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Formula Weight Prediction by Internal Reference Diffusion-Ordered NMR Spectroscopy (DOSY)

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Abstract: Formula weight (FW) information is important to characterize the composition, aggregation number, and solvation state of reactive intermediates and organometallic complexes. We describe an internal reference correlated DOSY method for calculating the FW of unknown species in different solvents with different concentrations. Examples for both the small molecule (DIPA) and the organometallic complex (aggregate **1**) yield excellent correlations. We also found the relative diffusion rate is inversely proportional to the viscosity change of the solution, which is consistent with the theoretical Stokes−Einstein equation. The accuracy of the least-squares linear prediction r^2 and the percentage difference of FW prediction are directly related to the density change; greater accuracy was observed with decreasing density. We also discuss the guidelines and other factors for successful application of this internal reference correlated DOSY method. This practical method can be conveniently modified and applied to the characterization of other unknown molecules or complexes.

Introduction

Formula weight (FW) of reactive intermediates and organometallic complexes is important to characterize their composition, aggregation number, and solvation state. This information can be used to rationalize the reactivity and reaction pathways of these reactive species. The FW of a complex is intuitively correlated to its volume or size and hence to its diffusion in solution. Diffusion-ordered NMR spectroscopy (DOSY) is a 2D NMR experiment developed by Johnson in 1992 to measure diffusion coefficients and deduce the hydrodynamic radii of molecules in solution.[1] In this two-dimensional NMR technique, one dimension represents typical chemical shift information and the second dimension distinguishes species by diffusion coefficient, which is related to their particle sizes. Recent review articles surveyed the current state of the art of the DOSY technique and its application in chemistry and biological sciences.[2] Upon discovering the benefits of DOSY, it is easy to appreciate why one polymer chemist has called this technique "chromatography by NMR" because NMR spectra of individual components of a complex mixture are easily resolved on the basis of their diffusion properties.[3]

Diffusion coefficients and hydrodynamic radii[4] are correlated theoretically by the Stokes−Einstein relation[2e] (eq 1, $D = (kT)/(6\pi\eta r)$).[5] However, the Stokes−Einstein equation is based on the assumption of free diffusion, which requires the diffusion coefficient to be determined at infinite dilution.[6−9] It is also known that the diffusion coefficient varies considerably with the concentration of the diffusate thus it is difficult to calculate the exact FW of a species from a single DOSY experiment at a fixed concentration. DOSY spectra also include artifacts generated by temperature fluctuation, convection, and viscosity change.[10] Hence, the internal reference method strives to eliminate the complications of these effects while taking advantage of the benefits of DOSY spectra.[11−16] Consequently, one can in principle determine the FW and even the aggregation number and solvation state of a reactive intermediate accordingly. In this respect, FW determination by DOSY has a similar function to mass spectroscopy. The internal reference-correlated

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[5] $D = (kT)/(6\pi\eta r)$, where D is the diffusion coefficient, k is the Boltzmann constant, T is the temperature in K, η is the viscosity of the solution, and r is the radius of the molecular sphere.

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DOSY technique incorporates both separation and FW determination and is a good complement to the LC-MS technique. This DOSY methodology will extend and simplify the current NMR techniques such as analysis of quadrapole coupling,[17] multiplicity and bimolecular exchange,[18] HMPA titration,[19] and Job plots[20] that have been employed for determining aggregation states of reactive intermediates in solution.

In the first part of this paper, we present the correlation between diffusion coefficient and FW and the relevant properties of the solvent, such as viscosity and density. In the second part, we demonstrate the protocol of the internal reference system and discuss its requirements and limitations.

Results and Discussion

Part I. Diffusion Coefficient and FW Correlation. Theory of the Correlation between Diffusion Coefficient and FW. The Stokes−Einstein equation (eq 1) correlates the diffusion coefficients and hydrodynamic radii. Comparison of numerous solid state X-ray crystal structures carried out in our laboratory and structures in the Cambridge Crystal Database reveals that the densities of most organolithium aggregates are very similar, i.e., ~1.0 g/cm^3. Also we note that all the aggregates we have determined by crystal structures are relatively spherical. Hence, we can make the assumption that the volumes of these organolithium aggregates are proportional to their FW. Thus there should be a linear correlation between the diffusion coefficient determined by DOSY and FW as originally noted by Johnson via eq 2: $D = AM^{\alpha}$.[21] This equation is easily linearized by taking the logarithm of both sides (eq 3: $\log D = a \log \mathrm{FW} + b$) where we substitute FW for molar mass (M).

According to eq 3, there will be a linear correlation between the logarithm of measured diffusion coefficients and the logarithm of FWs of molecules in solution. Extending this relationship to a set of cautiously chosen molecules, we were able to use the FW of known molecules to establish a calibration curve. Thus, empirical FW of unknown aggregates can be interpolated and/or extrapolated from the curve thereby providing us with a rapid and convenient determination of these values.

