Analytical Methods

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Quantitative Interpretation of Diffusion-Ordered NMR Spectra: Can We Rationalize Small Molecule Diffusion Coefficients?**

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Diffusion-ordered spectroscopy (DOSY), which separates NMR signals according to diffusion coefficient, is finding increasing use for the analysis of mixtures of small- to medium-sized molecules.[1–4] The diffusion information in DOSY spectra is usually used purely qualitatively, to identify signals that come from the same species, in part because there is no simple relationship between diffusion coefficient and molecular structure. Here we describe a simple method for rationalizing small molecule diffusion coefficients, sufficiently accurate to allow common questions—roughly what is the molecular weight (MW) of this unknown? is this species a monomer or a dimer? are these species associating?—to be addressed.

Figure 1 shows the result of applying the new model to the interpretation of a 500 MHz ^{1}H DOSY spectrum of a mixture, technical grade "monoacetin", in D_2O. At the left of the spectrum is the conventional scale showing the experimental diffusion coefficient, at the right the calculated molecular weight scale. The dashed lines show the experimental diffusion coefficients of the main species present, the two isomers of monoacetin, the two isomers of diacetin, triacetin, and glycerol. The filled circles show the actual MWs of the four types of species, and confirm that the new model shows sufficiently good agreement with experiment to be of direct use in spectral interpretation.

The basis of the new model is as follows. At first sight the relationship between molecular size and diffusion coefficient is straightforward. The Stokes–Einstein Equation (1)[5]

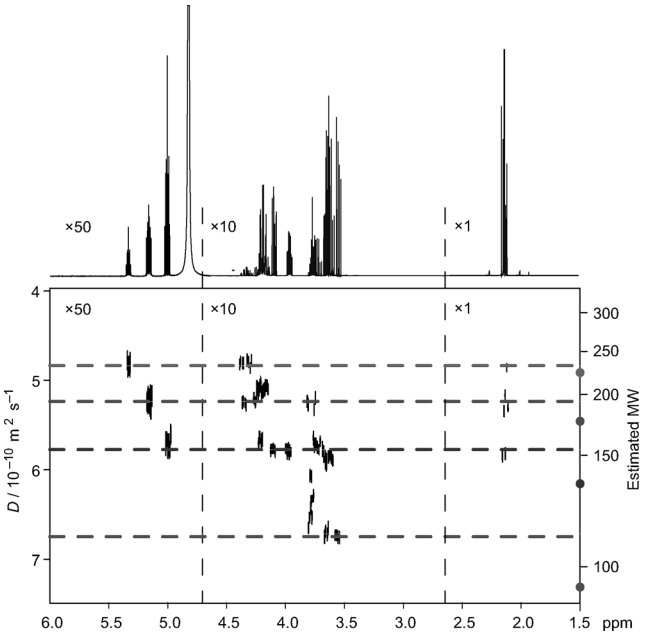

Figure 1. 500 MHz ^{1}H DOSY spectrum of technical grade monoacetin in D_2O. Vertical scales of the 1D (top) and DOSY (bottom) spectra are expanded as indicated to show all significant signals clearly. Dashed lines show the experimental diffusion coefficients and solid circles the diffusion coefficients predicted for the four types of species present (see section 7 in the Supporting Information).

$$D = \frac{k_B T}{6\pi\eta r f} \quad (1)$$

balances the thermal energy of random molecular motion against the friction acting on a hard sphere of hydrodynamic radius r moving through a continuum fluid of viscosity η at temperature T, where k_B is the Boltzmann constant and the friction factor f is unity for a hard sphere.

