JOURNAL OF MAGNETIC RESONANCE, Series A 102, 375–377 (1993)

A Sample-Shuttle Nuclear-Magnetic-Relaxation-Dispersion Spectrometer

SCOTT D. SWANSON*,† AND SCOTT D. KENNEDY ‡

*Department of Radiology, The University of Michigan, Ann Arbor, Michigan 48109; and ‡Department of Biophysics, University of Rochester, Rochester, New York 14642

Received February 12, 1993

Nuclear-magnetic-relaxation-dispersion spectrometers (NMRD) provide a measure of the spin–lattice relaxation rate of a nucleus as a function of the Larmor frequency (*1*). NMRD profiles contain information about the molecular dynamics of small complexes in solution, electron relaxation properties in paramagnetic systems (*2*), polymer dynamics (*3*), and magnetic cross relaxation in heterogeneous systems (*4*). In more complicated systems such as biological tissue, NMRD profiles help elucidate relaxation mechanisms (*5*). NMRD profiles are also important for the development of contrast agents for medical applications of diagnostic MRI (*6*).

The current NMRD hardware involves cycling the magnetic field by changing the current in a resistive electromagnet (*7–9*). Field cycling NMRD (FCNMRD) provides magnetic field switching rates on the order of 2 MHz/ms and measures relaxation rates up to 200 s^{-1} with Larmor frequencies of 10 kHz to 60 MHz. The instrumental apparatus is, however, rather elaborate and available in a limited number of laboratories throughout the world.

This Communication describes the development of a prototype sample-shuttle NMRD (SSNMRD) spectrometer. Sample shuttling has previously been used in magnetic resonance to measure low-field spin–lattice relaxation rates with the high sensitivity available in a large magnetic field (*10–12*) and to perform zero-field NMR and NQR with high sensitivity by moving the sample from a high-field region to zero field, where the Zeeman Hamiltonian vanishes and the internal Hamiltonians dominate (*13–15*). The SSNMRD spectrometer is conceptually outlined in Fig. 1 and discussed in the legend. This spectrometer uses sample shuttling and the axially inhomogeneous, peripheral magnetic field in the z direction of a superconducting solenoid. A spatially homogeneous sample moves back and forth between the high-field, homogeneous, isocenter of the magnet and a low-field, inhomogeneous, peripheral magnetic field. When the sample is in the inhomogeneous peripheral magnetic field the spins relax to new equilibrium magnetizations at all field strengths across the sample. The relaxation information is spatially encoded in the sample and measured by 1D imaging when the sample is transported back to the high-field, homogeneous region of the magnet. The Larmor frequency resolution of the experiment is determined by the spatial resolution of the image.

The prototype spectrometer was built around a four-way, solenoid-actuated valve (Automatic Switch Co., New Jersey, Part 8340-A-1) which diverts pressure either to port A or to port B and moves the sample either into or out of the isocenter of the magnet, respectively. The valve is switched by a TTL control line available from the Bruker 2T CSI (Fremont, California). The timing of the sample transport and data acquisition is conveniently programmed within the NMR pulse sequence and the relaxation information collected in a synchronized manner as shown in Fig. 2. The valve is normally in the A position and the sample is normally in the NMR probe in the homogeneous region of the magnet. When the valve is actuated the sample is transported into the peripheral field of the magnet. The sample rests for a variable time, τ, in the peripheral field and then returns to the isocenter of the magnet, where a profile of the magnetization is recorded. The "dead time" of the instrument, the time to complete a round-trip shuttle cycle, cannot be much longer than the T_1 of the sample. If the dead time is much longer than the T_1, the magnetization of the sample will be proportional to the field strength throughout the trajectory and nearly independent of the time τ. The nominal dead time for the prototype SSNMRD spectrometer is one second.

The sample is contained in a glass or acrylic tube, 15 cm long, with Teflon glides on either end. This sample tube travels inside an acrylic transport tube with an i.d. slightly larger than the o.d. of the Teflon glides (15 mm). The sample is stopped by acrylic tubes, held in place by wooden clamps, at positions A and B. O rings between the stopping tubes and the transport tube provide a seal for the high pressure. The nominal operating pressure of the device is 30 psi.