Another way to correlate diffusion coefficient and molecular radii is to combine the Stokes−Einstein equation ($D = (kT)/(6\pi\eta r_{\mathrm{H}})$) and the relationship between FW and molar radius−r_{M} (eq 4: $M = (4\pi r_{\mathrm{M}}{}^3 \rho N)/3$, M is the FW, r_{M} is the molecular radius of the species, ρ is the density of the liquid, and N is the Avogadro constant). Others have shown that the r_{H} and r_{M} are similar to each other for small molecules.[2a] Following eq 4, we can derive the following relationship as eq 5: $\log D = -1/3 \log M + 1/3 \log \rho - \log \eta - 1/3 \log (162\pi^2)/(k^3T^3N)$. We note that the Stokes−Einstein equation is based upon the assumption that the solution is infinitely diluted and the solute molecule is much larger in size than the solvent molecule. If we apply eq 5 to molecules that have a similar size as the solvent molecule, the constants for $\log M$, $\log \rho$, and $\log \eta$ may vary correspondingly. Hence, we postulate that the diffusion coefficient of a molecule/aggregate is related to its own FW and also to the density and viscosity of the corresponding solution it is in.

Sample Systems. To investigate the relationship between the diffusion coefficient and FW and the density and viscosity of the solution, we first chose a small molecule, diisopropylamine (DIPA), as a representative example of a relatively small but typical unknown compound. We also chose 1-tetradecene (TDE), cyclooctene (COE), and benzene as the internal references due to their chemical and NMR properties including solubility in different solvents, low reactivity, or coordination with other species, chemical shift dispersion, and desirable FW distribution. Then, we applied our internal reference correlated-DOSY method utilizing four typically utilized deuterated solvents. These four solvents are toluene-d_8 (TOL), cyclohexane-d_{12} (CyHex), dichloromethane-d_2 (CD_2Cl_2), and chloroform-d ($CDCl_3$), as they are routine solvents for organometallic complex investigation and they also bear very different density and viscosity properties. We prepared samples of 1.12 M DIPA and three internal references in each solvent and made consecutive dilutions by doubling the volume to each subsequent sample (Table 1).[22] Then, we carried out a ^{1}H DOSY experiment on each sample until the sample became too diluted to provide a distinguishable ^{1}H NMR signal.

Viscosity and Density Variations. Initially, we would like to consider the density and viscosity change as we dilute the sample. DIPA and the three internal references are all liquids at room temperature and their viscosities and densities are shown in Table S1. The viscosities of the 28 samples are summarized in Table 1, and the densities of these samples are presented in Table 2. Entries for the density and viscosity of the pure solvents are also provided in the corresponding table. Assuming the total volume of a solution is the sum of all individual components,

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(22) Detailed information about sample preparation is presented in the Experimental Section.

Table 1. Viscosity of Solutions at Different Concentration with Four Solvents

concn (M)	viscosity (Cp) TOL	CyHex	$CDCl_3$	CD_2Cl_2
1.120	0.589	0.738	0.577	0.481
0.560	0.560	0.822	0.554	0.439
0.280	0.556	0.862	0.546	0.427
0.140	0.552	0.882	0.542	0.422
0.070	0.550	0.891	0.541	0.419
0.035	0.549	0.896	0.540	0.418
0.018	0.549	0.899	0.539	0.418
average	0.558	0.856	0.548	0.432
pure	0.548	0.901	0.539	0.417

Table 2. Density of Solutions at Different Concentration with Four Solvents

concn (M)	density (g/cm^3) TOL	CyHex	$CDCl_3$	CD_2Cl_2
1.120	0.813	0.780	1.042	0.984
0.560	0.840	0.779	1.261	1.155
0.280	0.853	0.779	1.371	1.240
0.140	0.860	0.779	1.425	1.283
0.070	0.864	0.779	1.453	1.304
0.035	0.865	0.779	1.466	1.315
0.018	0.866	0.779	1.473	1.320
average	0.852	0.779	1.336	1.229
pure	0.867	0.779	1.480	1.326

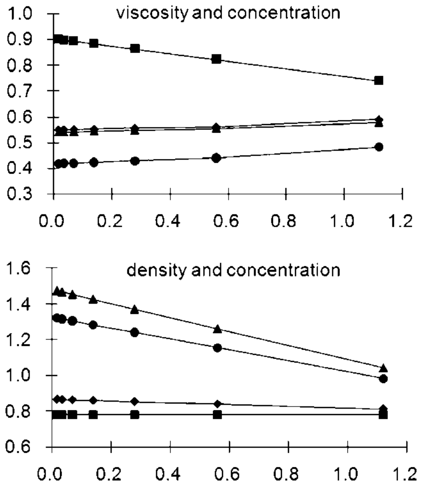

Figure 1. Viscosity (top, Cp) and density (bottom, g/cm^3) variations (y axis) as a function of changes of the concentration (M) of a solution (x axis). Diamond (◆) represents toluene, square (■) represents cyclohexane, triangle (▲) represents $CDCl_3$, and circle (●) represents CD_2Cl_2.

the viscosity ($\eta \approx 1/(\chi_a/\eta_a + \chi_b/\eta_b + ...)$ and density ($\rho = \rho(i)_a + \rho(i)_b + ...$) can be theoretically calculated for each individual solution. These results are depicted graphically in Figure 1.

