

On the Interpretation of Water Nuclear Magnetic Resonance Relaxation Times in Heterogeneous Systems

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Abstract: Evidence is presented for the existence of large magnetic field inhomogeneities in heterogeneous systems which make it difficult to analyze nmr wide-line and relaxation results correctly using current theoretical and experimental techniques. Spin-echo and null-point techniques have been employed to measure transverse and longitudinal relaxation times of deuterons for a series of deuterium oxide-glass bead systems. The average diameter of the beads ranged from 20 to 661 μ in seven different groups. Relaxation times were affected by the existence of the large surface area and decreased as the surface to volume ratio increased. No effects of surface character on relaxation times were detected. Accepted interpretation of the results in terms of fast exchange of water molecules between bulk and surface water indicates that there is practically no surface water in rotationally restricted form at the surface of glass beads: uncoated, coated with hydrophilic membranes, or coated with a hydrophobic silicone compound. However, further analysis of the experimental results indicates that any surface effects could have been masked by the large magnetic inner field inhomogeneities created by the heterogeneous nature of the samples. A simple model is used to show the existence of large field gradients in heterogeneous systems. Theoretical values of field inhomogeneity agree well with experimental values. It was also observed that paramagnetic substances bound at the surface of the glass beads enhanced the longitudinal relaxation rate of the deuterium over the same number of ions in an aqueous solution of the same available volume. The results have important implications for the evaluation of similar data arising from heterogeneous biological samples. It is possible, however, that measurements of relaxation times in uniform heterogeneous systems (such as the beads) could be used to determine diffusion coefficients in liquids.

In spite of its importance, the state of water at foreign surfaces is not well understood. It has, however, been suggested that there might exist strongly hydrogen bonded water molecules at hydrophilic surfaces.¹ Recently, there have been numerous studies of water by nmr, but the interpretation of the experimental results is in controversy.² One of the controversial points is the magnitude of the nuclear spin transverse relaxation (T_2) that is due to molecular diffusion. It is well known that T_2 is decreased by molecular diffusion through inhomogeneous local magnetic fields.³ These can be created by microscopic heterogeneity in the samples or inhomogeneities in the applied external field. Hansen and Lawson^{2a} and Cooke and Wien^{2g} reported the existence of large diffusion effects in heterogeneous systems. Hazelwood and his coworkers^{2b-e} claimed that no diffusion effects are observed and that the line broadening of the proton spectrum of cellular water is caused solely by the "ordered water" existing in biological tissues. Packer⁴ has attempted to show that the diffusion effect caused by heterogeneity in striated muscle has a negligible contribution to the transverse relaxation of water protons. On the other hand, we believe that the application of currently available theories, which were

derived under simple restricted conditions, to complex heterogeneous systems such as biological systems without any modification is not appropriate.

Previously, one of us pointed out that the line broadening of proton and deuterium spectra in heterogeneous systems could be caused by the exchange of molecules in the bulk phase and those which exist near suspended matter.⁵ As a continuation of this work, we report here the results of relaxation time measurements and wide-line spectra of deuterium for a series of deuterium oxide-glass bead systems. Other beads such as styrene-divinyl benzene copolymer beads and hollow glass microballoons were also used in this work. The glass beads studied have four different surface characters in eight different sizes. This is a unique system by which we may examine the effects of the surface-volume ratio, surface character, magnetic field inhomogeneity, and paramagnetic ions. The purpose of the work is to furnish a better understanding of the nmr results on water in heterogeneous systems and clear up some of the controversial points. A simple sphere model will be considered in order to show the existence of large inner field inhomogeneity in heterogeneous systems.

Experimental Section

The Carr-Purcell-Meiboom-Gill^{3a,6} technique was employed to measure transverse relaxation times with a 180° pulse period, τ_{mg} from 0.143 sec down to 0.009 sec. The longitudinal relaxation time was measured by the null-point method.^{3a} The ²H pulse experimental unit used a Varian HA-60 high-resolution magnet equipped with an external proton field locking system. The measurements were made with regular 5-mm o.d. nmr sample tubes at a resonance frequency of 9.21 MHz. The sample temperature was 31.5°. The deuterium oxide was from Thompson Packard, Inc. and was 99.8% isotopically pure. The average diameter of the glass beads (Microbeads, Cataphote Corp., Jackson, Miss.), made of

(1) (a) R. E. Hester, K. Krishnan, and C. W. J. Scaife, *J. Chem. Phys.*, **49**, 1100 (1968); (b) J. J. Lim and M. H. Shamos, *Biophys. J.*, **11**, 648 (1971); (c) R. D. Schultz and S. K. Asunmaa, *Recent Progr. Surface Sci.*, **3**, 291 (1970).

(2) (a) J. R. Hansen and K. D. Lawson, *Nature (London)*, **225**, 542 (1970); (b) D. C. Chang, C. F. Hazelwood, B. L. Nichols, and H. E. Roschach, *ibid.*, **235**, 171 (1972); (c) C. F. Hazelwood, B. L. Nichols, and N. F. Chamberlain, *ibid.*, **222**, 747 (1969); (d) D. C. Chang, H. E. Roschach, B. L. Nichols, and C. F. Hazelwood, *Ann. N. Y. Acad. Sci.*, **204**, 434 (1973); (e) H. E. Roschach, D. C. Chang, C. F. Hazelwood, and B. L. Nichols, *ibid.*, **204**, 444 (1973); (f) F. W. Cope, *Nature (London)*, **237**, 215 (1972); (g) R. Cooke and R. Wien, *Biophys. J.*, **11**, 1002 (1971).

(3) (a) H. Y. Carr and E. M. Purcell, *Phys. Rev.*, **94**, 630 (1954); (b) N. Bloembergen, E. M. Purcell, and R. V. Pound, *Phys. Rev.*, **73**, 678 (1948).

(4) K. J. Packer, *J. Magn. Resonance*, **9**, 438 (1973).

(5) J. A. Glasel, *Nature (London)*, **227**, 704 (1970).

(6) S. Meiboom and D. Gill, *Rev. Sci. Instrum.*, **29**, 688 (1958).

