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Communication

Molecular mass estimation by PFG NMR spectroscopy

Christopher A. Crutchfield, Douglas J. Harris *

Cytec Industries, Inc., Research and Development, 1937 West Main Street, Stamford, CT 06904, USA

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Abstract

A simplified PFG NMR diffusion analysis method was developed to estimate the molecular mass of small molecules in dilute aqueous and organic solutions. Internal referencing was utilized to improve the experimental robustness and simplify the data analysis. Specifically, tetramethylsilane (TMS) and HDO were chosen for the organic and aqueous reference molecules, respectively. Relative diffusivity–molecular mass correlations were empirically developed in the range of 2–1280 g/mol for dilute $CDCl_3$ and D_2O solutions. The median error in the predicted molecular mass was found to be 10 rel%. The utility of the method was demonstrated by analyzing the major and minor components in olive oil.

Keywords: Molecular mass; Diffusion; DOSY; Relative diffusivity; PFG NMR

1. Introduction

Diffusion ordered spectroscopy (DOSY) [1–4], obtained through pulsed field gradient (PFG) NMR experiments [5,6], separates components on the basis of chemical shift and self-diffusion coefficient, D. The friction factor in the Stokes–Einstein equation [7] leads to an expected inverse relationship between D and both effective hydrodynamic radius, R_H, and solution viscosity. There is a *qualitative* understanding that bigger molecules have larger values of R_H and hence diffuse slower. This work empirically establishes *quantitative* correlations between diffusion and molecular mass for common small organic and water-soluble molecules in the two most frequently used NMR solvents, $CDCl_3$ and D_2O.

Estimation of molecular mass by PFG NMR cannot have the precision of mass spectrometry, but nevertheless has great utility in structure determination and resonance assignment. In regards to the former, molecular mass is beneficial for identifying symmetric molecules, e.g. cyclohexane or diethyl ether. In regards to the latter, spectral resonances observed in complex chemical mixtures can be assigned from the estimated molecular mass in conjunction with knowledge obtained from multidimensional NMR spectra, mass spectrometry, or the reaction chemistry.

For routine application, the PFG NMR molecular mass estimation method must be facile and the typical accuracy should be better than 30%. In the authors' opinion, the currently practiced methodology cannot achieve either requirement. Therefore, the method was optimized to maximize robustness and minimize experiment time. Internal reference molecules, such as tetramethylsilane (TMS) or water, are conveniently present in common NMR solvents. Diffusion analysis can be simultaneously conducted on both the reference and the analyte, leading to improved accuracy without additional experiment time.

The internal references provide several additional benefits. As shown in prior work [8,9], the ratio of diffusion coefficients for the reference molecule and an analyte is independent of solution viscosity. Jones et al. [8] defines this ratio, properly termed *relative diffusivity,* D_{rel} as:

$$D_{rel,ref} = D_{ref}/D_X \quad (1)$$

where ref and X correspond to the referencing species and the analyte, respectively. Expressed in this fashion, the

* Corresponding author. Fax: +1 203 321 2979.
E-mail address: douglas.harris@cytec.com (D.J. Harris).

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ratio approximately corresponds to the relative size of the analyte in comparison to the reference: $D_{rel,ref} \approx R_{H,X}/R_{H,ref}$. Determination of D_{rel} does not require knowledge of gradient strength, gyromagnetic ratio, gradient pulse duration, or diffusion time. This leads to experimental simplicity. Furthermore, the observed values of D_{rel} are typically in a numerical range suitable for human cognition.

This work presents a facile, accurate, and sensitive PFG NMR method for measuring relative diffusivity. The robustness of the relative diffusivity measurement was demonstrated for analytes in 10 common organic NMR solvents. Values obtained for a plethora of molecules in dilute $CDCl_3$ and D_2O solutions were used to establish relative diffusivity–molecular mass calibration curves. Finally, the utility of the method was demonstrated by characterizing major and minor components in a complex mixture, specifically olive oil.