For the pure solvents, dichloromethane has the lowest viscosity (0.42 Cp). Chloroform has a similar viscosity (0.54) to toluene (0.55), and cyclohexane bears the highest viscosity (0.90).[23] From the top graph in Figure 1, we notice that the viscosity of both toluene and chloroform solutions remain nearly constant. By comparing toluene and chloroform solutions at the most diluted concentration (0.018 M) to the most concentrated sample (1.120 M), the viscosity changes are only −6.8% and −6.5%, respectively. The change (−13.2%) of the dichloromethane solution is higher than the previous two solutions. However, for the solutions of cyclohexane, the viscosity change is quite significant (+21.7%).

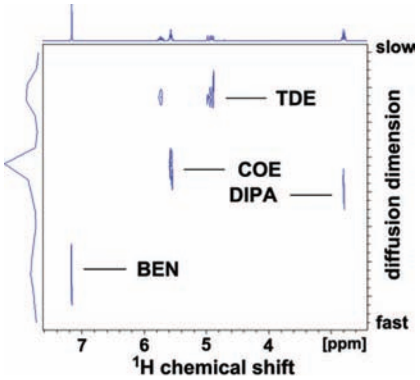

Figure 2. ^{1}H DOSY spectrum of DIPA and three internal references in toluene-d_8 (1.12 M).

For density of the pure solvents, cyclohexane has the smallest value (0.779 g/cm^3), toluene has a similar density (0.867 g/cm^3) to cyclohexane, dichloromethane is heavier than the former two (1.326 g/cm^3), and chloroform bears the highest density (1.480 g/cm^3).[14] The bottom graph in Figure 1 shows that the density of both toluene and cyclohexane solutions remain nearly constant over the concentration range used in our experiments. By comparing the solutions of toluene and cyclohexane at the most diluted concentration (0.018 M) to the most concentrated sample (1.120 M), the density changes are only +6.6% and −0.1%. However, for the solutions of chloroform and dichloromethane, the density changes are quite significant. The ratios are +41.4% and 34.1%, respectively.

These calculations as presented in Figure 1 are important because in the following sections we note that the viscosity and density changes are directly related to the relative diffusion rate and the accuracy of FW prediction.

DOSY Separations. For each of the four solvents, we prepared samples at seven different concentrations. Thus, we carried out a total of 28 DOSY experiments to correlate the diffusion coefficient and the FW of our sample unknown compound (DIPA). For example, the ^{1}H DOSY spectrum of DIPA with the three aforementioned internal references in toluene-d_8 solution (1.12 M) separates into four components in the diffusion dimension. These are clearly identifiable in the DOSY spectrum reproduced in Figure 2. In increasing order of diffusion coefficient (decreasing radii) these are the TDE ($C_{14}H_{28}$, FW 196 g/mol), COE (C_8H_{14}, FW 110 g/mol), DIPA ($C_6H_{15}N$, FW 101 g/mol), and benzene (C_6H_6, FW 78 g/mol). It is noteworthy that diffusion dimension separation of these components was achieved, especially for COE and DIPA as they have similar FW and are observed to exhibit a 13.7% difference in relative diffusion coefficient.

Diffusion Coefficient Distributions. Diffusion coefficients of DIPA and the three internal references were calculated for each of the 28 DOSY experiments. Diffusion coefficients of the four components in toluene-d_8 at different concentrations are listed in Table 3 as an example. The diffusion coefficients of the four components in other three solvents are presented in Table S2 to S4 in the Supporting Information. Several features of these diffusion coefficients are noteworthy. As expected, in any given sample, the diffusion coefficient is inversely proportional to the FW, i.e., the smaller the FW of a component the larger diffusion coefficient it has. For example, in the sample of 1.12 M solution in toluene-d_8, the diffusion rate of benzene (FW 78.1 g/mol) is

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(23) These data are from the Knovel online databases.

Table 3. Diffusion Coefficients of DIPA and Three Internal References in Toluene-d_8 at Different Concentrations

	D (10^{-10} m^2/s)			
concn (M)	BEN	DIPA	COE	TDE
1.124	22.06	17.87	15.43	11.01
0.562	24.07	19.68	18.76	12.84
0.281	22.91	20.81	19.33	13.08
0.141	22.24	18.60	17.83	12.84
0.070	22.35	18.22	17.74	13.52
0.035	20.99	18.49	17.85	14.07
0.018	21.32	20.14	18.20	15.95
average	22.28	19.12	17.88	13.33

Table 4. Average Diffusion Coefficient (10^{-10} m^2/s) of DIPA, Three Internal References, Solvents, and r^2 and FW Prediction Differences in Four Solvents

entry		TOL	CyHex	$CDCl_3$	CD_2Cl_2
1	D^a (BEN)	22.41	17.51	22.82	31.81
2	D^a (COE)	17.88	12.55	16.68	22.09
3	D^a (TDE)	13.33	8.78	12.48	15.06
4	D^a (DIPA)	19.12	13.89	17.69	23.90
5	D average of solvent	19.66	20.66	23.23	34.52
6	r^2 average	0.980	0.983	0.948	0.964
7	FW diff (%)	4.9	1.6	10.6	7.3

[a] D represents average diffusion coefficient for the molecule at all concentrations.