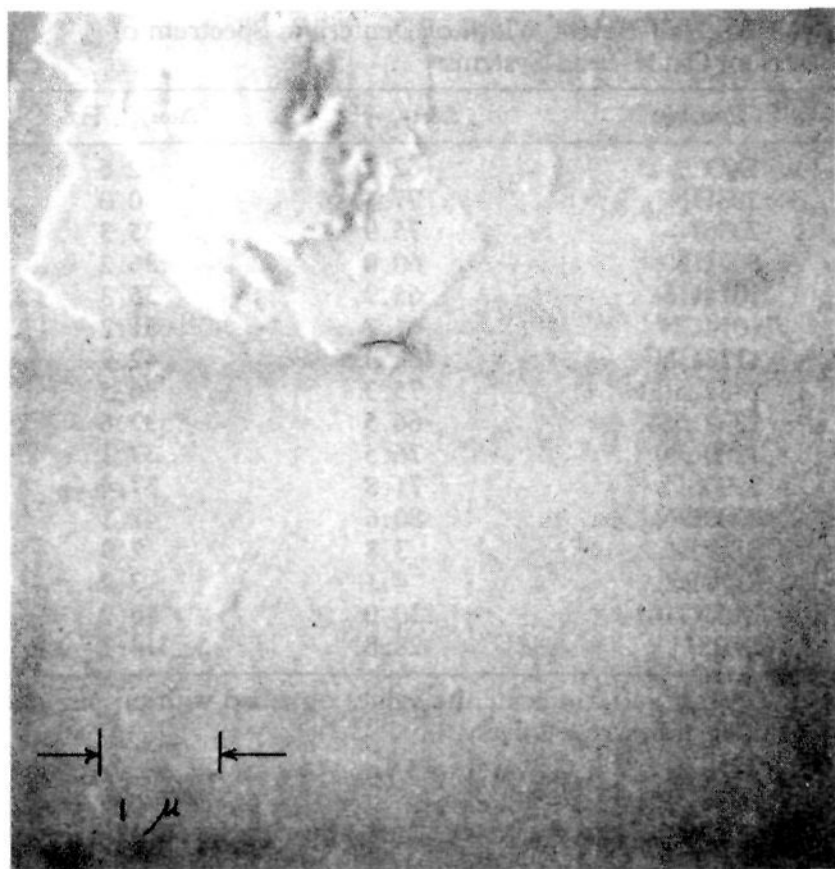


Figure 1. Scanning electron microgram of the uncoated glass bead surface, $\times 5000$.

optical crown glass, ranged from 661 down to 20 μ in seven different sizes. The glass beads were boiled in 0.01 *M* NaOH solution for 30 min and washed with distilled water. To eliminate any paramagnetic dust the washed beads were put into 6 *M* HCl solution and left overnight at 40°. The beads were cleaned again with distilled water and dried. These are designated uncoated beads. Under light and electron microscopic examination they are very smooth spheres with a quite narrow size distribution within the specified limits by the manufacturer (Figure 1). The standard deviation of the bead diameter is less than 20% of the average diameter for all sizes of beads.

The glass beads were coated with three different materials: silicone compound (Siliclad, Becton Dickinson and Co., Parsippany, N. J.), cellulose nitrate, and cellulose acetate. The siliconed beads were prepared by the procedure suggested by the manufacturer. The membrane coated beads were prepared by the following procedure. The technique is to wet the glass bead surface with membrane polymer solution and precipitate the polymer on the bead surface in a precipitant phase. This is done by dropping the glass beads into a column which contains a membrane polymer solution in the top and a precipitant in the bottom. A 125-ml flask with a stem (15 cm long, 1 cm i.d.) was filled with ethylene glycol up to the middle of the stem. Then a membrane polymer solution was poured carefully on the top of the ethylene glycol. The two liquid columns were separated by a clear interface and no interfacial precipitation was formed. When the glass beads were dropped into the liquid column from the top, the polymer solution film coated on the based surface in the polymer solution phase precipitated on the surface in the ethylene glycol phase. The coated beads being collected in the flask were gently stirred by a magnetic stirrer in order to disperse the solvent coming out of the membrane film on the bead surface. The coating process was performed at constant temperature $25 \pm 0.5^\circ$. The cellulose acetate solution was 3.45 wt % solution of Eastman Kodak E-398-3 in acetone and the cellulose nitrate solution was collodion (J. T. Baker Chemical Co., N. J.) diluted by 0.5 by volume with ethyl ether and 1.5 with ethyl alcohol.

The coated beads were washed with distilled water and later with deuterium oxide to replace the small amount of water remaining in the membrane matrix by deuterium oxide. Optical and scanning electron micrograms show that the coatings are very smooth with a thickness of approximately 2 μ for No. 203 beads and even thinner for small beads (Figure 2). It is estimated that the volume of the coated membrane is less than 1% of the total sample volume. The cleaned beads were packed into the sample tubes and empty space was filled with deuterium oxide. The packing was done very carefully to produce the same degree of compactness for all samples.



Figure 2. Scanning electron microgram of the glass bead surface coated with cellulose nitrate membrane, $\times 2600$.

Air bubbles trapped inside the sample tubes were eliminated by evacuation. The fraction of the total sample volume occupied by the uncoated beads was $62 \pm 0.5\%$ for all sizes of beads.

Results

The observed values of T_1 and T_2 are given in Tables I and II. In both T_1 and T_2 measurements the magnitude of the total magnetization observed decayed exponentially within experimental error with a lower limit for the 180° pulse interval of 0.009 sec. This indicates that there is one $(T_1)_{\text{obsd}}$ and one $(T_2)_{\text{obsd}}$ for each sample.

Table I. $(T_1)_{\text{obsd}}$ of Deuterium in Deuterium Oxide-Bead Systems (Reproducibility $\pm 2\%$)

Sample bead ^a	Bead diameter, μ	$(T_1)_{\text{obsd}}$, sec
D ₂ O		0.528
203UN	661	0.515
203SI		0.515
203CN		0.515
203CA		0.515
507UN	252	0.510
507SI		0.510
1014UN	122	0.486
1014SI		0.486
1014CN		0.487
1014CA		0.487
1723UN	65	0.475
1723SI		0.475
2332.5UN	53	0.475
2332.5SI		0.476
3200UN	33	0.436
3200SI		0.436
4000UN	20	0.404
400SI		0.408
SDVB1	250	0.520
SDVB2	63	0.500
MBIG101	40	0.410
MBSI	40	0.470

^a UN = uncoated, SI = siliconed, CN = cellulose nitrate membrane coated, CA = cellulose acetate membrane coated, SDVB = styrene-divinylbenzene copolymer bead, MBIG101 = sodium borosilicate glass microballoon, MBSI = silica microballoon.

Table II. $(T_2)_{\text{obsd}}$ of Deuterium in Deuterium Oxide-Bead Systems (Reproducibility $\pm 5 - 10\%$, $(T_2)_{\text{D}_2\text{O}} = 0.529$)

Sample bead	Pulse interval, $2\tau_{\text{mg}}$, sec					
	0.286	0.143	0.104	0.071	0.053	0.036
203UN		0.24		0.22	0.22	0.29
203SI		0.24		0.22	0.24	0.29
203CN		0.24		0.22	0.23	0.29
203CA		0.24		0.22	0.23	0.29
507UN		0.15		0.16		0.15
507SI		0.15		0.16		0.15
1014UN				0.078		0.066
1014SI				0.078		0.066
1014CN				0.078		0.066
1014CA				0.078		0.066
1723UN						0.049
1723SI						0.049
2332.5UN						0.034
2332.5SI						0.034
3200UN						0.027
3200SI						0.027
4000UN						
4000SI						
SDVB1	0.50					
SDVB2	0.40					
MBIG101					0.08	
MBSI					0.05	
	0.028	0.026	0.018	0.013	0.009	
203UN			0.27			
203SI			0.27			
203CN			0.27			
203CA			0.27			
507UN			0.15			
507SI			0.15			
1014UN			0.074	0.076		
1014SI			0.074	0.076		
1014CN			0.074	0.076		
1014CA			0.074	0.076		
1723UN			0.042	0.046	0.050	
1723SI			0.042	0.046	0.050	
2332.5UN	0.034		0.034	0.040	0.045	
2332.5SI	0.034		0.034	0.040	0.045	
3200UN		0.027	0.020	0.023	0.029	
3200SI		0.027	0.020	0.023	0.029	
4000UN		0.019	0.017	0.016	0.017	
4000SI		0.019	0.017	0.016	0.017	