2. Experimental

Experiments utilized a Bruker Avance-400 spectrometer operating at a ^1H frequency of 400.1 MHz, equipped with a 5-mm broadband observe (BBO) z-axis gradient probe capable of generating 55 G/cm field strengths. *Samples were not spinning during the analyses*. The motion caused by sample spinning leads to erroneous diffusion measurements. The probe temperature, calibrated with neat 1,2-ethanediol [13], was maintained at 30 °C. The sample temperature gradient was determined to be $\leqslant$0.03 °C/cm by ^{59}Co NMR analysis of $K_3Co(CN)_6$ [14]. A 1D convection-compensating double stimulated-echo pulse sequence [10] with three 0.6 ms sinusoidal-shaped spoiler gradient pulses (*dstegp3s1d* in the standard Bruker pulse sequence library) was used to obtain the spectra. Sinusoidal gradient pulses with durations of 2.5 ms were used for each of the four diffusion pulses, δ. The diffusion time, Δ, was 50 ms. Gradient strengths of 6% and 45% of maximum power were used to obtain spectral pairs with acquisition times of 2 s and recycle delays of 8 s. The experiment time to acquire a pair of PFG NMR spectra with eight scans and four dummy scans was 4 min, not including a 5-min temperature-equilibration delay.

3. Results

To illustrate the accuracy and simplicity of the modified relative diffusivity determination method, Fig. 1 shows the Stejskal–Tanner attenuation plot [5] for TMS, methanol, and dodecanol obtained using a convection-compensating PFG NMR pulse sequence [10]. Linear regression of the data obtained the following relative diffusivity values: methanol, $D_{rel,TMS} = slope_{TMS}/slope_{methanol} = -5.42/-8.27 = 0.66$; dodecanol, $D_{rel,TMS} = -5.42/-3.47 = 1.56$. Data analysis using non-linear least squares yielded similar, but likely more accurate values: 0.67 and 1.64, respectively.

Observe that utilization of a convection-compensated PFG NMR pulse sequence produces excellent linearity

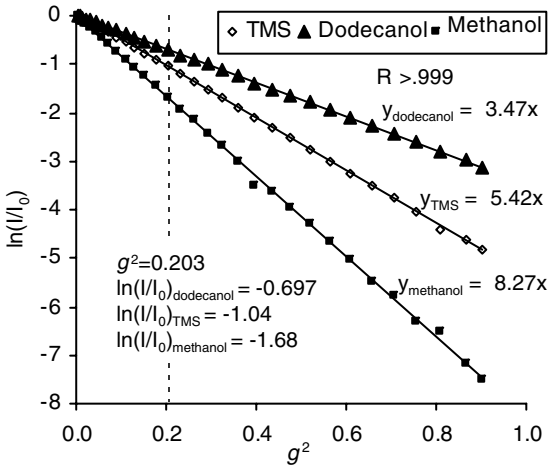

Fig. 1. Stejskal–Tanner plot of signal attenuation in dilute $CDCl_3$. g represents fraction of maximum gradient field strength, 55 G/cm.

for signal attenuation. Because of this linearity, relative diffusivity can be accurately determined from two spectra obtained at low and high gradient strengths, g:

$$D_{rel} = \frac{D_{ref}}{D_X} = \frac{\ln(I_{ref,g=high}/I_{ref,g=low})}{\ln(I_{X,g=high}/I_{X,g=low})} \quad (2)$$

where I is the height or area of the peaks in question. Appropriate choice of high gradient field strength enables precise measurements of both D_{ref} and D_X. Optimal precision was achieved when the high gradient strength leads to attenuation in the range of $e^{-0.3}$ to e^{-2}. In Fig. 1, the best data was obtained at $g^2 = 0.2$. Eq. (2) was used to determine $D_{rel,TMS} = 0.67$ and 1.61 for methanol and dodecanol, respectively. These values are within 2% of those found from non-linear regression. In addition to retaining high accuracy, this simplified method also achieves good precision, typically ±2 rel%. Acquisition of just two spectra reduces the experiment time or improves sensitivity. The data in the following sections was obtained using this two spectra method.

