermore that they were fast wave devices, and, consequently can generate large amounts of CW power. Thus, gyrotrons satisfied many of the requirements for MAS/DNP experiments at high frequencies. Concurrently, David Singel, who had perform 60 MHz DNP experiments with Nino Yannoni at IBM, arrived in the Chemistry Department at Harvard as an Assistant Professor and was also interested in continuing DNP. Thus, this series of events catalyzed the start of a new effort to initiate DNP at higher frequencies. It culminated in the 1986 submission of a successful grant application to NIH to construct a DNP spectrometer operating at 140 GHz or 211 MHz for ^{1}H. In addition, since so little was known about high field EPR spectra it was decided to also construct an EPR spectrometer to operate at the same frequency. We note that this grant was funded without any preliminary results and without a clearly demonstrated application – something that would be unlikely in today's funding climate.

Thus, our initial effort at DNP/MAS was focused on a 140 GHz instrument and our first papers appeared a few years later with a study using a borrowed EIO on a 1D conductor. Later, Lino Becerra and Gary Gerfen managed to get the gyrotron functioning and to enhance ^{13}C MAS spectra of polymers doped with BDPA [28]. In 1995 we reported our first low temperature experiments using TEMPO as a polarizing agent where we achieved and enhancement $\varepsilon = 185$ at 20 K [29], and we managed to polarize a protein [30], virus particles [31], and perform some 2D experiments [32]. However, as we stated above our ultimate idea for DNP was that it would be a signal enhancement technique applicable to problems in structural biology and therefore we were motivated to move the experiments to higher field. In addition, there were many things technically incorrect with the 140 GHz/ 211 MHz system that made it difficult to operate in a CW basis. For example, the vacuum system on the tube was poor, and the cathode would poison easily. In addition, our low temperature probes, while advanced for the time, lacked many important features such as sample exchange systems operating at low temperature. Thus, beginning in late 1990s we initiated an effort to address some of these issues and to move DNP to higher frequencies. The first step in the process was to construct a system that would operate at approximately double the frequency or 400 MHz for ^{1}H. In addition, we wanted a spectrometer that would be stable and permit long term dipole recoupling experiments useful for structural biology. The result was the 250 GHz gyrotron and a low temperature MAS probe that would spin stably at $\sim$100 K for extended periods. The initial results with this system are described in the paper by Bajaj et al. [33], which is the one that triggered the present writeup. In particular, we showed there that at 20 K it was possible to obtain $\varepsilon = 170$ on static glycine sample and we were able to perform the initial double quantum DNP enhanced MAS ^{13}C—^{13}C recoupling experiments using the SPC-5 sequence.

Subsequently, this spectrometer was improved in many ways. For example, we added a corrugated waveguide [34], the probe was reconstructed for triple resonance experiments and includes a sample exchange system [35]. These improvements permitted us to trap photocycle intermediates of bacteriorhodopsin and to detect four L-intermediates that had never been observed previously [36,37]. A complete description of the gyrotron oscillator appeared in a subsequent JMR paper [38]. In addition, the success with the 250 GHz system stimulated work on higher frequency DNP gyrotrons and we have successfully constructed devices at 330 GHz [39]and 460 GHz [40,41]}. The latter corresponds to a 700 MHz ^{1}H frequency and we have recently obtained our initial results with this system. In addition, solid state spectrometers are now commercially available which utilize gyrotrons for DNP. Collectively, these results suggest that high frequency DNP will likely be an important ingredient of MAS and other types of NMR and imaging experiments in the future.

References

[1] A. Pines, M.G. Gibby, J.S. Waugh, Proton-enhanced NMR of dilute spins in solids, J. Chem. Phys. 59 (1973) 569–590.
[2] J. Schaefer, E.O. Stejskal, ^{13}C nuclear magnetic resonance of polymers spinning at the magic angle, J. Am. Chem. Soc. 98 (1976) 1031–1032.
[3] R. Haberkorn, J. Herzfeld, R.G. Griffin, High-resolution P-31 and C-13 NMR-spectra of unsonicated model membranes, Biophys. J. 21 (3) (1978). A204–A204.
[4] G.S. Harbison, J. Herzfeld, R.G. Griffin, Solid-state ^{15}N nuclear magnetic-resonance study of the schiff-base in bacteriorhodopsin, Biochemistry 22 (1) (1983) 1–5.
[5] R.G.S. Spencer, K.J. Halverson, M. Auger, A.E. McDermott, R.G. Griffin, P.T. Lansbury, An unusual peptide conformation may precipitate amyloid formation in alzheimers-disease – application of solid-state NMR to the determination of protein secondary structure, Biochemistry 30 (43) (1991) 10382–10387.
[6] P.T. Lansbury, P.R. Costa, J.M. Griffiths, E.J. Simon, M. Auger, K.J. Halverson, D.A. Kocisko, Z.S. Hendsch, T.T. Ashburn, R.G.S. Spencer, B. Tidor, R.G. Griffin, Structural model for the b amyloid fibril: interstrand alignment of an antiparallel b sheet comprising a C-terminal peptide, Nat. Struct. Biol. 2 (1995) 990–997.
[7] W.P. Aue, D.J. Ruben, R.G. Griffin, Two-dimensional rotational spin-echo nmr in solids – resolution of isotropic and anisotropic chemical-shifts, J. Magn. Reson. 43 (3) (1981) 472–477.
[8] W.P. Aue, D.J. Ruben, R.G. Griffin, Uniform chemical-shift scaling in rotating solids, J. Magn. Reson. 46 (2) (1982) 354–357.
[9] J.E. Roberts, S. Vega, R.G. Griffin, Two-dimensional heteronuclear chemical-shift correlation spectroscopy in rotating solids, J. Am. Chem. Soc. 106 (9) (1984) 2506–2512.
[10] M.G. Munowitz, R.G. Griffin, Two-dimensional nuclear magnetic-resonance in rotating solids – an analysis of line-shapes in chemical shift-dipolar spectra, J. Chem. Phys. 76 (6) (1982) 2848–2858.