$(T_2)_{\text{obsd}}$ was measured as a function of pulse period, but little variation of the $(T_2)_{\text{obsd}}$ with pulse interval over the range mentioned in the previous section was observed. $(T_2)_{\text{obsd}}$ decreased rapidly with decreasing bead size whereas $(T_1)_{\text{obsd}}$ decreased very slowly. Coating hardly affected either $(T_1)_{\text{obsd}}$ or $(T_2)_{\text{obsd}}$. The reproducibility was $\pm 2\%$ for $(T_1)_{\text{obsd}}$ and $\pm 5-10\%$ for $(T_2)_{\text{obsd}}$. $(T_1)_{\text{D}_2\text{O}}$ and $(T_2)_{\text{D}_2\text{O}}$ are the same within the experimental error limit and agree well with a literature value.⁷ The half-height width of the wide-line deuterium spectrum is listed in Table III for most samples.

Discussion

System Analysis. The system described is a heterogeneous one composed of solid beads and liquid deuterium oxide. Therefore, there is a strong possibility of a diffusion effect on T_2 measurements by the spin-echo technique. However, since little variation of $(T_2)_{\text{obsd}}$ was seen at widely different pulse intervals, first the results will be analyzed neglecting any diffusion effect, according to the accepted form used by many previous workers. On the other hand, as will be discussed in a later part, the field inhomogeneity effect could be enormous and mask all other surface effects.

(7) D. E. Woessner, *J. Chem. Phys.*, **40**, 234 (1964).

Table III. Half-Height Width of Deuterium Spectrum of Deuterium Oxide-Bead Systems^a

Sample	$\Delta\omega_{1/2}$, Hz	$\Delta\omega_{1/2}'$, Hz
D ₂ O	2.7	2.6
203UN	77.1	30.0
203SI	75.9	35.3
507UN	60.0	36.2
1014UN	65.9	35.3
1014CN	61.2	31.2
1723UN	82.4	42.9
2332.5UN	75.3	36.5
2332.5SI	66.5	37.6
3200UN	76.5	37.1
3200SI	71.8	37.4
4000UN	80.6	47.1
SDVB1	3.8	2.9
SDVB2	4.1	3.2
MBIG101	30.0	16.2
MBSI	28.8	14.7

^a $\Delta\omega_{1/2}$ and $\Delta\omega_{1/2}'$ indicate the values obtained with unspun and spun sample tubes, respectively.

Since it is clearly shown that the relaxation time decreases as the surface-volume ratio increases, it is reasonable to believe that the water molecules near the foreign surface have different properties from those in the bulk phase. There might be a gradual change in physical properties from the water molecules at the surface to those in the bulk phase as the distance from the surface increases. However, we may divide the total water into two groups: the surface water and the bulk water. The water molecules at the surface layer undergo relatively restricted motion. There will be rapid exchange of water molecules between the surface layer and the bulk phase and within the bulk phase. Since only one T_1 and one T_2 were observed, this indicates that the fast exchange limit applies and the exchange of water molecules between the bulk and surface water is much faster than the deuterium relaxation rates. This system can then be described by the following equation⁸

$$(1/T_j)_{\text{obsd}} = P_{a,i}/T_{j,a} + P_{b,i}/T_{j,b} \quad (1)$$

$$i = 1, 2, \dots, i - n$$

$$j = 1, 2$$

$P_{a,i}$ and $P_{b,i}$ are the probabilities that the exchanging molecules are in states a and b with relaxation times $T_{j,a}$ and $T_{j,b}$, respectively. Since $P_{b,i}$ is proportional to the total surface area of beads in the sample of bead size i , eq 1 can be expressed as a function of bead radius. The fact that the total surface area of beads in a given sample volume is inversely proportional to the average diameter of the sample beads (Appendix III) was applied in the derivation of the following equation.

$$(1/T_j)_{\text{obsd},i} = (r_1/r_i)P_{b,i}(1/T_{j,b} - 1/T_{j,a}) + 1/T_{j,a} \quad (2)$$

$$j = 1, 2$$

$(T_j)_{\text{obsd},i}$ is the observed relaxation time of the sample with a bead size i , r_1 is the average radius of reference bead, r_i is the average radius of the sample beads, and $P_{b,i}$ is the fraction of surface water of the reference sample. Any size bead can be taken as a reference. In this analysis, we will take the largest bead (No. 203) as a reference.

(8) J. R. Zimmerman and W. E. Brittin, *J. Phys. Chem.*, **61**, 1328 (1957).

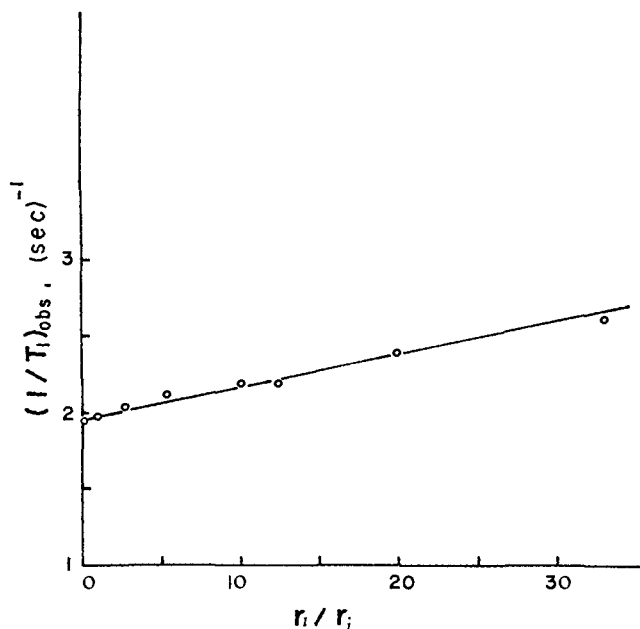


Figure 3. Observed longitudinal relaxation rate of deuterium in deuterium oxide-glass bead systems is shown as a function of bead size. r_1/r_i is the ratio of the radius of reference bead (No. 203), 330.5 μ , to that of sample beads.

Equation 2 indicates that $(1/T_j)_{\text{obsd},t}$ is a linear function of (r_1/r_i) with a slope of $P_{b,i}(1/T_{j,b} - 1/T_{j,a})$. Figures 3 and 4 show the plots of $(1/T_1)_{\text{obsd}}$ and $(1/T_2)_{\text{obsd}}$ of uncoated bead samples as a function of the ratio of the sample bead diameters. The curves have slopes of 0.02 and 1.75 sec^{-1} , respectively, and pass through $1/T_{1,j,a}$; i.e., $1/T_{D_2O}$ at $r_1/r_i = 0$. Since the water molecules at the surface of beads can be considered to be undergoing restricted motion and the observed relaxation times decrease as the surface-volume ratio increases, we may assume the $T_{j,b}$ is much smaller than $T_{j,a}$. With this assumption, the ratio of relaxation times at the surface layer, $T_{1,b}/T_{2,b}$, can be obtained by taking the ratio of the slopes from Figures 3 and 4.