Unfortunately, real molecules in real solvents are not hard spheres moving through a continuum fluid. Figure 2 shows a comparison between diffusion coefficients of a range of small organic molecules estimated using the Stokes–Einstein equation and those measured using pulsed field gradient (PFG) NMR techniques. Species were chosen to be representative of those commonly encountered in synthetic and pharmaceutical NMR laboratories, but excluding those with heavy (> Cl) atoms or known to aggregate in dilute solution; hydrodynamic radii were estimated assuming spherical molecules (see sections 1, 2, and 4 in the Supporting Information for further information). The data show that the Stokes–Einstein equation is a poor guide to small molecule diffusion,

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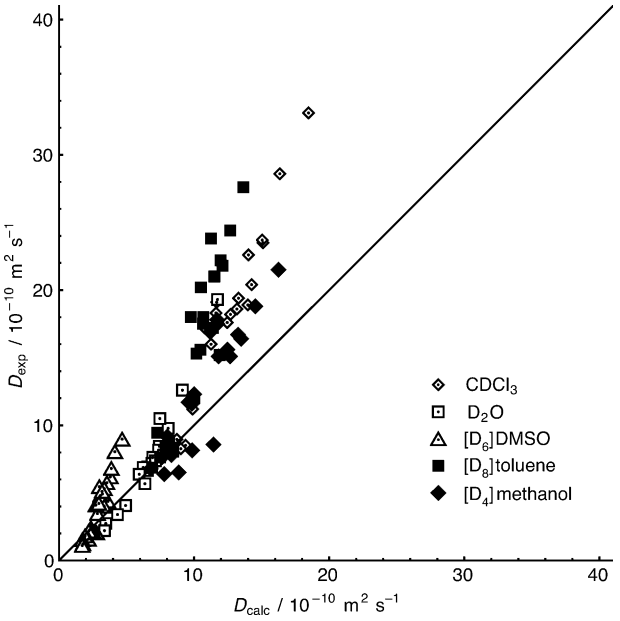

Figure 2. Measured diffusion coefficient plotted against diffusion coefficient calculated using the Stokes–Einstein equation for 109 samples of 44 small molecules in five deuterated solvents, with a solid line of unit slope (DMSO = dimethyl sulfoxide).

but there are clear systematic trends that it should be possible to rationalize. (The latter become even clearer if the data are scaled by solvent viscosity before plotting; see section 4 in the Supporting Information).

The reasons for the failure of Equation (1) are multiple. They include breakdown of the continuum approximation, since the solvent molecules are not infinitely small compared to the solute; nonspherical geometry and/or flexibility of solute molecules; and solvation, that is, association of the solvent with the solute. It follows therefore that any simple framework for interpreting small molecule diffusion will require a more or less brutal simplification, treating some or all of these problems empirically.

Analytical theories exist both for shape effects and for the breakdown of the continuum approximation. The classic analysis of shape effects by Perrin[6] shows that these are relatively small for typical small molecules, only becoming important when aspect ratios are high (e.g. for long rigid linear molecules or wide thin disks). For aspect ratios less than three, changes in D are less than 10%. The finite size of solvent molecules, on the other hand, has a big effect on small molecule diffusion. As was shown by Gierer and Wirtz,[7] the effect of moving from negligible to large solvent molecule size is to change the friction factor f in Equation (1) to get Equation (2),

$$f_{GW} = \left(\frac{3r_S}{2r} + \frac{r}{r + r_S}\right)^{-1} \tag{2}$$

where r and r_S are the solute and solvent radius, respectively, effectively reducing the numerical factor in the Stokes–Einstein denominator. For equal sizes of solute and solvent the effect is to reduce the factor from 6 to 3, well beyond the transition from "stick" to "slip" boundary conditions (changing the factor from 6 to 4) sometimes invoked for small solutes; in the case of H_2 in water, for example, the numerical factor is reduced over fivefold.[8] While the effects of flexibility in long chains are well-understood,[9] in small molecules neither conformational flexibility nor solvation effects are amenable to analytical treatment. Both lead to slower diffusion than would be expected for a hard sphere.