Fig. 2 shows relative diffusivity for dilute solutions in deuterated solvents containing a mixture of acetonitrile, dodecanol, and 5-methylsalicylic acid. The observed

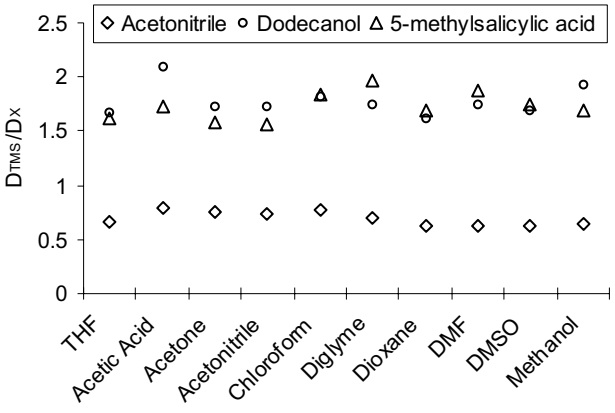

Fig. 2. Variation of relative diffusivities in 10 deuterated solvents.

relative diffusivity values are within a range of 25 rel% and the average calculated standard deviation was 11 rel%, excellent consistency considering the variation of the properties for the 10 solvents. The results demonstrate the reduced solvent dependence of the relative diffusivity method.

Relative diffusivity–molecular mass calibration curves were determined in the two most common NMR solvents, $CDCl_3$ and D_2O. A wide variety of samples ranging in molecular mass from 2 to 1280 g/mol were analyzed at 0.5 wt% concentration in these solvents. The compounds included aromatic, aliphatic, cyclic, and linear molecules. The materials were of interest to our specialty chemical company. TMS [9] and water (HDO) were chosen as internal references. Fig. 3 (bottom) shows relative diffusivity–molecular mass calibration curves generated from the experimental results. Power-law relationships between relative diffusivity and molecular mass are drawn with a best-fit mass fractal dimension value of 1.72:

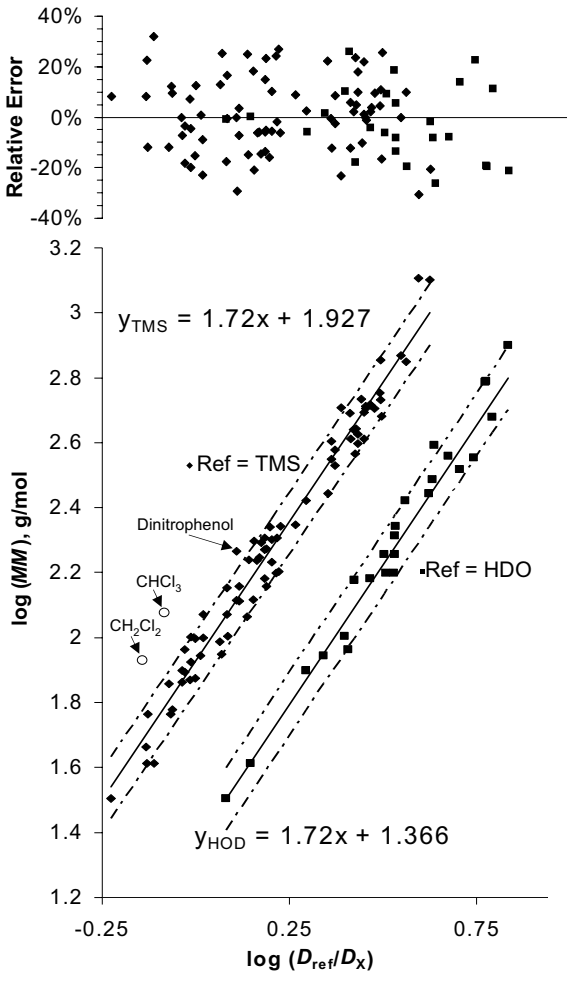

Fig. 3. Calibration curves relating molecular mass and relative diffusivity; diamonds signify the use of TMS as an internal reference ($CDCl_3$ solutions), squares signify HDO (D_2O solutions). Molecules of abnormally high density, CH_2Cl_2 and $CHCl_3$, or low density, H_2 (not shown, $D_{TMS}/D_{H_2} = 0.18$), are not included in the fit. The upper graph is a residual analysis plot that retains the same symbols.