the fastest (22.6 × 10^{-10} m^2/s) and TDE (FW 196 g/mol) is the slowest (11.01 × 10^{-10} m^2/s). The FW of COE (FW 110 g/mol) is 8.9% bigger than DIPA (FW 101 g/mol) and the diffusion rate of COE is 13.7% smaller than DIPA. (15.43 × 10^{-10} m^2/s for COE and 17.87 × 10^{-10} m^2/s for DIPA). In toluene-d_8 solution, the average diffusion coefficients of each of the four components at different concentrations show a similar trend: BEN > DIPA > COE > TDE (Table 3). The average diffusion coefficient of all components in the other three solvents are similar to these observations (Table S2 to S4 in the Supporting Information). At each concentration, the diffusion coefficients of each component retain their relative order, although their magnitudes vary at each different concentration. We do not have an explanation for the observed variance although we attribute this to a systematic experimental error possibly due to slight temperature differences or convection within the individual samples due to the variation in experimental conditions.

For each solution, the diffusion coefficient of components remains inversely proportional to their FWs. Thus, it is of interest to compare the diffusion coefficient of the same molecule in different solvents. This comparison is relevant to the relationship between the diffusion coefficient and the viscosity or density of a solution. Hence, we determined the average diffusion coefficient of the four components at different concentrations in the four different solvents (Table 4, entry 1−4) and we depicted the relationship between these average diffusion coefficient and the average viscosity (Figure 3, top) and average density (Figure 3, bottom) changes in the four solvents. We observed an inverse relationship between the average diffusion rate and the average viscosity, but no relationship with the average density. This means that in a lower viscosity solvent, a higher diffusion rate is observed, which is consistent with the idealized Stokes−Einstein equation. We also calculated the average diffusion coefficient of the solvent itself. The average diffusion coefficients of the four solvents are listed in Table 4, entry 5. The diffusion rate trend is similar to the above observations of the solutes, i.e., the diffusion rate is determined by viscosity (Figure 4, top) and not by density (Figure 4, bottom). The range of diffusion coefficient in all samples is from 8.42 × 10^{-10} (TDE in cyclohexane) to 37.46 × 10^{-10} m^2/s (benzene in CD_2Cl_2). This provides a good range for the diffusion coefficients and FW/viscosity correlations.

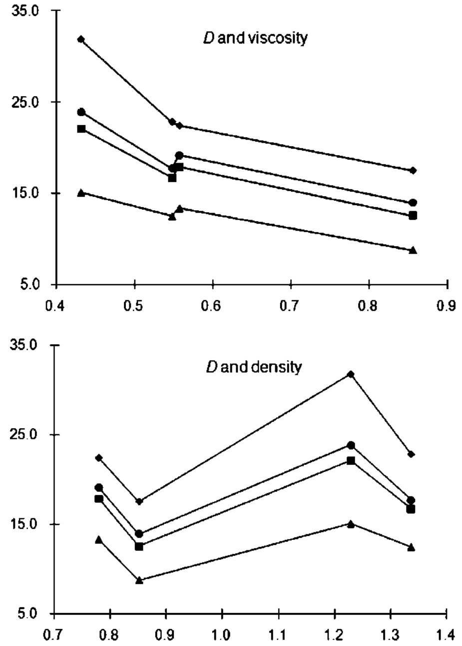

Figure 3. Correlation between average diffusion coefficient of all solutes (y axis, 10^{-10} m^2/s) and average viscosity (top, Cp) and average density (bottom, g/cm^3) (x axis) in the four solvents. Diamond (◆) represents BEN, circle (●) represents DIPA, square (■) represents COE, and triangle (▲) represents TDE.

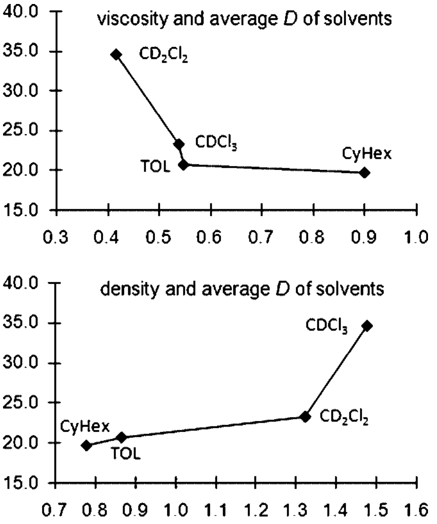

Figure 4. Correlation between average diffusion coefficient (y axis, 10^{-10} m^2/s) and average viscosity (top, Cp) and average density (bottom, g/cm^3) (x axis) of the four solvents.

