

Quantitative Interpretation of Diffusion-Ordered NMR Spectra: Can We Rationalise 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 rationalising 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 ¹H DOSY spectrum of a mixture, technical grade "monoacetin", in D₂O. 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]

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

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.

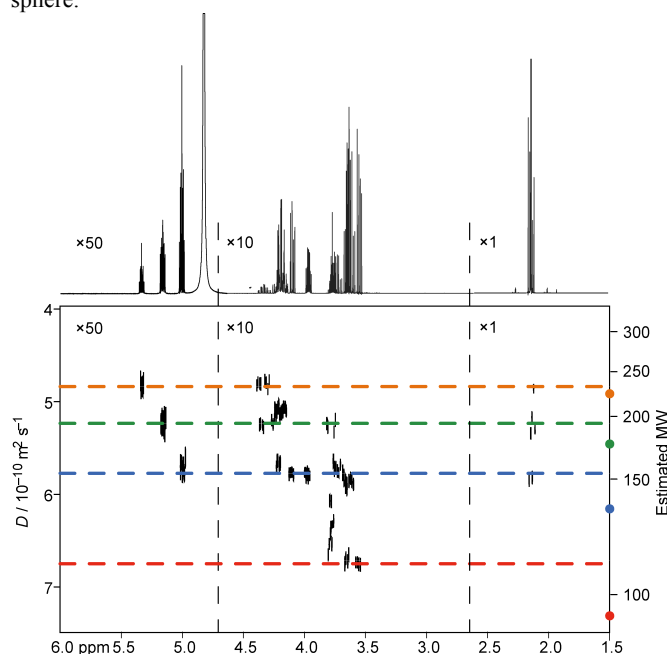


Figure 1 500 MHz ¹H DOSY spectrum of technical grade monoacetin in D₂O. Vertical scales of the 1D (top) and DOSY (bottom) spectra are expanded as indicated in order to show all significant signals clearly. Dashed lines show the experimental diffusion coefficients, and solid circles the diffusion coefficients predicted for the four types of species present (S1-S6, see Supporting Information SI 7).

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 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 Supporting Information [SI] 1, 2 and 4 for further information). The data show that Stokes-Einstein equation is a poor guide to small molecule

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[**] This work was supported by the Engineering and Physical Sciences Research Council [grant numbers EP/H024336/1 and EP/E05899X/1] and by Givaudan Flavors and Fragrances. AMcL thanks AstraZeneca for a studentship.

Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.

diffusion, but there are clear systematic trends that it should be possible to rationalise. (The latter become even clearer if the data are scaled by solvent viscosity before plotting - see SI 4).

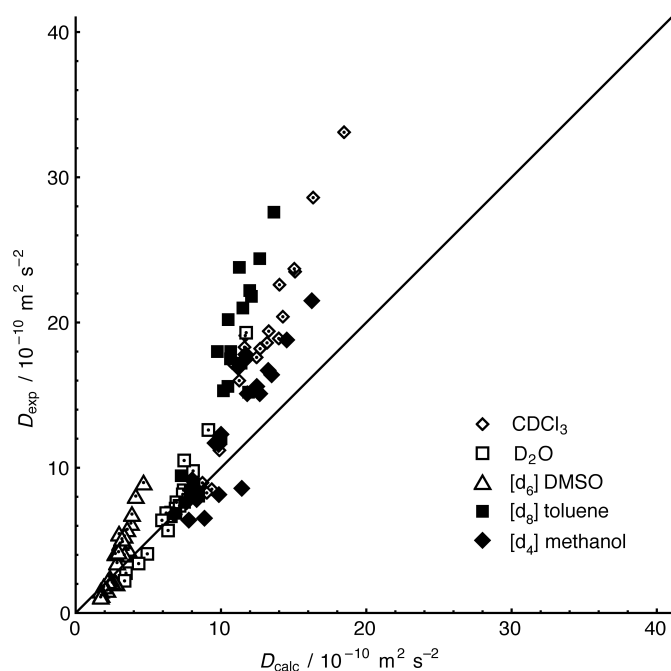


Figure 2. Measured diffusion coefficient plotted against diffusion coefficient calculated using the Stokes-Einstein equation for 110 samples of 44 small molecules in 5 deuteriated solvents, with a solid line of unit slope.

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; non-spherical geometry and/or flexibility of solute molecules; and solvation, i.e. 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 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 eq. (1) to

$$f_{\text{GW}} = \left(\frac{3r_{\text{S}}}{2r} + \frac{r}{r+r_{\text{S}}} \right)^{-1} \quad (2),$$

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 MW have been proposed. For condensed species with $\text{MW} > 1000$, 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 SI 4). This equation has proved popular, particularly for organometallic applications^[12,13], despite being parameterised for a very restricted set of compounds (for which r is indeed not expected to scale as $\text{MW}^{1/3}$ ^[9]); for the set of small molecules studied here it consistently overestimates D (see SI 4). Probably the most powerful class of relations is those that express D ^[13-16] or relative diffusion coefficient^[17] as proportional to an empirical power of MW. Such power laws can give excellent results if parameterised for a specific class of compound, e.g. 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 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 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:

$$D = \frac{k_{\text{B}}T \left(\frac{3\alpha}{2} + \frac{1}{1+\alpha} \right)}{6\pi\eta \sqrt[3]{\frac{3\text{MW}}{4\pi\rho_{\text{eff}}N_{\text{A}}}}}, \quad \text{where } \alpha = \sqrt[3]{\frac{\text{MW}_{\text{S}}}{\text{MW}}} \quad (3)$$

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 SI 4 for further details and 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 SI 9).