An NMR probe was constructed based on the design of Alderman and Grant (*16*). The probe is 15 cm in length and 2.6 cm in diameter and resonates at 85.5 MHz with a Q of approximately 170. The probe provided good homogeneity and sensitivity for the long, cylindrical samples used in the SSNMRD spectrometer.

† To whom correspondence should be addressed.

1064-1858/93 $5.00

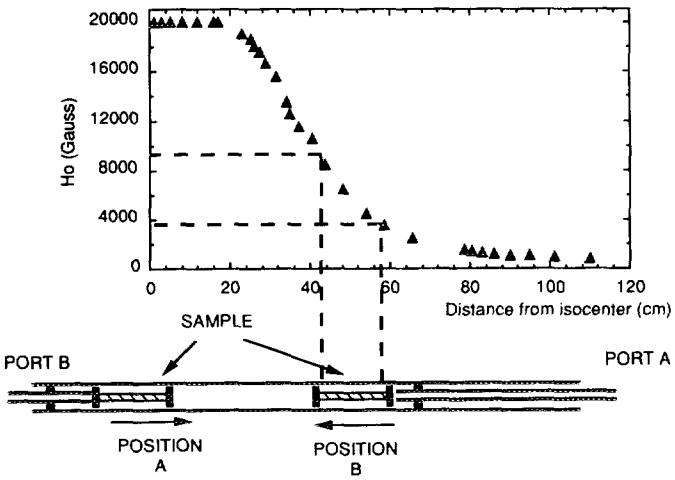

FIG. 1. The magnitude of the magnetic field measured with a gaussmeter, along the z axis of the 2 T, 31 cm bore magnet and a schematic of the sample-shuttle concept. The spatially homogeneous sample is moved quickly from position A to position B. While at position B, different portions of the sample are in different magnetic fields and the nuclear magnetization in the sample relaxes to new equilibrium values. In this example the proton Larmor frequencies vary from 16 to 41 MHz continuously across the sample. The sample remains at position B for a variable time, τ, and is moved quickly back to position A, and a 1D image of the magnetization is immediately obtained. The cycle is repeated with multiple values of τ to record, with high resolution in ω_0, the spin–lattice decay of magnetization as a function of Larmor frequency from 16 to 41 MHz. Position B can be moved to another location in the peripheral field and the relaxation rates measured over another segment of ω_0 values. This cycle is repeated until a complete dispersion profile is generated with a maximum Larmor frequency dictated by the field strength of the magnet and a minimum Larmor frequency determined by the most remote placement of position B.

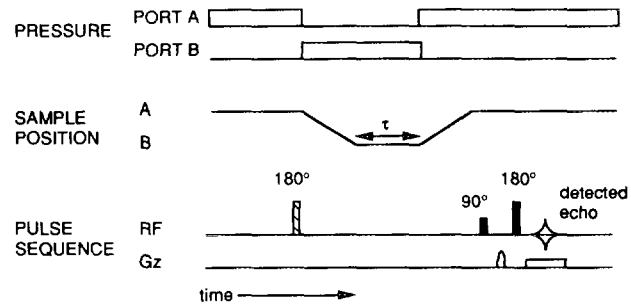

FIG. 2. Timing diagram of the prototype SSNMRD. Pressure is normally applied to port A, which holds the sample in the isocenter of the magnet. A TTL pulse from the CSI actuates the four-way valve, applying pressure to port B and moving the sample to a desired position in the peripheral magnetic field, where it is held for a variable time τ. When the logic signal goes low, pressure is again applied to port A and the sample is transported back to the isocenter of the magnet, where a 1D spin-echo image is recorded. This trajectory is completed for 32 different values of τ and the dispersion profile recorded. When relaxation rates at magnetic fields greater than 0.5 T are measured, a 180° inversion pulse is applied to the spins prior to moving the sample to maximize the dynamic range of the experiment.

The NMRD profile of a 150 μM solution of $MnCl_2$ is shown in Fig. 3. The high-field dispersion observed is due to modulation of the dipole–dipole interaction between the metal electron and the water protons by rotational motion of manganese aquo ion complex. Relaxation rates from 3 to 85 MHz were measured by stopping the sample at seven different positions in the external magnetic field.