Values of *a* and *b*. In the above section, we derived eq 3 (log $D = a$ log FW $+ b$) to correlate the observed diffusion coefficient of an unknown molecule in solution to its FW. For each individual sample, values of a and b in eq 3 were calculated from the least-squares linear prediction of the diffusion coefficients and FWs of the three internal references. All the values of a and b of the total 28 experiments are listed in Table 5. The relationship between the change in the a/b values versus concentration change in toluene-d_8 is depicted Figure 5, first row. We observed that the a value for toluene-d_8 solutions

Table 5. Values of *a* and *b* of the Four Solvents at Different Concentrations

	TOL		CyHex		$CDCl_3$		CD_2Cl_2	
concn (M)	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
1.124	−0.74	−7.27	−0.73	−7.38	−0.62	−7.55	−0.80	−7.13
0.562	−0.67	−7.35	−0.75	−7.34	−0.64	−7.48	−0.50	−7.66
0.281	−0.63	−7.44	−0.75	−7.34	−0.61	−7.51	−0.83	−6.91
0.141	−0.59	−7.55	−0.77	−7.31	−0.54	−7.66	−0.78	−7.03
0.070	−0.53	−7.67	−0.76	−7.32	−0.61	−7.49	−0.79	−7.01
0.035	−0.43	−7.87	−0.74	−7.39	−0.71	−7.29	−0.92	−6.70
0.018	−0.35	−8.00	−0.62	−7.65	−0.64	−7.42	−0.82	−6.95
average	−0.56	−7.59	−0.73	−7.39	−0.62	−7.49	−0.78	−7.06

increases from −0.74 (1.120 M) to −0.35 (0.018 M) as the concentration decreases. The starting value −0.74 is similar to the number in the literature reported by Johnson[21] for the FW prediction of polymers. It is especially noteworthy that at the low concentration range the *a* value −0.35 approaches −1/3, which is the value for infinitely diluted solution in eq 5. On the other hand, the *a* value is limited to a small range for cyclohexane (−0.77 to −0.62, see Figure 5, second row) and $CDCl_3$ (−0.71 to −0.54, see Figure 5, third row) where no correlation can be drawn between *a* values and concentrations. This is also the case for CD_2Cl_2, where the *a* values are distributed irregularly (−0.92 to −0.50, Figure 5, fourth row) and no connection of *a* values between consecutive concentrations is observable. For all of the four solvents, the *b* values exhibit an opposite trend to the *a* values as the concentration changes (see Figure 5).

If we consider the viscosity and density changes of the four solvents from the most diluted samples (0.018 M) to the most concentrated solutions (1.120 M), we found only toluene shows small changes for both viscosity (−6.8%) and density (+6.6%). For cyclohexane, the density only changes by 0.1% but the viscosity changes by +21.7%. For $CDCl_3$, the viscosity only changes by −6.5% but the density changes by +41.4%. For CD_2Cl_2, both the viscosity (−13.2%) and the density (+34.1%) change significantly. We feel that these changes for viscosity and density may provide an explanation for changes of *a*/*b* values depicted in Figure 5. Hence, model behavior of *a*/*b* values will only be observed if both the viscosity and the density remain constant throughout the dilutions; if either the viscosity or the density changes, the *a*/*b* values show poor correlation. Fortunately, the accuracy of the FW prediction is not affected by the degree of *a*/*b* values correlations. The overall average values of the 28 experiments for *a* is −0.67 and for *b* is −7.38 and we suggest that these numbers should be used as a guide for FW predictions in routine DOSY experiments.

FW Predictions: r^2 and FW prediction. After we calculated diffusion coefficients of individual components in a solution, we set up a relationship between diffusion coefficient and FW (eq 3: $\log D = a \log \text{FW} + b$), and we have applied this correlation to study several organometallic complexes.[24] The above DOSY results strongly support the robustness of this method and indicate that the diffusion coefficients and FWs (Table 3) of DIPA and the three internal references, TDE, COE, and benzene, can indeed be utilized to define a linear correlation between the relative log *D* (diffusion coefficient) and log FW. The correlation between log FW and log *D* from the linear least-squares fit to reference points of all components in these mixtures is extremely high, $r^2 > 0.900$ (Table 6). The range of r^2 is from 0.904 to 1.000 and the average r^2 is 0.969. These remarkable results highlight the ability to use suitable internal references in ^{1}H DOSY experiments to interpolate relative diffusion coefficients and FWs. We also calculated the average r^2 for the seven samples of one of the four solvents (Table 6), and we depicted the relationship between r^2 and viscosity change (Figure 6, top) and density change (Figure 6, bottom). We found that the r^2 is related to the change of the density and not to the viscosity. This proves that the accuracy of r^2 is not dependent on the diffusion rate, which is related to the viscosity of the solution. These results are also consistent to other researchers' suggestions that the halogenated solvents, such as $CDCl_3$ and CD_2Cl_2, may yield poor FW predictions.[2a]

For an individual sample, we calculated *a* and *b* values from the least-squares linear prediction by correlating the diffusion coefficient and FW of the three internal references and utilized these values in eq 3 to calculate the FW of the unknown compound, DIPA. The calculated FW and the difference between the calculated FW and the actual FW are listed in Table 7. The range of the difference between the predicted and the authentic FW is from 0.2% (for cyclohexane solution) to 17.8% (for $CDCl_3$ solution), and the average is 6.1%. These results highlight the applicability of using suitable internal references in ^{1}H DOSY experiments to interpolate relative diffusion coefficients and FWs. We found a similar trend between the FW difference and r^2 by comparing the average FW difference to viscosity and density. We observed that the FW difference is related to the density change of the solvent (Figure 7, bottom) but not significantly to the viscosity change (Figure 7, top). This provides evidence that the accuracy of FW prediction is independent of the diffusion rate or the viscosity of the solution.