$$MM = k_{ref} * D_{rel}^{1.72} \quad (3)$$

where $k_{TMS} = 84._5$ and $k_{HDO} = 23._2$ g/mol.

As seen in the residuals graph in Fig. 3 (top), the median error in the predicted molecular mass was found to be 10 rel%. Most of the calculated masses are within ±30 rel% from the true values. The largest source of error arises from the dependence of diffusivity on factors including molecular architecture, density, and solvent quality. Therefore, the simplistic equations fail to *fully* predict molecular mass for some samples, and large errors were observed for dense compounds such as halogenated alkanes. More complex calibration equations could be hypothesized to account for molecular structure. The sophisticated relationships would require knowledge of the molecule *a priori*, a necessity that typically cannot be fulfilled for unknown compounds.

The relative diffusivity methodology was applied to examine the major and minor components of virgin olive oil, a substance previously studied by NMR techniques [11,12]. Fig. 4 shows a ^1H PFG NMR spectrum of olive oil. For the main component, oleic triglyceride, $D_{rel,TMS} = 3.8$. Application of Eq. (3) leads to an estimated molecular mass of 820 g/mol, only 7% less than the true molecular mass of 885 g/mol. The identities of the aldehyde components, present at low abundance, have not been well established in the literature. In this sample, the average molecular mass for the alkanal with a sharp peak at $\delta^1H = 9.74$ ppm, $D_{rel,TMS} = 2.3$, was estimated from Eq. (3) to be 350 ± 100 g/mol. This mass range is far greater than expected from the tentative assignment as hexanal and heptanal [11]. The relative diffusivity value, $D_{rel,TMS} = 2.8$, of the alkanal compounds with resonances in the region $\delta^1H =$ 9.4–9.6 ppm leads to estimated average masses of 480 ± 150 g/mol. This range is outside the possible values for oxidized fragments of oleic acid. More work is required to confirm this finding, and to identify the species.

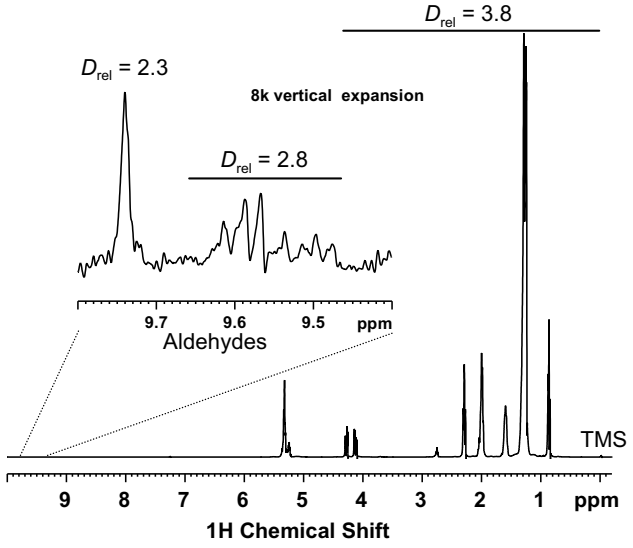

Fig. 4. ^1H PFG NMR spectrum of 5 wt% olive oil in $CDCl_3$, obtained at $g = 6\%$; 8k scans. Inlay shows scaled expansion of alkanal spectral region.

4. Discussion and conclusions

In a few minutes, this simple and robust relative diffusivity PFG NMR method can quantitatively estimate molecular mass with a typical accuracy of 10 rel%, making it a valuable addition to the NMR spectroscopist's toolbox. The calibration equations were developed for molecules in dilute $CDCl_3$ and D_2O solutions, but can be applied to analytes in other solvents, albeit with an expected lower accuracy. The described method is suitable for automated spectral analysis and molecular mass estimation routines.