Carver and Richards⁹ have plotted the ratio of T_1 and T_2 as a function of $\omega\tau$ for various relaxation mechanisms including a quadrupolar mechanism for spin = 1 nuclei such as the deuteron. These values are calculated from the ratio of the spectral density functions for T_1 and T_2 for given mechanisms. From this relationship $\omega\tau_b = 9.7$ was obtained where τ_b is the molecular correlation time of the surface water. Since the resonance frequency used in this work was 9.21 MHz, τ_b is easily obtained: 1.67×10^{-7} sec. This is very close to the correlation time of water molecules in ice, $\tau = 10^{-6}$ sec.^{3b} The longitudinal relaxation time of a deuteron in the surface water can be calculated from the following equation.¹⁰

$$1/T_1 = 3/80(1 + (\eta^2/3)) \left(\frac{e^2qQ}{\hbar} \right)^2 \{J(\omega) + 4J(2\omega)\} \quad (3)$$

$$J(\omega) = 2\tau/(1 + \omega^2\tau^2)$$

Assuming that the asymmetry factor η is zero and using

(9) J. P. Carver and R. E. Richards, *J. Magn. Resonance*, **6**, 89 (1972).

(10) A. Abragam, "The Principles of Nuclear Magnetism," Oxford University Press, London, 1961, p 314.

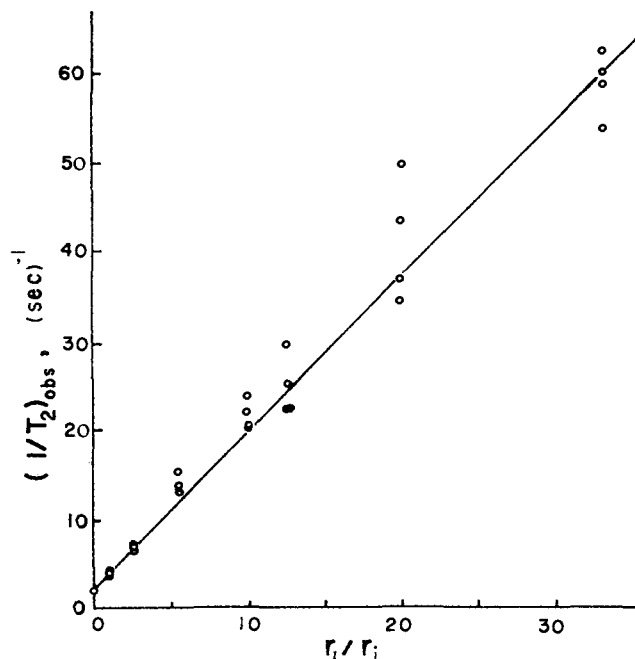


Figure 4. Observed transverse relaxation rate of deuterium in deuterium oxide-glass bead systems is given as a function of bead size. r_1/r_i is the ratio of the diameter of reference bead (No. 203), 330.5 μ , to that of sample beads.

the quadrupole coupling constant, 220 kHz,¹¹ a calculated $T_{1,b} = 1.98 \times 10^{-3}$ sec was obtained. $T_{2,b} = 2.26 \times 10^{-5}$ sec was also obtained from the ratio of $T_{1,b}$ and $T_{2,b}$. The fraction of the surface water in the reference sample, $P_{1,b}$, was easily obtained from either of the slopes in Figures 3 and 4. It was 3.96×10^{-5} . Thus, the surface molecules have the correlation time of 1.67×10^{-7} sec, $T_{1,b}$ of 1.98×10^{-3} sec, and $T_{2,b}$ of 2.26×10^{-5} sec.

Since the packing density of the sample beads is known (62%), the fraction of surface water can be expressed as a function of an average bead radius and thickness of the surface water (Appendix I). In eq 4

$$P_{b,i} = 7.3(m/r_i)g10^{-8} - 13.8(m/r_i)^2g10^{-16} \quad (4)$$

is the number of surface water layers in terms of the diameter of water molecules (1.5 Å), and g is a geometrical factor for the surface which is 1.0 for a perfectly smooth spherical surface and greater than 1.0 otherwise. The average coordination number of each bead was assumed to be 9 because the packing density 62% is very close to packing density 61% with a coordination number 9.^{12a} The second term in eq 4 is practically negligible in comparison with the first term, and the thickness of the surface water layer, $17.9(1/g)$ layers, is obtained.

Shereshesky and Weir^{12b} reported a g value of 54.5 for the surface of similarly treated glass beads. Therefore, the thickness of the surface water at the surface of uncoated glass beads can be estimated as 0.33 layers. As shown by scanning electron micrograms in Figures 1 and 2, the membrane-coated bead surface is much more irregular than that of the uncoated bead surface. Therefore, the g value of the membrane-coated bead surface

(11) J. A. Glasel, "Water, A Comprehensive Treatise," Vol. 1, Plenum Press, New York, N. Y., 1973, p 246.

(12) (a) J. Clarke, *Nature (London)*, **240**, 408 (1972); (b) J. L. Shereshesky and C. E. Weir, *J. Phys. Chem.*, **60**, 1162 (1956).

can be expected even greater than that of the uncoated ones. This leads to the conclusion that there is no surface water in rotationally restricted form which has properties different from those of bulk water. If the surface water exists at the bead surface, then it should be affected by the character of the surface material. However, as shown in Tables I and II, there is no difference in relaxation times between the beads uncoated, coated with hydrophilic cellulose derivative membranes, and coated with hydrophobic silicone compound. The differences previously reported⁵ were evidently due to lack of uniformity of packing in the samples. In order to see whether or not the shortening of the relaxation times was caused by any soluble paramagnetic ions, on the surface of the samples, the sample beads were leached in sample amounts of pure D₂O for several days and then D₂O was extracted. The relaxation times of this extracted deuterium oxide were exactly the same as those of pure deuterium oxide. This indicates that there were no easily soluble paramagnetic ions on the samples.

The effect of bead size on the relaxation times can be explained by the following two facts, regardless of the existence of structured water at the surface. First, the water molecules will go through a reorientation when they hit the bead surface. Therefore, the existence of the foreign surface itself will affect the observed relaxation times of water molecules. The effect of the surface character might be relatively small and probably within the experimental error limit. Second, as mentioned at the beginning, there is a field inhomogeneity effect in this heterogeneous system even when the external field is extremely homogeneous. The magnitude of this effect in heterogeneous systems is not well understood.