From the above observations, we can conclude that r^2 and the accuracy of FW prediction difference are highly dependent on the changes of density, but not on viscosity changes. For halogenated solvents, such as $CDCl_3$ or CD_2Cl_2, both r^2 and FW prediction difference are not as good as hydrocarbon solvents. Hence, we suggest that DOSY experiments designed to determine FW are best conducted in nonhalogenated solvents.

Organometallic Complex Investigations: Mixed Trimeric Complex 1. After utilizing DIPA as an unknown compound and establishing a good correlation between FW and diffusion coefficient in the above experiments, we applied the internal reference system to an organometallic complex, mixed trimeric complex **1**[25,26] (Scheme 1), to test the applicability of our DOSY methodology. Since the FW of complex **1** (678.1 g/mol) is much higher than the three internal references, the prediction of FW for complex **1** required an extrapolation from eq 3. We observed a similar diffusion coefficient distribution (Table S5) and excellent results for r^2 and FW prediction: the average r^2 is 0.997 and the average FW prediction difference is only 0.9%. These results strongly corroborate the suitability of our internal reference correlated DOSY method for the FW prediction of organometallic complexes.

Part II. Guidelines to Internal Reference Correlated DOSY Method. In the above sections, we displayed the effectiveness of three internal reference correlated DOSY

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[24] (a) Li, D.; Hopson, R.; Li, W.; Liu, J.; Williard, P. G. *Org. Lett.* **2008**, *10*, 909–911. (b) Jacobson, M. A.; Keresztes, I.; Williard, P. G. *J. Am. Chem. Soc.* **2005**, *127*, 4965–4975. (c) Keresztes, I. Ph.D. Dissertation, Brown University, Providence, RI, 2002.

[25] (a) Williard, P. G.; Sun, C. *J. Am. Chem. Soc.* **1997**, *119*, 11693–11694. (b) Li, D.; Sun, C.; Liu, J.; Hopson, R.; Li, W.; Williard, P. G. *J. Org. Chem.* **2008**, *73*, 2373–2381. (c) Liu, J.; Li, D.; Sun, C.; Williard, P. G. *J. Org. Chem.* **2008**, *73*, 4045–4052.

[26] Hilmersson, G.; Malmros, B. *Chem.—Eur. J.* **2001**, *7*, 337–341.

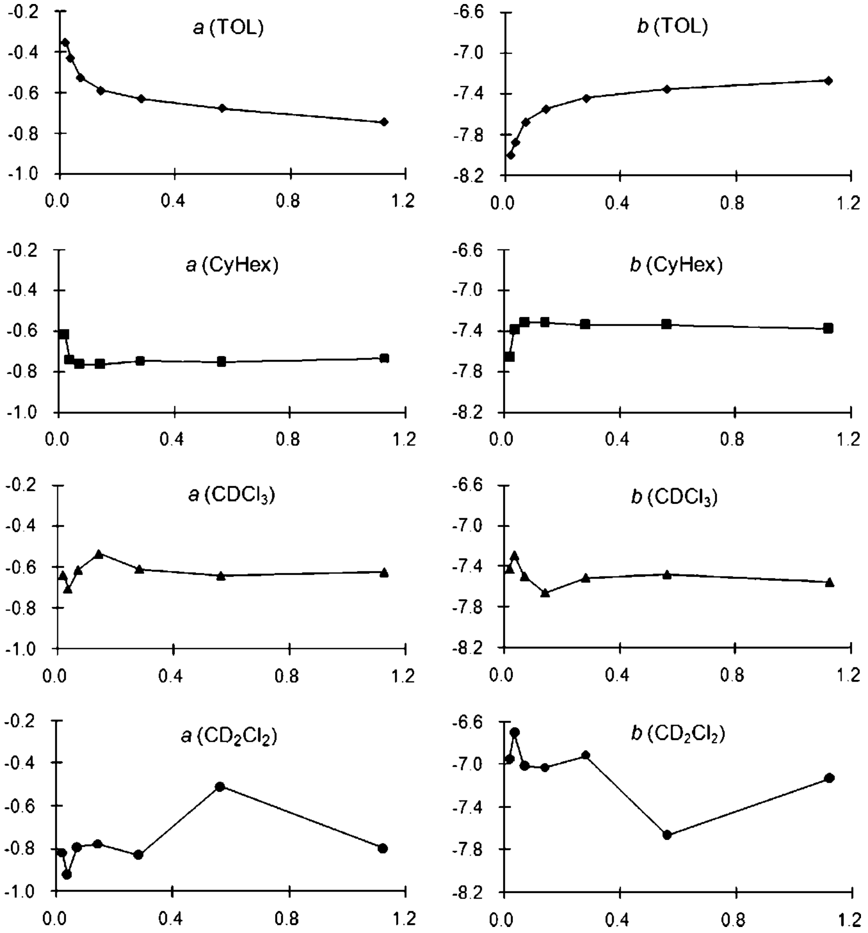


Figure 5. Values of *a* and *b* of the four solvents at different concentrations.