A number of empirical methods for relating diffusion coefficients to the MW have been proposed. For condensed species with a MW larger than 1000, Polson[10] suggested a hard-sphere model with an empirical density of 825 $kg\,m^{-3}$, noting though that this model fails for smaller species. Chen and Chen[11] suggested an empirical expression adapted from the Gierer–Wirtz–Stokes model to fit experimental data for crown ethers in alcohols (see section 4 in the Supporting Information). This equation has proved popular, particularly for organometallic applications,[12,13] despite being parameterized for a very restricted set of compounds (for which r is indeed not expected to scale as $MW^{1/3}$);[9] for the set of small molecules studied here it consistently overestimates D (see section 4 in the Supporting Information). Probably the most powerful class of relations are those that express D[13–16] or the relative diffusion coefficient[17] as proportional to an empirical power of MW. Such power laws can give excellent results if parameterized for a specific class of compounds, for example, a homologous series, in a single solvent. In contrast to Flory-Huggins theory, the exponent, or fractal dimension, reflects all of the complicating factors noted above, rather than being determined solely by chain flexibility and solvation.

Here in contrast we seek an approximate but general expression relating diffusion coefficient to the MW for a wide range of small molecules for any solvent. The starting points are that the single largest source of error in the simple Stokes-Einstein approach, the breakdown of the continuum model, can and should be treated analytically; that for a general relation between D and MW, shape factors will have to be ignored and species assumed to be spherical; and that, as implicitly acknowledged by Polson, the average effects of flexibility, solvation, and asphericity can be approximated by a reduction in the effective density of the solute molecule. One complication is that the Gierer–Wirtz model assumes knowledge of the solvent molecule radius r_S, but since the solute radius is to be calculated assuming that all small molecules have the same effective density, the same logic can be applied to the solvent. The model then requires just a single parameter ρ_{eff}, the effective density of a small molecule, allowing for packing effects, geometry, solvation, and flexibility, together with the viscosity, η, the molecular weight MW_S of the solvent used, and N_A, the Avogadro number, to give an estimate of D for a given solute MW [Eq. (3)].

$$D = \frac{k_B T\left(\frac{3\alpha}{2} + \frac{1}{1+\alpha}\right)}{6\pi\eta\sqrt[3]{\frac{3MW}{4\pi\rho_{eff}N_A}}}, \text{ where } \alpha = \sqrt[3]{\frac{MW_S}{MW}} \tag{3}$$

This model has the advantages of simplicity, generality of application, a simple physical interpretation with a well-

established theoretical basis, and a single adjustable parameter (see section 4 in the Supporting Information for further details and an explanation of the model). An Excel spreadsheet allowing D to be estimated from MW and vice versa is available for download from the authors' web site (see section 9 in the Supporting Information).

Figure 2 shows the result of calculating D for the 109 different combinations of 44 solutes and 5 deuteriated solvents studied. Numerical optimization gave an effective density $\rho_{\text{eff}} = 619\ \text{kg m}^{-3}$, giving a root-mean-square (rms) difference between estimated and experimental diffusion coefficients of 15%. Remarkably, the quality of agreement is similar to that obtained by fitting the data to a different power law for each solvent, using a total of 10 adjustable parameters (see section 4 in the Supporting Information). This is in part because the power law approach makes no direct allowance for the known effects of the breakdown of the continuum approximation (which also materially reduces the effectiveness of using tetramethylsilane (TMS) as a diffusion reference).

Clearly no method that fails to account explicitly for factors such as shape, flexibility, and solvation can hope to reproduce experimental diffusion coefficients accurately. Nevertheless, the simple method outlined above performs sufficiently well to give chemically useful answers to all of the questions posed in the first paragraph of this article. Significant homomolecular or heteromolecular associations will give rise to experimental D values less than those estimated, allowing such association to be detected. The performance of the method could be improved, at the expense of some added complication, by making allowance for the presence of heavy atoms, particularly in the case of chloroform where the assumption of light atoms is stretched to breaking point, giving rise to the significant deviations from ideal performance at the top right of Figure 3. One such possibility is to treat chloroform as a special case and assign to it a reduced effective MW_s (see section 8 in the Supporting Information). Clearly the conclusions derived here apply only to isotropic unconstrained diffusion, as encountered in DOSY experiments; in constrained diffusion, as exploited in diffusion-weighted magnetic resonce imaging,[18] further complications arise.