Field Inhomogeneity Effect. The magnetic field inhomogeneity inside the heterogeneous samples is created by the difference in magnetic susceptibilities between the water and the materials in the samples. The degree of inhomogeneity depends on *both* the difference in the susceptibilities of system materials in the sample tube and the geometry of the particulates in the system. As a simple model, a single sphere of radius r_0 , which is located in a uniform magnetic field established in a continuous medium, will be considered. The field near the sphere will be deformed by the existence of this sphere unless the sphere has exactly the same magnetic susceptibility as the medium. The local magnetic field strength at any point outside the sphere will be a function of the coordinates of the point in three dimensions. The volume average field gradient of the z component around the sphere is (Appendix II)

$$\bar{G}_z = (9H_0A/4)(1/(r^2 + rr_0 + r_0^2))(1/rr_0) \quad r > r_0 \quad (5)$$

$$A = (4\pi(\chi_{v,s} - \chi_{v,m}))/[3 + 4\pi(\chi_{v,s} + \chi_{v,m})]r_0^3$$

$\chi_{v,s}$ and $\chi_{v,m}$ are the magnetic volume susceptibilities of the sphere and the medium. Equation 5 indicates that the field gradient near the surface of the sphere is approximately proportional to the inverse of the sphere radius and a function of the difference of the magnetic susceptibilities of the sphere and the medium. The gradient could be very large for an extremely small sphere even though there is a small difference in the susceptibilities between sphere and water. When there is a large difference in the susceptibilities, the effect will be even greater. For instance, if the medium is water and

the uniform field strength H_0 is 14,000 G (which is used in these experiments), the average gradient \bar{G}_z at the surface of a silica sphere with radius 10μ is 1.3×10^3 G/cm. In this work, the samples were made of a large number of beads and the exact solution will be much more complicated than eq 5. However, it is clear that the bead size and the susceptibility difference will affect the local field gradient in the same direction as the case of the single sphere model; *i.e.*, the field gradient will increase as the particle size decreases and the susceptibility difference increases. If so, this can explain the results shown in Tables I and II. $(T_1)_{\text{obsd}}$ decreases very slowly as the bead size gets smaller because T_1 is not affected by field inhomogeneity^{3a} but only by the existence of surface area. On the other hand, T_2 is affected by field inhomogeneity and decreases substantially as the surface-volume ratio increases. Cotts and his coworkers^{13a} measured T_2 for lithium in solid lithium particles ($\sim 20 \mu$) suspended in mineral oil by the spin-echo technique and observed this effect.

In order to see the effect of different bead materials, several samples were made of styrene-divinylbenzene copolymer (SDVB) beads, silica microballoons (MBSI), and sodium borosilicate glass microballoons (MBIG-101). The SDVB beads were supplied by Dow Chemical Co. and contained 8% divinylbenzene. The microballoons supplied by Emerson and Cuming, Inc., are smooth surfaced hollow beads with a wall thickness of $\sim 2 \mu$. As shown in Table II, the observed relaxation times of these samples are greater than those of solid glass bead samples of similar sizes. In particular, the $(T_2)_{\text{obsd}}$ of SDVB samples are much longer than others. It may be argued that the difference is caused by "structured water" at the hydrophilic glass surface and "non-structured water" at the hydrophobic surface of the SDVB beads. But this could not explain the fact that there is no difference in the relaxation times between the glass bead samples: uncoated, coated with hydrophilic membranes, and coated with hydrophobic silicone compound. It also could not explain the dependency of $(T_2)_{\text{obsd}}$ of SDVB samples on the size of the beads. However, all the observations can be explained by diffusion effects caused by the local inhomogeneity which is a function of the bead size and the susceptibility difference of the beads and water.

A series of wide-line nmr spectra were taken for most of the samples. In Table III the half-height widths of the spectra are given for both spun ($\Delta\omega_{1/2}'$) and unspun ($\Delta\omega_{1/2}$) sample tubes. Little difference between $\Delta\omega_{1/2}$ and $\Delta\omega_{1/2}'$ of pure D₂O indicates that the external field is very homogeneous. As expected from the values of $(T_2)_{\text{obsd}}$, the solid glass beads gave broad spectra and SDVB beads gave the sharpest spectra. On the model outlined above, the field inhomogeneity created by the hollow spheres is expected to be smaller than the one made by solid spheres. This is shown in Tables II and III. $(T_2)_{\text{obsd}}$ and $\Delta\omega_{1/2}$ of the microballoon samples are longer and narrower respectively than those of solid glass bead samples of similar sizes. The T_2 's calculated from the half-height width $\Delta\omega_{1/2}$ of the wide-line spectra are always much smaller than the one obtained from spin-echo experiments at any available pulse intervals.

(13) (a) D. Zamir, R. C. Wayne, and R. M. Cotts, *Phys. Rev. Lett.*, **12**, 327 (1964); (b) L. E. Drain, *Proc. Phys. Soc., London*, **80**, 1380 (1962).

This is because the wide-line spectra are broadened mainly due to the inner inhomogeneity of the local magnetic field while in the spin-echo measurements the inhomogeneity effect is reduced by a certain amount in the Carr-Purcell experiment. However, the important observation is that in particulate systems where field gradients are nonlinear, the independence of T_2 with 180° pulse spacing must *not* be taken to indicate the absence of diffusion effects. The field inhomogeneity in our system can be estimated roughly by eq 5. The factor A is approximately equal to $4\pi(\chi_{v,s} - \chi_{v,m})r_0^3/3$ because the term $4\pi(\chi_{v,s} + 2\chi_{v,m})$ is much smaller than 3 for most cases. Since the beads are closely packed, we may assume $r \approx r_0$ as an estimate. Then, eq 5 becomes

$$\bar{G}_z \approx (\pi H_0)(\chi_{v,s} - \chi_{v,m})/r_0 \quad (6)$$

and the field inhomogeneity ΔH_z created by this average gradient can be estimated by multiplying by the radius of the beads used as distance parameter. Equation 7

$$\Delta H_z \approx \bar{G}_z \cdot r_0 \approx (\pi H_0)(\chi_{v,s} - \chi_{v,m}) \quad (7)$$

is very similar to the approximate expression due to Drain^{13b} for paramagnetic solid samples. He showed that, in powdered samples, there is a line broadening caused by the magnetization of small particles and concluded that the approximate expression for this contribution is $\Delta H \approx 3H_0\chi_{v,s}$.

Equation 7 shows that ΔH is independent of bead diameter while \bar{G}_z is inversely proportional to r_0 . The $\Delta\omega_{1/2}$ of solid glass bead samples in Table III do not show any appreciable size variation. This observation is the same for SDVB bead samples. The volume susceptibilities of the glass beads and the SDVB beads were measured with a Vibrating Sample Magnetometer (Princeton Applied Research, Princeton, N. J.). They are $+0.640 \times 10^{-6}$ emu/cm³ for glass beads and -0.648×10^{-6} emu/cm³ for SDVB beads. The paramagnetic susceptibility shown by the glass beads is due to the small amount of iron content in the glass. The surfaces of the glass beads were completely cleaned by acid. The esr spectrum of the acid cleaned glass beads (JEOL ESR, Model JES-ME-IX) showed two sharp peaks, one large and one small one. The large peak gave an electron g value of $g \approx 3.88$ which corresponded to divalent iron and the small peak gave $g \approx 2.30$ corresponding to that of trivalent iron. Therefore, it is believed that the major paramagnetic component doped into the glass is Fe^{2+} and a much smaller amount is in the form of Fe^{3+} . The sharp esr peaks are also an indication that Fe^{2+} and Fe^{3+} are uniformly distributed in the glass.