A sample of cross-linked bovine serum albumin (BSA) was prepared by mixing 200 μl of a 25% glutaraldehyde solution with 20 ml of a 20 wt% solution of BSA. The glutaraldehyde was thoroughly mixed with the protein solution at 5°C in a beaker to ensure sample homogeneity. The mixture was poured into the sample tube and became semisolid within two hours due to the cross-linking reactions.

Figure 4 shows the NMRD profile of cross-linked BSA obtained from the sample-shuttle NMRD spectrometer. This profile was obtained in a manner analogous to the $MnCl_2$ sample except that the relaxation rates at 10 positions in the peripheral field were recorded. The most striking feature in this dispersion profile is the peaks present at 2.2 and 2.9 MHz. These peaks are due to the relaxation sink for proton magnetization created by proton–nitrogen dipole–dipole coupling when the proton energy-level splittings match the ^{14}N energy-level splittings (*17*). The ^{14}N energy-level splittings at low magnetic fields, particularly those involved in amide bonds, are dominated by nuclear electric quadrupole interactions. This feature in immobilized protein samples has been observed many times before with field-cycling NMRD spectrometers and recorded with high resolution to

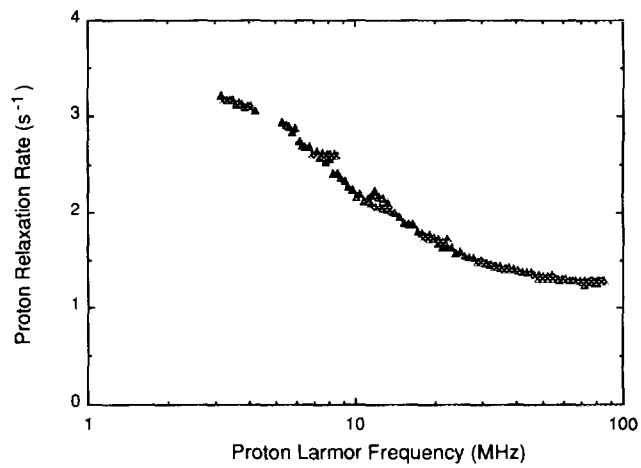

FIG. 3. NMRD profile of 150 μM $MnCl_2$. The high-field dispersion of Mn(II) due to the rotational correlation time of the manganese aquo ion complex is observed. This NMRD profile was recorded with the sample at seven different positions in the peripheral magnetic field. The data at each position were obtained in about five minutes. The 1D spin-echo image was obtained with one acquisition per time point, a block size of 256, a spectral width of 40 kHz, a field of view of 20 cm, an echo time of 10 ms, and a recycle time of 5 s. Much of the Larmor frequency resolution available by this experiment was not utilized. The NMRD profile presented shows only every 10th data point of the acquired NMRD profile to minimize clutter.

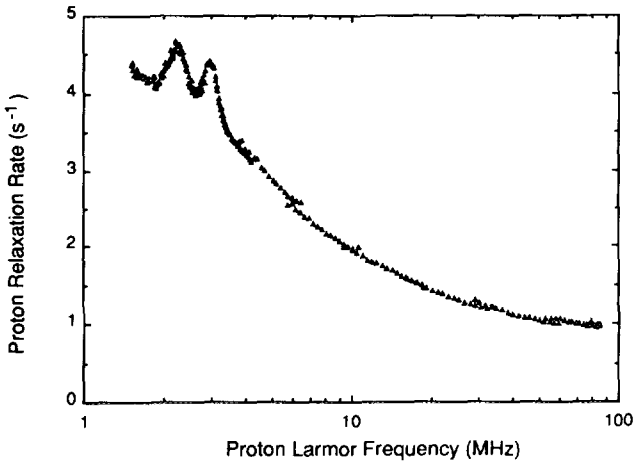

FIG. 4. NMRD profile of cross-linked bovine serum albumin (BSA). The peaks present at 2.2 and 2.9 MHz occur because the magnitude of the proton Zeeman Hamiltonian matches the magnitude of the ^{14}N quadrupole coupling constant, which becomes a relaxation sink for the proton magnetization at these field strengths. The NMRD profile was recorded with the sample at 10 different positions in the peripheral magnetic field. Every 10th data point was used above 4 MHz and every 4th data point was used below 4 MHz. Other parameters are as given in the legend to Fig. 3.

observe the fine structure of the dispersion profile in this region (*18*). The ^{14}N peaks seen here were recorded with the sample at two positions in the peripheral field and acquired in approximately 10 minutes. A field-cycling spectrometer would take approximately four hours to measure the relaxation rates in this region with a Larmor frequency resolution of 50 kHz per data point. The SSNMRD spectrometer is an ideal machine for measuring the ^{14}N peaks present in the dispersion profiles of high-molecular-weight, cross-linked, and denatured proteins.