[11] M.G. Munowitz, R.G. Griffin, G. Bodenhausen, T.H. Huang, Two-dimensional rotational spin-echo nuclear magnetic resonance in solids: correlation of chemical shift and dipolar interactions, J. Am. Chem. Soc. 103 (1981) 2529.
[12] D.P. Raleigh, M.H. Levitt, R.G. Griffin, Rotational resonance in solid state NMR, Chem. Phys. Lett. 146 (1988) 71–76.
[13] T.G. Oas, R.G. Griffin, M.H. Levitt, Rotary resonance recoupling of dipolar interactions in solid-state nuclear magnetic-resonance spectroscopy, J. Chem. Phys. 89 (2) (1988) 692–695.
[14] T. Gullion, J. Schaefer, Rotational-echo double-resonance NMR, J. Magn. Reson. 81 (1989) 196–200.
[15] C.M. Rienstra, L. Tucker-Kellogg, C.P. Jaroniec, M. Hohwy, B. Reif, M.T. McMahon, B. Tidor, T. Lozano-Perez, R.G. Griffin, De novo determination of peptide structure with solid-state magic-angle spinning NMR spectroscopy, Proc. Natl. Acad. Sci. USA 99 (16) (2002) 10260–10265.
[16] F. Castellani, B. van Rossum, A. Diehl, M. Schubert, K. Rehbein, H. Oschkinat, Structure of a protein determined by solid-state magic-angle-spinning NMR spectroscopy, Nature 420 (6911) (2002) 98–102.
[17] G. Bodenhausen, D.J. Ruben, Natural abundance N-15 NMR by enhanced heteronuclear spectroscopy, Chem. Phys. Lett. 69 (1) (1980) 185–189.
[18] L. Zheng, K.W. Fishbein, R.G. Griffin, J. Herzfeld, 2-Dimensional solid-state H-1-NMR and proton-exchange, J. Am. Chem. Soc. 115 (14) (1993) 6254–6261.
[19] B. Reif, R.G. Griffin, 1H detected 1H,15N correlation spectroscopy in rotating solids, J. Magn. Reson. 160 (2003) 78–83.
[20] D.H. Zhou, D.T. Graesser, W.T. Franks, C.M. Rienstra, Sensitivity and resolution in proton solid-state NMR at intermediate deuteration levels: quantitative linewidth characterization and applications to correlation spectroscopy, J. Magn. Reson. 178 (2) (2006) 297–307.
[21] A.W. Overhauser, Polarization of nuclei in metals, Phys. Rev. 92 (1953) 411–415.
[22] T.R. Carver, C.P. Slichter, Polarization of nuclear spins in metals, Phys. Rev. 92 (1953) 212–213.
[23] K.L. Felch, B.G. Danly, H.R. Jory, K.E. Kreischer, W. Lawson, B. Levush, R.J. Temkin, Characteristics and applications of fast-wave gyrodevices, Proc. IEEE 87 (1999) 752–781.
[24] R.A. Wind, M.J. Duijvestijn, C. Vanderlugt, A. Manenschijn, J. Vriend, Applications of dynamic nuclear-polarization in C-13 NMR in solids, Prog. Nucl. Magn. Reson. Spectrosc. 17 (1985) 33–67.
[25] M. Afeworki, R.A. Mckay, J. Schaefer, Selective observation of the interface of heterogeneous polycarbonate polystyrene blends by dynamic nuclear-polarization C-13 NMR-spectroscopy, Macromolecules 25 (16) (1992) 4084–4091.
[26] M. Afeworki, S. Vega, J. Schaefer, Direct electron-to-carbon polarization transfer in homgeneously-doped polycarbonates, Macromolecules 25 (1992) 4100–4106.
[27] D.J. Singel, H. Seidel, R.D. Kendrick, C.S. Yannoni, A spectrometer for EPR, DNP, and multinuclear high-resolution NMR, J. Magn. Reson. 81 (1989) 145–161.
[28] L.R. Becerra, G.J. Gerfen, R.J. Temkin, D.J. Singel, R.G. Griffin, Dynamic nuclear polarization with a cyclotron resonance maser at 5 T, Phys. Rev. Lett. 71 (21) (1993) 3561–3564.