Table 6. r^2 for log *D* − log FW Correlation to the Changes of Concentration in Different Solvents

	r^2			
concn (M)	TOL	CyHex	$CDCl_3$	CD_2Cl_2
1.124	0.974	0.992	0.934	0.992
0.562	0.998	0.981	0.998	1.000
0.281	0.986	0.982	0.914	0.994
0.141	0.997	0.962	0.972	0.977
0.070	0.978	0.987	0.941	0.905
0.035	0.998	0.977	0.944	0.978
0.018	0.932	0.999	0.932	0.904
average	0.980	0.983	0.948	0.964

method for predicting the FW of an unknown species. Our attempts at using fewer than three internal references introduced greater error in FW predictions. We would also caution against using the solvent as an internal reference, as it does not fit the criterion specified for application of the Stokes−Einstein equation. We conclude that to predict accurate FW by the internal reference DOSY method, there are several chemical and NMR guidelines for sample preparation.

Chemical Properties. The internal reference should have no reaction (e.g., no OH/NH groups) and no coordination (e.g., no ether/carbonyl oxygen atoms) with the unknown species. They also should have good solubility in the chosen NMR solvents, good FW distributions (such as BEN (78 g/mol)/COE (110 g/mol)/TDE (196 g/mol)), and appropriate melting points for low-temperature DOSY experiments. Hence, we typically chose hydrocarbons as our internal references.

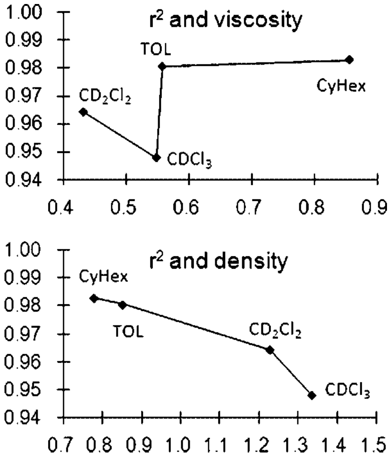


Figure 6. Correlation between average r^2 (*y* axis) and viscosity (top, Cp) and density (bottom, g/cm^3) (*x* axis) of the four solvents.

NMR Properties. The individual NMR spectrum of any internal reference should have no overlap with the signature peaks of the unknown compound. The three internal references we chose have no overlap to signature peaks of DIPA, complex **1** or some other organometallic species. Other researchers utilized TMS as a reference, but it sometime overlaps with the unknown complex in our studies, such as LiHMDS complexes. To achieve the best resolution of the diffusion dimension separations, the integrations of the fingerprint peaks of both the internal references and the unknown species should have similar

Table 7. FW Prediction of DIPA in All Samples and the Differences between the Exact FW of DIPA

	TOL		CyHex		$CDCl_3$		CD_2Cl_2	
concn (M)	FW[a]	diff. (%)	FW[a]	diff. (%)	FW[a]	diff. (%)	FW[a]	diff. (%)
1.120	98.5	2.7	103.3	2.1	93.7	7.4	98.2	3.0
0.560	104.2	3.0	103.8	2.6	105.0	3.8	101.7	0.6
0.280	93.8	7.3	100.9	0.3	118.8	17.4	107.3	6.1
0.140	104.4	3.2	104.7	3.5	111.0	9.7	110.3	9.0
0.070	110.5	9.2	100.9	0.3	114.9	13.6	117.2	15.8
0.035	103.6	2.4	101.4	0.2	105.9	4.6	97.8	3.4
0.018	94.9	6.2	103.4	2.2	119.2	17.8	114.5	13.2
average		4.9		1.6		10.6		7.3

[a] FWs are predicted from eq 3.

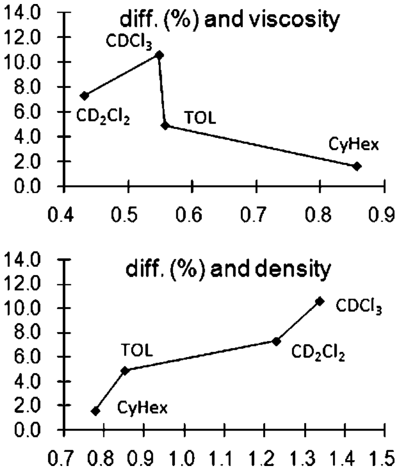
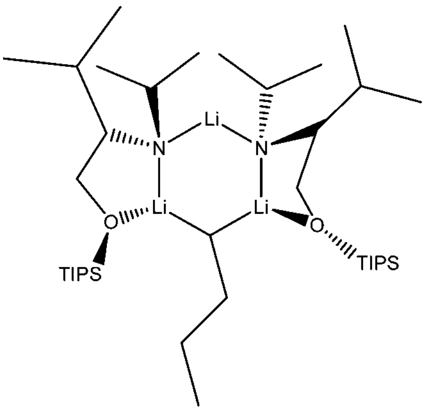


Figure 7. Correlation between average FW prediction difference (%) of DIPA (*y* axis) and viscosity change(top, Cp) and density change (bottom, g/cm^3) (*x* axis) of the four solvents.

