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Preliminary communication

Measurement of self-diffusion coefficients of carbohydrate molecules in solution using an unmodified high resolution n.m.r. spectrometer

LAURANCE D. HALL and STANLEY D. LUCK

Department of Chemistry, University of British Columbia, Vancouver, British Columbia, V6T 1Y6 (Canada)

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The complete characterization of the properties of any molecule in solution includes, in addition to the determination of its primary structure and solution geometry, measurement of its rate of tumbling about its molecular axes and of its translational diffusion through the medium in which it is dissolved. The use of commercially available high resolution n.m.r. instrumentation to evaluate all but the last parameter is commonplace. We now demonstrate that the same spectrometer and probes can be used without modification to measure translational diffusion coefficients for many carbohydrate derivatives; such measurements are illustrated here by studies of α,β-D-glucose in aqueous ethanolic solution.

We have adopted the "pulsed field-gradient" method of Stejskal and Tanner[1], summarized in Fig. 1. In essence, this is the two-pulse spin-echo sequence of Hahn, with the addition of a linear field gradient applied equally during both the "dephasing" and "refocusing" periods. The crucial point is that the molecules under study are at all times changing their positions within the sample due to Brownian motion. In the absence of the applied gradients, the 180° pulse causes a refocusing, during the second Δ-period, of the magnetization which has become dephased during the first Δ-period as a result of the residual inhomogenieties (ΔB_0) in the static magnetic field (B_0). This refocusing effect is incomplete because of spin–spin relaxation and the translational motion of the molecules in the inhomogeneous field. The linewidth of 4.4 Hz from a sample of water in an n.m.r. tube, measured in our magnet after it is shimmed, corresponds to an effective background gradient of 28 ±6 $\mu T \cdot m^{-1}$ (ref. 2); the natural linewidth of water is 0.13 Hz. In

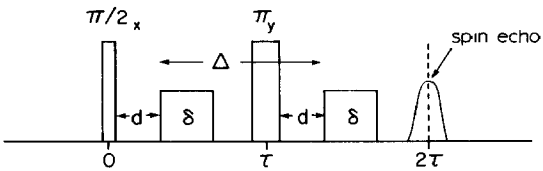

Fig. 1. Pulse sequence showing the duration of the gradient, δ, which is incremented, the variable delay, d, which is decremented to keep Δ fixed, and the time, 2τ, at which the spin echo is obtained. For a flip angle of 90°, the duration of the rf pulse was 9.0 μs. The measured rise of the gradients was 10 ms, and δ ranged from 30 to 300 ms (Δ = 350 ms).

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the measurement of self-diffusion coefficients, the attenuation of the amplitude of the spin echo due to the translational motion of the molecules is dramatically enhanced by the application of large linear field gradients. The intensity (A) of the observed spin echo is related to the duration (δ) and field strength of the applied gradient (G), the time between the $\pi/2$ and π-pulses (Δ), and the translational diffusion coefficient (D); explicitly[3],

$$A = \text{const} \cdot f(\Delta) \cdot \exp\left\{\frac{2\Delta}{T} - \gamma^2 D G^2 \delta^2 (\Delta - \delta/3)\right\} .$$

The function $f(\Delta)$ represents any J-modulation of the spin echo. For a constant value of Δ, this simplifies to

$$A' = \text{const}' \cdot \exp\{-\gamma^2 D G^2 \delta^2 (\Delta - \delta/3)\} , \qquad (1)$$

so that a plot of $\ln A'$ versus $\delta^2(\Delta - \delta/3)$ has slope $-\gamma^2 D G^2$.

The gradients in this work were produced using the normal, room-temperature x-shim coils of our Oxford Instruments 6.2 T magnet, which are designed to produce linear corrections for field inhomogeneity and hence give linear gradients. The sample tube was not spun to avoid averaging of the gradient and to ensure Brownian motion of the molecules. The gradients were calibrated by performing the pulsed field-gradient experiment for a sample of known diffusion coefficient. Using published data for the self-diffusion of water[4], we found that gradients of up to ~ 1.0 mT·m^{-1} are generated by the x- and y-shim coils; these can be turned on under computer control to any value during a pulse sequence using either the "homospoil" control, or the appropriate shimming function. Coils can be constructed to produce much larger gradients[5].