Substituting the measured susceptibilities of the beads and susceptibility of water, -0.721×10^{-6} emu/cm³, into eq 7, we obtain $\Delta H_z = 6.0 \times 10^{-2}$ G for the glass bead system and $\Delta H_z = 3.2 \times 10^{-3}$ G for the SDVB bead system. The average values of $\Delta\omega_{1/2}$ of the glass bead and the SDVB systems are 74.1 and 3.95 Hz, respectively. These are equivalent to $\Delta H = 11.3 \times 10^{-2}$ and 6.1×10^{-3} G at resonance frequency 9.21 MHz and $H_0 = 14,000$ G. Therefore, the experimental values of ΔH are approximately twice that for ΔH_z predicted by eq 7. This difference could be attributed to the inhomogeneity created by the field gradients existing in other directions, to the difference between the complex sample system and the simple model, and to other relaxation mechanisms such as surface relaxation.

According to eq 7 the ratio of the inhomogeneity of the glass bead system to that of the SDVB system is the ratio of the susceptibility difference, *i.e.*

$$\frac{(\Delta H_z)_{\text{glass}}}{(\Delta H_z)_{\text{SDVB}}} = \frac{(\chi_{v,s} - \chi_{v,m})_{\text{glass}}}{(\chi_{v,s} - \chi_{v,m})_{\text{SDVB}}}$$

The ratio of $\Delta\omega_{1/2}$ of the glass bead system to that of the SDVB system is 18.8, which agrees well with the ratio of the susceptibility differences of the two different beads, 18.6.

Therefore, in spite of its simplicity, the model predicts well the experimental values and could be useful in predicting the inner field inhomogeneities created in other heterogeneous systems. If it were possible to numerically solve the problem of field gradient distribution within a sample of uniform beads uniformly packed, the effects investigated in this work could be used to measure diffusion coefficients in liquids. The field gradients attainable are much higher than currently available using external devices.

Recently, Hazelwood and his coworkers^{2e} estimated the field inhomogeneity in muscle samples by eq 8 which is derived for a sphere of radius r_0 in uniform field H_0 . They obtained eq 8 by a different approach from eq A7

$$G = 10^{-3}(H_0/r)(r_0/r)^3 \cos \theta \quad r > r_0 \quad (8)$$

(Appendix II). However, these two equations have the same relationship between r_0 , r , H_0 , and the field gradient. Here H_0 , r , and r_0 have the same meaning as in eq 5. By substituting r_0 of 5 Å and r of 250 Å (the myosin-actin spacing in muscle cell), they obtained $G = \sim 10^{-1}$ G/cm and concluded that the field inhomogeneity is negligible. However, the gradient thus obtained is the *minimum* value. If the diameter of myosin filament, 160 Å, is considered as r_0 , the field gradient at the surface ($r = r_0$) of a sphere of a diameter of r_0 will be $\sim 10^{+5}$ G/cm according to eq 8. If the diameter of actin filament is used, the gradient will be even larger because the diameter of actin filament is much smaller than that of myofibril. Of course, this gradient at the surface is the maximum value. Therefore, eq 8 may be also used to indicate the probable existence of large field gradients in heterogeneous biological samples.

Theoretical analyses of diffusion effects on T_2 measurement by the spin-echo technique have been reported for several simple cases such as the linear field gradient^{3a,14} and a local field gradient distributed according to a Gaussian function.¹⁵ Robertson¹⁴ derived an approximate equation for spin-echo decay of spins diffusing in a bounded region. It was assumed that a linear external field gradient G exists only in the z direction and boundary surfaces in the sample system do not produce any magnetic field or distort the externally applied uniform field. It was also assumed that the spins are diffusing in a compartment which is infinite in size in the x and y directions but has a limited distance a in the z direction between walls. Under those conditions he arrived at the following limiting equations. For $t \ll a^2/(\pi^2 D)$

$$F(t) = e^{-\gamma^2 G^2 D t^3 / 12} \quad (9)$$

For $t \gg 2a^2/(\pi^2 D)$

$$F(t) = e^{-(a^4 \gamma^2 G^2 / 120 D)(t - 17a^2 / 56 D)} \quad (10)$$

(14) B. Robertson, *Phys. Rev.*, **151**, 273 (1966).

(15) K. A. Valiev and M. M. Bil'danov, *Zh. Strukt. Khim.*, **7**, 834 (1966).

$F(t)$ is the factor contributing to spin-echo decay due to the diffusion effect, γ is the magnetogyric ratio, and t is the time between the 90° pulse and the first echo. Equation 9, which is exactly the same as the one derived by Carr and Purcell,^{3a} and eq 10 represent unbounded and bounded diffusions, respectively.

Although our situation is entirely different from the conditions under which these equations were derived, it is interesting to see if such a model could be useful to describe our system. Assuming that $a = 2(\bar{r} - r_i)$ (Appendix III) and the diffusion coefficient of water is $\sim 10^{-5}$ cm²/sec, we obtain $a^2/\pi^2 D \simeq 1.7$ sec for No. 203 beads, and it is much larger than the pulse interval, 0.018 to 0.143 sec. Therefore, it satisfies the condition for unbounded diffusion by eq 9. The field gradient in eq 9 can be estimated by eq 7 and $\Delta\omega_{1/2}$ from Table III. The relaxation rate calculated by eq 9 is found to be ~ 40 sec⁻¹ which is much larger than the observed values of $3 \sim 5$ sec⁻¹. $(T_2)_{\text{obsd}}$ of No. 203 bead samples shows no general increase as the pulse interval gets shorter and can be considered constant within the experimental error limits. For No. 4000 beads we obtain $2a^2/\pi^2 D = 3 \times 10^{-3}$ sec, which also satisfies the inequality condition for eq 10. The relaxation rate contributed by this bounded diffusion is found to be $\sim 10^{-3}$ sec⁻¹, which is negligible in comparison with the observed values in Table II. Packer⁴ made a similar calculation for striated muscle. If eq 10 can be applied to the No. 4000 bead system, then the calculated value indicates that the observed relaxation rate of No. 4000 bead samples is entirely due to the bead surface whether it is caused by the interaction between the surface and water or by rotationally restricted water at the surface. However, as mentioned earlier, the latter case is not very likely because Table II does not show any difference at all between diverse surfaces. It is more likely that eq 9 and 10 cannot describe such complex systems as our or other heterogeneous systems and in particular are not applicable to biological samples.

Contrary to the assumptions Robertson¹⁴ made in the derivation of eq 9 and 10, in our system or other heterogeneous systems such as biological ones, the field gradient is created internally by the heterogeneity of the system itself and the gradient is *not* linear. The geometry of the particles is far from the parallel barrier model and, therefore, it is natural that eq 9 and 10 cannot predict diffusion phenomena in these complex systems. Furthermore, it is worthwhile to mention here that Wayne and Cotts,¹⁶ in their model experiment to test Robertson's theory, observed an increased shortening of proton relaxation times of CH₄ as the distance between barriers decreased, in other words as the number of barriers increased. This was observed *without* an externally applied field gradient. Robertson¹⁴ found that the surface relaxation rate in that case could not account for the observed results. We suggest here that this also could be explained by the field inhomogeneity created in that system. Since the barriers were located normal to the z direction, it is not likely that there was any substantial gradient created near the center, but it could still be possible that large gradients could have been created near the corners of the barriers because the total sample box was small enough to be located in the receiver coil.¹⁷

(16) R. C. Wayne and R. M. Cotts, *Phys. Rev.*, **151**, 264 (1966).