[29] G.J. Gerfen, L.R. Becerra, D.A. Hall, R.G. Griffin, R.J. Temkin, D.J. Singel, High-frequency (140 GHz) dynamic nuclear-polarization – polarization transfer to a solute in frozen aqueous-solution, J. Chem. Phys. 102 (24) (1995) 9494–9497.
[30] D.A. Hall, D.C. Maus, G.J. Gerfen, S.J. Inati, L.R. Becerra, F.W. Dahlquist, R.G. Griffin, Polarization-enhanced NMR spectroscopy of biomolecules in frozen solution, Science 276 (1997) 930–932.
[31] M. Rosay, A.C. Zeri, N.S. Astrof, S.J. Opella, J. Herzfeld, R.G. Griffin, Sensitivity-enhanced NMR of biological solids: dynamic nuclear polarization of Y21M fd bacteriophage and purple membrane, J. Am. Chem. Soc. 123 (5) (2001) 1010–1011.
[32] M. Rosay, V. Weis, K.E. Kreischer, R.J. Temkin, R.G. Griffin, Two-dimensional ^{13}C—^{13}C correlation spectroscopy with magic angle spinning and dynamic nuclear polarization, J. Am. Chem. Soc. 124 (13) (2002) 3214–3215.
[33] V.S. Bajaj, C.T. Farrar, M.K. Hornstein, I. Mastovsky, J. Vieregg, J. Bryant, B. Elena, K.E. Kreischer, R.J. Temkin, R.G. Griffin, Dynamic nuclear polarization at 9T using a novel 250 GHz gyrotron microwave source, J. Magn. Reson. 160 (2003) 85–90.
[34] P.W. Woskov, V.S. Bajaj, M.K. Hornstein, R.J. Temkin, R.G. Griffin, Corrugated waveguide and directional coupler for CW 250 GHz gyrotron DNP experiments, IEEE Trans. Microwave Theory Tech. 53 (2005) 1863–1869.
[35] A.B. Barnes, M.L. Mak-Jurkauskas, Y. Matsuki, V.S. Bajaj, P.C.A.v.d. Wel, R. DeRocher, J. Bryant, J.R. Sirigiri, R.J. Temkin, J. Lugtenburg, J. Herzfeld, R.G. Griffin, Cryogenic sample exchange NMR probe for magic angle spinning dynamic nuclear polarization, J. Magn. Reson. 198 (2009) 261–270.
[36] M.L. Mak-Jurkauskas, V.S. Bajaj, M.K. Hornstein, M. Belenky, R.G. Griffin, J. Herzfeld, Gradual winding of the bacteriorhodopsin chromophore in the first half of its ion-motive photocycle: a dynamic nuclear polarization-enhanced solid state NMR study, Proc. Natl. Acad. Sci. 105 (2008) 883–888.
[37] V.S. Bajaj, M.L. Mak-Jurkauskas, M. Belenky, J. Herzfeld, R.G. Griffin, Functional and shunt states of bacteriorhodopsin resolved by 250-GHz dynamic nuclear polarization-enhanced solid-state NMR, Proc. Natl. Acad. Sci. 106 (2009) 9244–9249.
[38] V.S. Bajaj, M.K. Hornstein, K.E. Kreischer, J.R. Sirigiri, P.P. Woskov, M.L. Mak-Jurkauskas, J. Herzfeld, R.J. Temkin, R.G. Griffin, 250 GHz CW gyrotron oscillator for dynamic nuclear polarization in biological solid state NMR, J. Magn. Reson. 189 (2) (2007) 251–279.
[39] A.C. Torrezan, S.T. Han, M.A. Shapiro, J.R. Sirigiri, R.J. Temkin. CW operation of a tunable 330/460 GHz gyrotron for DNP enhanced nuclear magnetic resonance, in: 33rd International Conference on Infrared, Millimeter and Terahertz Waves, Pasadena, CA, 2008.
[40] M.K. Hornstein, V.S. Bajaj, R.G. Griffin, K.E. Kreischer, I. Mastovsky, M.A. Shapiro, J.R. Sirigiri, R.J. Temkin, Second harmonic operation at 460 GHz and broadband continuous frequency tuning of a gyrotron oscillator, IEEE Trans. Electron Dev. 52 (5) (2005) 798–807.
[41] A.C. Torrezan, S.-T. Han, I. Mastovsky, M.A. Shapiro, J.R. Sirigiri, R.J. Temkin, A.B. Barnes, R.G. Griffin, Continuous-wave operation of a frequency tunable 460 GHz second-harmonic gyrotron for enhanced nuclear magnetic resonance, IEEE Trans. Plasma Sci. 38 (2010) 1150–1159.