Collection of spectra at only two different gradient strengths leads to more rapid data acquisition or to improved signal-to-noise compared to more standard approaches that involve the collection of a series of spectra. Even with optimal gradient settings, this approach limits the extent of relative diffusivities that can be precisely probed to those between $0.1 \leqslant D_{\mathrm{rel,ref}} \leqslant 10$. Analytes must have molecular mass below $\sim$1200 and $\sim$4000 g/mol for HDO and TMS references, respectively. Above this range, the more conventional approach must be applied. The topic of PFG NMR molecular mass determination for macromolecules is beyond the scope of this discussion, but has been extensively explored in published literature.

The authors of this work have successfully used the method in an industrial setting to identify or classify unknown compounds, even when present at trace levels in mixtures. This method also aided in assignment of observed spectral resonances to major and minor components observed in chromatography/mass spectrometry. In cases where parent ion peaks cannot be observed due to fragmentation, PFG NMR spectroscopy was more accurate than mass spectrometry!

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References

[1] S.J. Gibbs, C.S. Johnson Jr., A PFG NMR experiment for accurate diffusion and flow studies in the presence of eddy currents, J. Magn. Reson. 93 (1991) 395–402.
[2] K.F. Morris, C.S. Johnson Jr., Diffusion-ordered two-dimensional nuclear magnetic resonance spectroscopy, J. Am. Chem. Soc. 114 (1992) 3139–3141.
[3] B. Antalek, Using pulsed gradient spin echo NMR for chemical mixture analysis: how to obtain optimum results, Concepts Magn. Reson. 14 (2002) 225–258.
[4] C.S. Johnson Jr., Diffusion ordered nuclear magnetic resonance spectroscopy: principles and applications, Prog. NMR Spectrosc. 34 (1999) 203–256.
[5] E.O. Stejskal, J.E. Tanner, Spin diffusion measurements: spin echoes in the presence of a time-dependent field gradient, J. Chem. Phys. 42 (1965) 288–292.
[6] W.S. Price, Pulsed-field gradient nuclear magnetic resonance as a tool for studying translational diffusion: part 1. basic theory, Concepts Magn. Reson. 9 (1997) 299–336.
[7] A. Einstein, Investigation on the Theory of the Brownian Movement, Dover, New York, 1956.
[8] J.A. Jones, D.K. Wilkins, L.J. Smith, C.M. Dobson, Characterisation of protein unfolding by NMR diffusion measurements, J. Biomol. NMR 10 (1997) 199–203.
[9] E.J. Cabrita, S. Berger, DOSY studies of hydrogen bond association: tetramethylsilane as a reference compound for diffusion studies, Magn. Reson. Chem. 39 (2001) S142–S148.
[10] A. Jerschow, N. Müller, Suppression of convection artifacts in stimulated-echo diffusion experiments. double-stimulated-echo experiments, J. Magn. Reson. 125 (1997) 372–375.
[11] R. Sacchi, M. Patumi, G. Fontanazza, P. Barone, P. Fiordiponti, L. Mannina, E. Rossi, A.L. Segre, A high field ^{1}H nuclear magnetic resonance study of the minor components in virgin olive oils, J. Am. Oil Chem. Soc. 73 (1996) 743–758.
[12] G. Vlahov, Application of NMR to the study of olive oils, Prog. Nucl. Magn. Reson. Spectrosc. 35 (1999) 341–357.
[13] A.L. van Geet, Calibration of the methanol and glycol nuclear magnetic resonance thermometers with a static thermistor probe, Anal. Chem. 40 (1968) 2227–2229.
[14] W.J. Goux, L.A. Verkruyse, S.J. Saltert, The impact of Rayleigh–Benard convection on NMR pulsed-field-gradient diffusion measurements, J. Magn. Reson. 88 (1990) 609–614.