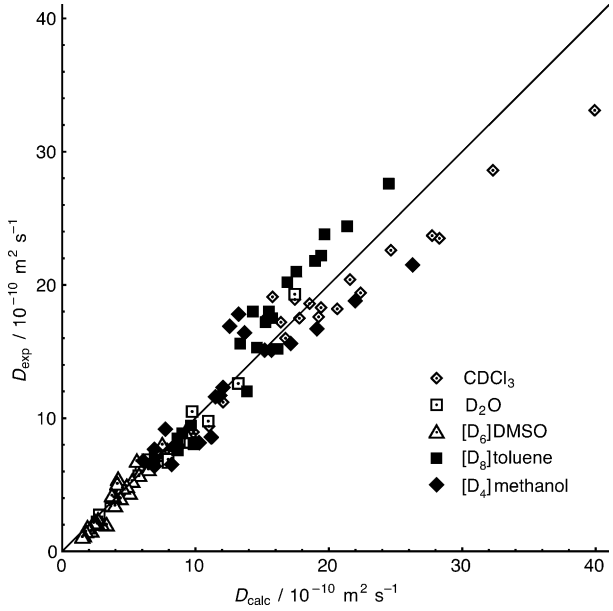

Figure 3. Measured diffusion coefficient plotted against diffusion coefficient calculated using Equation (3) for 109 samples of 44 small molecules in five deuterated solvents, with a solid line of unit slope.

Figure 1 flatters the new method somewhat: since it compares chemically cognate species, differing only in the degree of acetylation, the degree of agreement seen between prediction and experiment is considerably better than the 15% rms deviation found across the full chemical space spanned by the training set of species listed in the Supplementary Information. Nevertheless it is a realistic and effective illustration of just the sort of way in which the new method can be used. The degree of agreement is in fact even better than shown, in that no allowance has been made for the relatively high concentration of solute, which causes obstruction effects and reduces the experimental diffusion coefficients compared to predictions. The new method is appealing in its simplicity, offers chemically useful results with a minimum of effort, and should significantly enhance the use of DOSY measurements.

Experimental Section

The monoacetin sample consisted of 3% v/v technical grade monoacetin (Acros Organics) in D_2O with 92 mM sodium [D_4]trimethylsilylpropanoate (TSP) as reference. DOSY measurements were carried out nonspinning on a Varian VNMRS 500 MHz spectrometer at 296 K using the Oneshot[19] DOSY pulse sequence. Data were acquired with an array of 16 gradient amplitudes ranging from 3.0 G cm^{-1} to 27.0 G cm^{-1} in equal steps of gradient squared, using 512 transients, 65536 complex data points, a total diffusion encoding gradient of 1 ms, and a diffusion time of 0.3 s with a total experiment time of 20 h. DOSY spectra were constructed using correction for the effects of pulsed field gradient non-uniformity.[20]

The diffusion coefficients of 44 different solutes were measured as 1% w/w solutions in five deuteriated solvents, in a total of 109 diffusion samples. Diffusion measurements were carried out nonspinning using the Oneshot[19] sequence on a Varian Unity 400 MHz spectrometer equipped with a 30 G cm^{-1} gradient coil, using temperature control at 298 K. Samples were allowed to equilibrate to the specified temperature before any measurements were made. Thick-walled 5 mm NMR tubes were used to eliminate convection, and the sample temperature was calibrated using deuteriomethanol.[21] Processing was carried out using the manufacturer's VnmrJ software, modified to correct for spatially nonuniform pulsed field gradients.[20] Ten magnetic field gradient amplitudes from 3.0 to 27.3 G cm^{-1} were used and incremented in equal steps of gradient squared. For each gradient amplitude, 32 transients of 32768 complex data points were acquired. The values of diffusion delay (Δ) and gradient pulse duration (δ) were chosen appropriately for the value of D, as described in section 6 of the Supporting Information.

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