Scheme 1. Structure of Mixed Trimeric Complex **1**



ratio. One example of the ^1H NMR of DIPA with three internal references in toluene-d_8 (1.12 M) is shown in Figure S1. The ratio between the aromatic protons of benzene, the olefin protons of COE, the terminal olefin protons of TDE, and the methine protons of DIPA are 1.00:0.96:1.04:1.03. This near-perfect ratio yields good separation of these components on the diffusion dimension. The same requirements for obtaining quality data from any DOSY experiment are applicable here; the length of the gradient pulse and diffusion time should be optimized to generate exponential signal attenuation vs gradient strength in the Stejskal−Tanner plot[27] (see Figure S16 in the Supporting Information) for diffusion coefficient calculations. We also routinely conducted these internal reference correlated DOSY to other nuclei, such as ^{13}C and ^7Li. These heteronuclei DOSY experiments provided similar accuracy of FW prediction as ^1H DOSY.

Conclusions

In the above sections, we describe an internal reference correlated DOSY method for calculating the FW of unknown species in four different solvents with different concentrations. Both the small molecule (DIPA) and the organometallic complex (aggregate **1**) yield excellent results. We also found the relative diffusion rate is inversely proportional to the viscosity change of the solution, which is consistent with the theoretical Stokes−Einstein equation. We also derived an equation ($\log D = a \log \mathrm{FW} + b$) to calculate the FW of unknown compound from the observed diffusion coefficients. The distribution of constants *a* and *b* is related to changes in density and viscosity of a series of solutions, but the values of these constants was demonstrated to have no effect on the accuracy of the FW predictions. The accuracy of the least-squares linear prediction r^2 and the % difference of FW prediction are directly related to the density change; greater accuracy was observed with decreasing density. Any correlation of r^2 and FW prediction accuracy with viscosity remains unclear. We also discuss the guidelines for successful application of internal reference correlated DOSY experiments from perspective of the chemist and NMR spectroscopist. The derived information of FW of a complex is essential to the understanding of its solvation state and aggregation number, which are important to rationalize reaction pathways. This method could be easily tailored and applied to the characterization of other unknown molecules or complexes.

Experimental Section

General. All NMR samples were directly prepared in the NMR tubes. Toluene-d_8 was kept with 4 Å molecular sieves under argon. *n*-BuLi was obtained from Aldrich Chemical Co. (2.5 M in hexanes), and the exact concentration of the solution was titrated with diphenyl acetic acid in THF. All NMR experiments were recorded on a Bruker DRX 400 spectrometer with a variable-temperature unit. The data were processed with the Topspin 1.3 pl6 software. Standard ^1H and ^{13}C NMR spectra were recorded at 400.13 and 100.61 MHz, respectively. ^1H chemical shifts were referenced to the benzene signal at δ 7.16 ppm.

DOSY Experiments. DOSY experiments were performed on a Bruker DRX400 spectrometer equipped with an Accustar *z*-axis gradient amplifier and an ATMA BBO probe with a *z*-axis gradient coil. Maximum gradient strength was 0.214 T/m. The standard Bruker pulse program, dstebpgp3s, employing a double stimulated echo sequence, bipolar gradient pulses for diffusion, and three spoil gradients was utilized. Bipolar rectangular gradients were used with total durations 0.5−3 ms. Gradient recovery delays were 0.5−1 ms. Diffusion times were between 500 and 2000 ms. Individual rows of the quasi-2D diffusion databases were phased and baseline corrected.

Preparation of DOSY Samples. Prior to each experiment, seven NMR tubes were oven-dried and sealed with serum septa and parafilm. Each tube was placed under vacuum and flushed with nitrogen three times.

For DIPA, an initial sample was prepared by adding DIPA and internal references via syringe in a 3:1:3:3 molar ratio of DIPA/BEN/COE/TDE (95.2, 20.0, 88.0, 172.2 μL) with NMR solvent added via syringe to bring the total volume up to 600 μL (224.6 μL). The sample was injected into the spectrometer, locked, and shimmed, and the initial DOSY spectrum was taken.

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(27) Jerschow, A.; Müller, N. *J. Magn. Reson.* **1997**, *125*, 372–375.

The trimeric complex **1** was formed in situ. Uncoordinated ligand and internal references were added via syringe to an NMR tube as a 6:1:3:3 molar ratio of ligand/BEN/COE/TDE (272.1, 9.1, 40.2, 78.6 μL) with NMR solvent added via syringe to bring the total volume up to 600 μL (200 μL). *n*-Butyl lithium (20 μL, 2.5 M in hexanes) was added to the tube to form the trimeric complex **1**, bringing the total volume of the first sample to 620 μL. The sample was injected into the spectrometer, locked, and shimmed, and the initial DOSY spectrum was taken.

For each system, upon completion of the first spectrum, 300 μL of solution was extracted via syringe and injected into the next empty NMR tube. The corresponding NMR solvent (300 μL) was added via syringe to the tube to bring the volume up to 600 μL to effectively halve the concentration. This sample was injected into the spectrometer, locked, and shimmed, and the DOSY spectrum acquired. This process continued until all seven tubes had been used.

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Supporting Information Available: ^{1}H NMR and ^{1}H DOSY NMR spectra of free DIPA and complex **1** with internal references in different solvents; ^{1}H NMR spectra of benzene, COE, TDE, DIPA, and complex **1** in toluene-d_8; and the diffusion coefficients of DOSY experiments that are not listed in the main body of the paper. This material is available free of charge via the Internet at http://pubs.acs.org.

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