The results presented here demonstrate both the advantages and the disadvantages of the SSNMRD spectrometer. The main advantages are its low cost and relative ease of construction. The apparatus can be assembled in a matter of days to fit any existing NMR spectrometer with a z-gradient coil. Although not attempted in this study, it may be possible to perform the experiment with the first-order z-shim coil if a pulsed field gradient is not available. If the experiment is performed in the absence of any gradients during signal detection with small samples, the relaxation dispersion of individual resonances of solute molecules may be observed. The high magnetic field homogeneity available in superconducting magnets will allow, for instance, studies of site-specific dynamics in small proteins.

The main disadvantage of the prototype SSNMRD spectrometer is that it is not able to record proton relaxation rates at Larmor frequencies below 1 MHz. This may be surmounted by shielding the peripheral region with a large-diameter (20 cm) ferromagnetic tube, concentrating the magnetic flux density within the walls of the tube and creating a region of low field in the center of the tube. Another limitation of the current design is the relatively long dead time. The sample-shuttle speed can be increased by optimal choice of pneumatic valves and shuttle tubes. We estimate that the dead time can be reduced from 1 s to approximately 300 ms, allowing relaxation rates up to 20 s^{-1} to be measured.

This device is inexpensive and easy to construct. It may be implemented in any laboratory with NMR imaging capabilities. The sample-shuttle NMRD spectrometer will be very useful to scientists wishing to measure NMRD profiles without access to a field-cycling NMRD spectrometer.

ACKNOWLEDGMENTS

This work was partially supported by NIH Grants CA 54362 (S.D.S) and CA 11198 (S.D.K.). Discussions with R. G. Bryant are gratefully acknowledged.

REFERENCES

1. S. H. Koenig and R. D. Brown III, *Prog. NMR Spectrosc.* **22,** 487 (1990).
2. I. Bertini, X. Xia, and C. Luchinat, *J. Magn. Reson.* **99,** 235 (1992).
3. S. Ganapathy, V. P. Chacko, and R. G. Bryant, *Macromolecules* **19,** 1021 (1986).
4. C. C. Lester and R. G. Bryant, *Magn. Reson. Med.* **22,** 143 (1991).
5. S. H. Koenig, R. D. Brown III, M. Spiller, and N. Lundbom, *Magn. Reson. Med.* **14,** 482 (1990).
6. R. N. Muller, L. VanderElst, P. A. Rinck, P. Vallet, F. Maton, H. Fischer, A. Roch, and Y. Van Haverbeke, *Invest. Radiol.* **23,** S229 (1988).
7. F. Noack, *Prog. NMR Spectrosc.* **18,** 171 (1986).
8. K. Hallenga and S. Koenig, *Biochemistry* **15,** 4255 (1976).
9. S. H. Koenig and R. D. Brown III, *Invest. Radiol.* **23,** 495 (1988).
10. R. V. Pound, *Phys. Rev.* **81,** 156 (1951).
11. G. P. Jones, J. T. Daycock, and P. T. Roberts, *J. Phys. E* **2,** 630 (1969).
12. A. Abragam and W. G. Proctor, *Phys. Rev.* **109,** 1441 (1958).
13. D. T. Edmonds, *Phys. Rep. C* **29,** 233 (1977).
14. A. Bielecki, D. B. Zax, K. W. Zilm, and A. Pines, *Rev. Sci. Instrum.* **57,** 393 (1986).
15. D. B. Zax, A. Bielecki, K. W. Zilm, A. Pines, and D. P. Weitekamp, *J. Chem. Phys.* **83,** 4877 (1985).
16. D. W. Alderman and D. M. Grant, *J. Magn. Reson.* **36,** 447 (1979).
17. F. Winter and R. Kimmich, *Biochim. Biophys. Acta* **719,** 292 (1982).
18. S. H. Koenig, *Biophys. J.* **53,** 91 (1988).