Figure 2 shows the result of calculating D for the 109 different combinations of 44 solutes and 5 deuteriated solvents studied. Numerical optimisation gave an effective density $\rho_{\text{eff}} = 619 \text{ kg m}^{-3}$, giving an 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 SI4). 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 TMS as a diffusion reference).

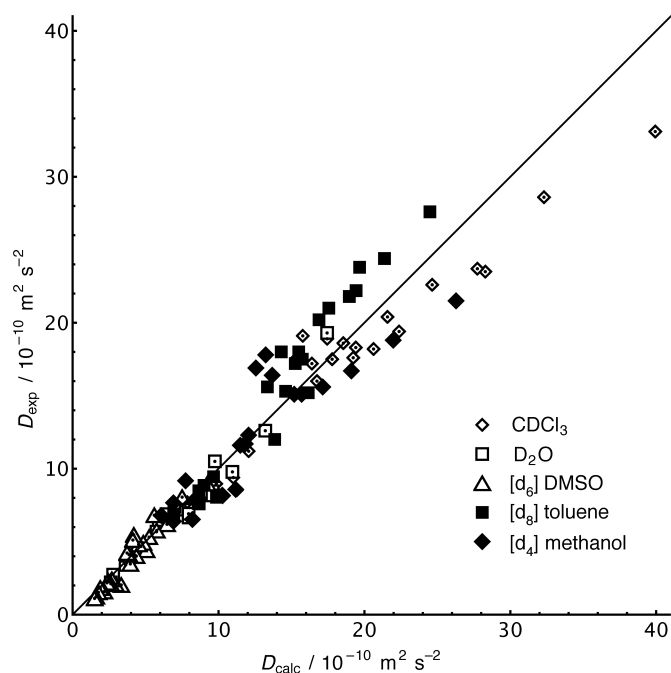


Figure 3. Measured diffusion coefficient plotted against diffusion coefficient calculated using eq. (3) for 110 samples of 44 small molecules in 5 deuteriated solvents, with a solid line of unit slope.

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 paper. Significant homomolecular or heteromolecular association 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 SI 8). Clearly the conclusions derived here apply only to isotropic unconstrained diffusion, as encountered in DOSY experiments; in constrained diffusion, as exploited in diffusion-weighted MR imaging^[18], further complications arise.

Figure 1 flatters the new method somewhat: since it compares chemically cognate species, differing only in 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 utility 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 non-spinning 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 5 deuteriated solvents, in a total of 110 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 non-uniform pulsed field gradients^[20]. 10 magnetic field gradient amplitudes from 3.0 to 27.3 G cm^{-1} were used, 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.

Received: ((will be filled in by the editorial staff))
Published online on ((will be filled in by the editorial staff))

Keywords: NMR Spectroscopy · Analytical Methods · DOSY · Diffusion · Molecular Weight

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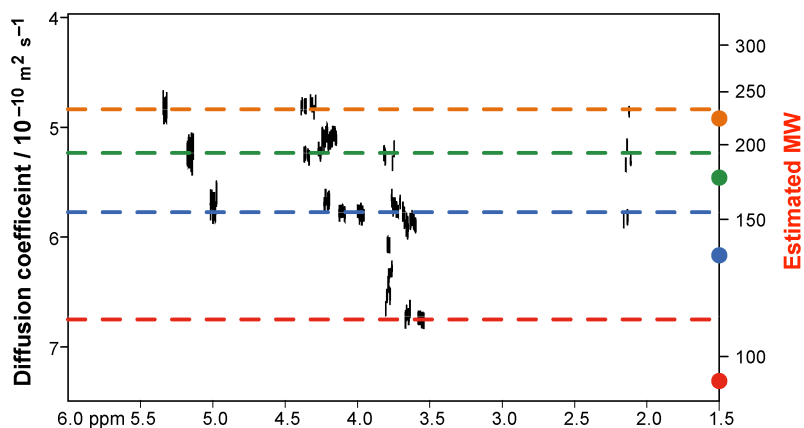
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Entry for the Table of Contents

Quantitative DOSY

Robert Evans, Zhaoxia Deng,
Alexandria K. Rogerson, Andy S.
McLachlan, Jeff J. Richards, Stephan
Haiber, Mathias Nilsson and Gareth A.
Morris _____ **Page – Page**

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



A new method is described that allows the diffusion coefficient of a small molecule to be estimated given only the molecular weight and the viscosity and molecular weight of the solvent, used allowing quantitative interpretation of the diffusion domain of DOSY spectra.