Paramagnetic Ion Effect. The glass beads cleaned only with 0.01 *M* NaOH solutions have paramagnetic material at their surfaces. The esr spectrum of these beads did not show any other substance except divalent and trivalent iron peaks, but the Fe³⁺ peak was broadened considerably in comparison with its breadth in HCl cleaned beads. This indicates that the paramagnetic material at the bead surface is essentially fully oxidized iron compounds among the glass components. Scanning electron micrograms (resolving power ~ 250 Å) of the glass bead surface are not able to detect any surface heterogeneity. This means the paramagnetic centers at the surface are quite uniformly distributed and their size is much smaller than ~ 250 Å. In order to find the concentration of the paramagnetic substance at the surface, known amounts of the beads were leached in 6 *M* HCl solution at 40° for 24 hr. A longer leaching period or use of 6 *M* HNO₃ solution did not make any difference in the following results, indicating that the paramagnetic substance is soluble in 6 *M* HCl solution and that a 24-hr period is sufficient to remove them. The dissolved metal ions were analyzed using a standard qualitative analysis procedure by precipitating them with H₂S gas. It was found that there were only Fe³⁺ ions in the solution thus confirming the result obtained from the esr spectrum. The Fe³⁺ content was analyzed gravimetrically by precipitation as Fe(OH)₃ and calcination in a furnace, and it was measured as Fe₂O₃. The average iron concentration at the surface was found to be 2.3×10^{-9} mol of Fe/cm².

In order to study the effects of the paramagnetic centers in the surface and of the ions in solution, nmr samples were made with the beads cleaned with only 0.01 *M* NaOH solution. The relaxation times of these samples are noted as $(T_1^*)_{\text{obsd}}$ and $(T_2^*)_{\text{obsd}}$ shown in Tables IV and V. On the other hand, the HCl solution,

Table IV. Pertinent Values of (T_1^*) and (T_1^+) are Listed^a

Sample	$(T_1^*)_{\text{obsd}}$, sec	$(T_1^+)_{\text{obsd}}$, sec	ϵ_t
203	0.488	0.523	4.5
1014	0.421	0.510	4.5
2332.5	0.293	0.453	4.1
4000	0.176	0.351	3.7

^a The measured relaxation enhancement coefficient ϵ_t is shown in the last column.

Table V. Pertinent Values of $(T_2^*)_{\text{obsd}}$ and $(T_2^+)_{\text{obsd}}$

Sample	$(T_2^*)_{\text{obsd}}$, sec	$(T_2^+)_{\text{obsd}}$, sec
203	0.31	0.440
1014	0.09	0.425
2332.5	0.04	0.337
4000	0.02	0.255

which contains Fe³⁺ ions dissolved from a known amount of the glass beads, was evaporated and then re-dissolved in the same amount of D₂O as the void fraction of the total bulk volume of the weighed glass beads. The deuterium relaxation times of these solutions are expressed as $(T_1^*)_{\text{obsd}}$ and $(T_2^+)_{\text{obsd}}$ shown in Tables IV and V.

Tables I and IV show that the paramagnetic centers at the bead surface shortened deuterium T_1 's considerably.

(17) R. M. Cotts, private communications, 1973.

However, Tables II and V show that the centers did not affect the observed values of T_2 . This is probably due to the fact that, as explained in the previous section, the effect of the large inner field inhomogeneity created in the system dominates even the effect of the paramagnetic centers at the surface.

Table IV shows that the paramagnetic centers bound at the bead surface enhanced the longitudinal relaxation of deuterium over a solution of the free ions in the same ionic concentration. The degree of the enhancement can be expressed as the enhancement factor ϵ_1 ¹⁸

$$\epsilon_1 = \frac{(1/T_1^*)_{\text{obsd}} - (1/T_1)_{\text{obsd}}}{(1/T_1^+)_{\text{obsd}} - (1/T_1)_{\text{D}_2\text{O}}} \quad (11)$$

Table IV shows that the enhancement factor ϵ_1 is virtually constant over a wide range of bead sizes. This result could be equally applied to other heterogeneous systems such as assemblies of biological macromolecules. In other words, paramagnetic ions bound at the surfaces of such assemblies could shorten the relaxation time of the proton or deuteron of water molecules by a greater extent than they are in solution.¹⁸

Conclusion

The transverse relaxation time of deuterons in the deuterium oxide-glass bead system is shortened very rapidly, while the longitudinal relaxation time decreases very slowly, as the size of the bead gets smaller. Effects due to changing the surface character of the beads were not detected. When the field inhomogeneity effect is neglected, the analysis of the experimental results by a fast exchange indicated that there is hardly any structured water at the surface of the glass bead whether it is uncoated, coated with hydrophilic membranes, or coated by hydrophobic silicone compound.

Mathematical analysis of a simple sphere model indicates, however, that there is a large inner field inhomogeneity in heterogeneous systems. This inhomogeneity is created by the difference of magnetic susceptibilities of the materials in the system and the geometry of the particulates in the system. The experimental results indicate that it is more likely that any surface effects are masked by the field inhomogeneities created in heterogeneous systems where the susceptibilities of the liquid phase and solid differ.

It was also observed that paramagnetic substances bound at the bead surface shortened the longitudinal relaxation time of deuterium by a greater amount than it is in the solution. This result could have significant meaning in the analyses the paramagnetic ion effects on nmr results in cellular systems.

Further analysis of the effects observed may enable them to be employed in the measurement of diffusion coefficients in liquids.

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(18) J. Eisinger, R. Shulman, and B. Szymanski, *J. Chem. Phys.*, **36**, 1721 (1962).

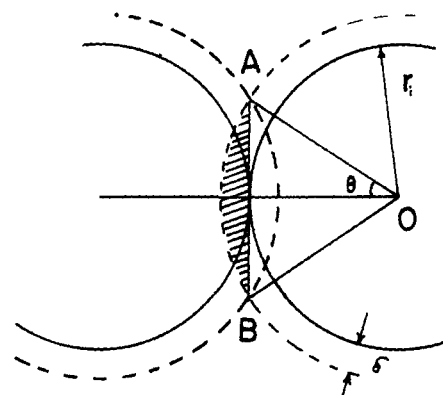


Figure 5. Coordinate system and symbols used in the calculation of $P_{b,i}$.

are implicit in a previous publication brought to our attention by one of its authors.¹⁹

Appendix I

Suppose two spheres of radius r_i have surface water of a thickness δ and are in contact as in Figure 5. Each sphere will lose a part of the space available for the surface water by the amount shaded in Figure 5. The shaded volume v can be obtained by subtracting the cone OAB from the partial sphere OAB.

$$\begin{aligned} v &= (\text{partial sphere OAB}) - (\text{cone OAB}) \\ &= (2\pi/3)(r_i + \delta)^3 \int_0^\theta \sin \theta \, d\theta - (\pi/3)(2r_i^2\delta + r_i\delta^2) \end{aligned}$$