Self-diffusion coefficients of benzene (2.15×10^{-9} m^2·s^{-1}) and ethanol (0.72×10^{-9} m^2·s^{-1}), measured using this system, are in reasonable agreement with published data[5,6] (2.23×10^{-9} and 1.0×10^{-9} m^2·s^{-1}, respectively), bearing in mind the lack of a temperature-controlling device. All experiments were done at 18–21°. The spectra and data for an aqueous solution of α,β-D-glucose (M) and ethanol (M) serve to illustrate the method (Figs. 2 and 3). The size of the water peak in the spectra is small because of chemical exchange with hydroxyl protons of ethanol and glucose. The unusual appearance of the peaks in the stacked plot for the anomeric region of the spectrum is J-modulation of the spin echo due to the various coupling constants. The full spectrum shown in Fig. 2 was obtained by calculation of the power spectrum. Linear, least-squares fit of the data to Eq. *1* yielded values for the diffusion coefficients of ethanol and α- and β-D-glucose (Fig. 3). Measurements of diffusion coefficients for molecules of the size of glucose (mol. wt. 180.16) are at the limit of the present system. It is too soon to claim categorically that the difference between the measured D values for α- and β-glucose is of structural significance. Nevertheless, we note that it is beyond error and draw attention to its potential importance as a means for differentiating between solvent–solute interactions. This and other extensions of this study are under active investigation.

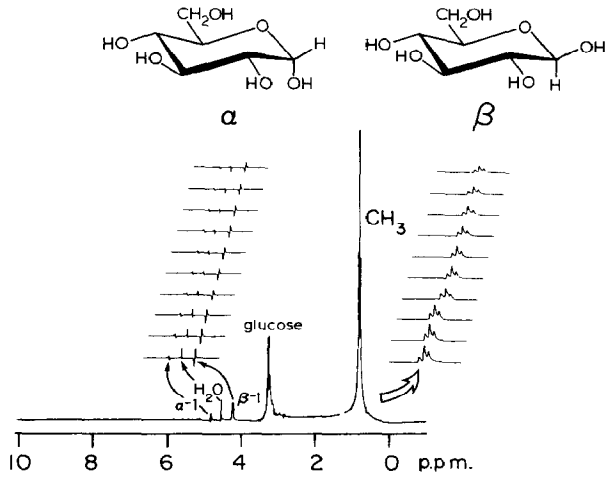

Fig. 2. Spectra of an aqueous solution of D-glucose (1.0M) and ethanol (1.0M) obtained using the sequence of Fig. 1.

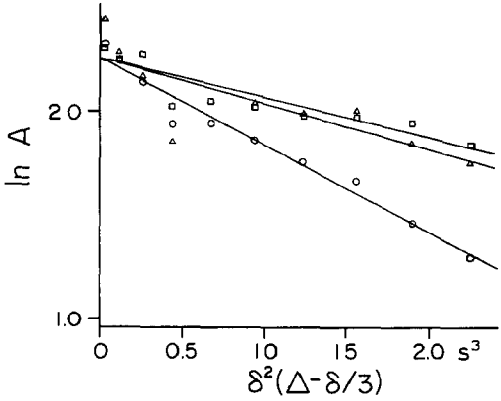

Fig. 3. Echo-attenuation data for H-1α (□), H-1β (△), and -CH_3 (○) in an aqueous solution of D-glucose (M) and ethanol (M). All data were obtained at ambient temperature. The self-diffusion coefficients are respectively (0.27 ±0.09) × 10^{-9}, (0.32 ±0.17) × 10^{-9}, and (0.63 ±0.08) × 10^{-9} $m^2 \cdot s^{-1}$.

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