Since $\cos \theta = r_i/(r_i + \delta)$

$$v = (\pi\delta^2/3)(3r_i + 2\delta)$$

If the total number of beads packed in the detectable sample space V is N_i and the coordination number of the packed bead is k , the total surface water V_s is

$$V_s = (4\pi N_i/3)((r_i + \delta)^3 - r_i^3) - (\pi\delta^2/3)(3r_i + 2\delta)N_i k \quad (A1)$$

The total water V_t in the sample volume V is

$$V_t = V(1 - \epsilon) = (4\pi r_i^3/3)(1 - \epsilon)/\epsilon \quad (A2)$$

ϵ is the fraction of the volume occupied by the beads or filling factor. Then, the fraction of the surface water $P_{b,i}$ is

$$P_{b,i} = V_s/V_t = 3(\delta/r_i)\{(\epsilon/1) - \epsilon\} + \delta/r_i\{(\epsilon/1) - \epsilon\} \times \{(3\delta/r_i) - (\delta/r_i)^2 - (3K/4)(\delta/r_i) - (K/2)(\delta/r_i)^2\} \quad (A3)$$

This equation is derived for a perfectly smooth spherical surface. For the surface which is not perfectly smooth, $P_{b,i}$ can be obtained by multiplying geometrical factor g which is 1 for a perfectly smooth surface and greater than 1 otherwise. $P_{b,i}$ can be expressed in terms of the number (m) of monolayers. Assuming the thickness of the monolayer of water molecules is 1.5 Å, and the coordination number is 9,^{12a} and substituting $\epsilon = 0.62$, eq A3 becomes

$$P_{b,i} = 7.3(mg/r_i)10^{-8} - 13.8(m/r_i)^2g10^{-16} \quad (A4)$$

(19) J. Clifford, J. Oakes, and G. J. T. Tiddy, *Spec. Discuss. Faraday Soc.*, No. 1, 175 (1970).

Appendix II

The distribution of magnetic field intensity \mathbf{H} for a sphere of radius r_0 in a uniform external field H_0 can be expressed as²⁰

$$r \geq r_0$$

$$\psi = -H_0 r \cos \theta +$$

$$\left\{ \mu_s - \mu_m \right\} / \left\{ \mu_s + 2\mu_m \right\} \frac{r_0^3}{r^2} H_0 \cos \theta \quad (\text{A5})$$

$$r \leq r_0$$

$$\psi = -3\mu_m H_0 r \cos \theta / \left\{ \mu_s + 2\mu_m \right\} \quad (\text{A6})$$

$$\mathbf{H} = -\nabla\psi$$

ψ is a scalar potential, γ is the distance from the center of the sphere O to the point of interest P , θ is the angle between the vector OP and the direction of the uniform field H_0 , and μ_s and μ_m are the magnetic permeabilities of the sphere and the medium, respectively. The field gradient exists three dimensionally. But for the purposes of approximation and mathematical simplicity, only the most important z component will be considered. The gradient of this component is considered as a major cause for the diffusion effect in spin-echo experiments. From eq A5 and A6

$$\frac{\partial H_z}{\partial Z} = G_z = (H_0 A / r_0^4) (9 \cos \theta - 15 \cos^3 \theta) \quad r \geq r_0 \quad (\text{A7})$$

where $A = (\mu_s - \mu_m)r_0^3 / (\mu_s + 2\mu_m)$. Or, since $\mu = 1 + 4\pi\psi_v$

$$A = 4\pi r_0^3 (\chi_{v,s} - \chi_{v,m}) / \{ 3 + 4\pi(\chi_{v,s} - 2\chi_{v,m}) \}$$

(20) D. Menzel, "Fundamental Formulas of Physics," Prentice-Hall, New York, N. Y., 1955, pp 315 and 325.

Here $\chi_{v,s}$ and $\chi_{v,m}$ are magnetic volume susceptibilities of the sphere and the medium, respectively. Coordinate transformation is necessary to obtain eq A7. The volume average field gradient from the surface ($r = r_0$) to a location r can be obtained by the following integration. Since the gradient is symmetric with respect to the plane of $\theta = \pi/2$, the integration has to be done over only half of the sphere.

$$\bar{G}_z = \frac{\int_{\pi/2}^{\pi} \int_0^{2\pi} \int_0^r G_z r^2 \sin \theta \, dr d\theta d\varphi}{\int \int \int r^2 \sin \theta \, dr d\theta d\varphi} = (9/4) H_0 A \left(\frac{1}{r^2 + rr_0 + r_0^2} \right) \left(\frac{1}{rr_0} \right) \quad (\text{A8})$$

Appendix III

The constant total sample volume detected by the receiver coil can be expressed as a function of bead radius r_1 , filling factor ϵ , and total number of beads N_t . The filling factor was 0.62 for all sizes of beads.

$$V = 4\pi r_1^3 N_t / 3\epsilon$$

The total surface area of all the beads in volume V is

$$A = 4\pi r_1^2 N_t = 3V\epsilon / r_1$$

A is inversely proportional to the diameter of the bead. The average thickness of the water (or void space) can be approximated by dividing the total void volume by total surface area.

$$(r - r_1) = V(1 - \epsilon) / A = 3V\epsilon / r = 0.204r_1 \quad (\text{A9})$$

Crystal and Molecular Structure of $C_{14}O_7NH_{21}$,

1-Acetyl-*trans*-3,*trans*-4-isopropylidenedioxy-*cis*-4-acetoxymethyl-2-acetoxypyrrolidine

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Abstract: The compound $C_{14}H_{17}NH_{21}$, a branched-chain sugar containing nitrogen as the heteroatom of the ring, shows an equilibrium between two conformers in which the 1-acetyl group bonded to N is *cis*, with the CH_3 near C_2 and O near C_5 , or *trans*, with the CH_3 near C_5 and O near C_2 . The structure of the more stable isomer, which is crystalline, is shown to have the *cis* conformation. The absolute configuration of the molecule was not established. There are four molecules in an orthorhombic unit cell having dimensions $a = 11.843$, $b = 16.393$, and $c = 8.246$ Å. The space group is $P2_12_12_1$, and the final value of $R = \Sigma ||F_o| - |F_c|| / \Sigma |F_o|$ is 0.084 for the 1153 independent X-ray diffraction maxima.

As part of an effort to synthesize *N*-acetylpyrrolidines and their nucleosides, Halford, Ball, and Long have prepared a branched-chain sugar having nitrogen as the heteroatom of the ring,^{1,2} 1-acetyl-*trans*-3,*trans*-

(1) M. H. Halford, D. H. Ball, and L. Long, *Chem. Commun.*, 255 (1969).

4-isopropylidenedioxy-*cis*-4-acetoxymethyl-2-acetoxypyrrolidine (NAP). This molecule contains an N-C-O linkage characteristic of amides and polypeptides. Two isomers exist in solution, related by a high barrier

(2) D. H. Ball, F. A. Carey, I. L. Klundt, and L. Long, *Carbohydr. Res.*, 10, 121 (1969).