Physically Separated References for Diffusion Coefficient-Formula Weight (D-FW) Analysis of Diffusion-Ordered NMR Spectroscopy (DOSY) in Water
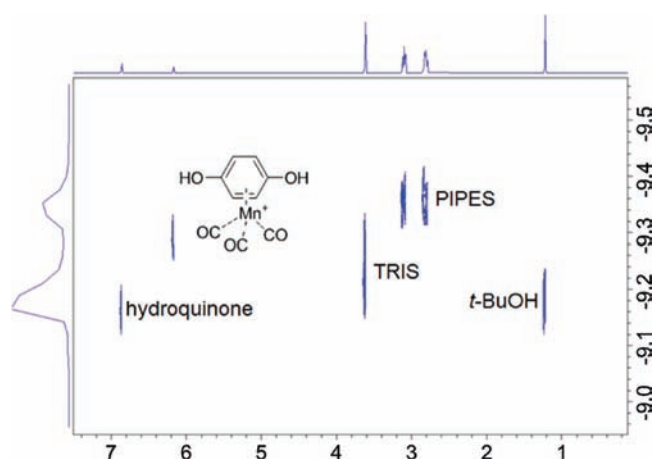
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ABSTRACT

Development and application of physically separated references for aqueous ^{1}H DOSY diffusion coefficient-formula weight (D-FW) correlation analysis is reported. Commercially available biological buffers (Tris and HEPES) and a water-soluble alcohol (*tert*-butanol) were used as physically separated references for a Ru and a Mn complex in D_2O. This extension of DOSY D-FW analysis expands its applicability to a wide variety of water-soluble molecules or metal complexes, with particular application to green chemistry.

Diffusion-ordered NMR spectroscopy has been previously established as an efficient and important means for the identification of molecular size and formula weights of compounds in solution.[1] Our group has successfully developed internal references for ^{1}H, ^{13}C, and ^{31}P DOSY, as well as diffusion coefficient-formula weight (D-FW) correlation analysis for solution-state structural identification of organometallic complexes.[2] We have also developed physically separated reference systems in order to generate formula weight (fw) information without contact between the reference and analyte solutions.[3]

Development of a set of DOSY references for aqueous solution is important for green chemistry and biochemistry research in which water is frequently used as solvent.

The requirements of references for DOSY D-FW correlation analysis in aqueous phase are similar to those in organic phase. They must have a reasonable range of formula weights, a good chemical shift distribution, solubility at experimental temperatures, and lack of reactivity or affinity toward the analyte.

Tetraalkylammonium salts were the first compounds to be chosen as DOSY references. To differentiate their chemical shifts, tetramethylammonium chloride (cation fw 74.0 g
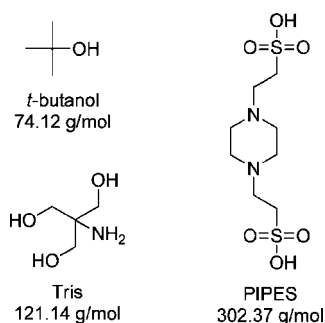
Figure 1. Structures of aqueous DOSY references.

Figure 2. ^{1}H DOSY spectrum of DOSY references.

mol^{-1}), triethylphenylammonium chloride (cation fw 192.2 g mol^{-1}), and tetrahexylammonium chloride (cation fw 354.4 g mol^{-1}) were adopted as the first set of references. Although these had a reasonable formula weight distribution, tetrahexylammonium chloride was barely soluble in water. Tetraalkylammonium salts with higher formula weights were insoluble. As a result, we decided to use a set of biological buffers as DOSY references. The set of Tris (fw 121.1 g mol^{-1}), MES (fw 195.2 g mol^{-1}), HEPES (fw 238.3 g mol^{-1}), and PIPES (fw 302.4 g mol^{-1}) have a broad range of formula weights and good solubility in water. However, as a result of their structural similarities, their major chemical shifts overlapped within the region of 2−4 ppm in the ^{1}H NMR spectrum. It is difficult for DOSY to separate data in the diffusion dimension for different compounds with the same chemical shifts, and therefore the above set was restricted to *t*-BuOH (FW 74.122 g mol^{-1}), Tris, and PIPES (Figure 1).

The ^{1}H DOSY spectrum (Figure 2) was consistent with the expected diffusion order of compounds in solution, with lighter compounds diffusing more rapidly than heavier compounds. Signal attenuation curves from signature chemical shifts of compounds were used to generate diffusion coefficients. With optimization of experimental parameters, full signal attenuation was attained after 16 steps of increasing gradient strength from
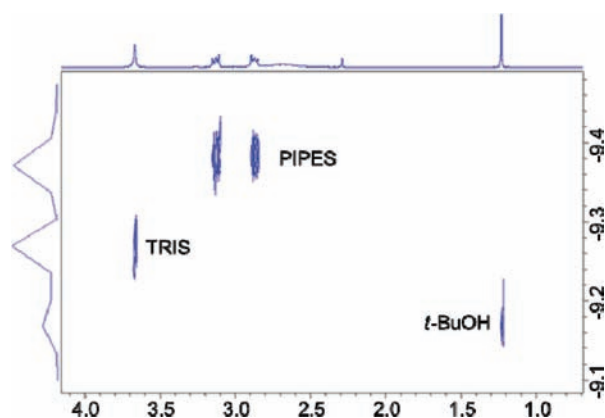
2% to 95% linearly. D-FW correlation analysis gave an excellent r^2 value (0.998) based on the linear line of best fit of log D = A log(fw) + C of a plot of the logarithm of the diffusion coefficient versus the logarithm of the fw, with less than 4% error for the predicted fw (fw*) for each of the DOSY references (Table 1). This is well within the typical 10% limit of these experiments.
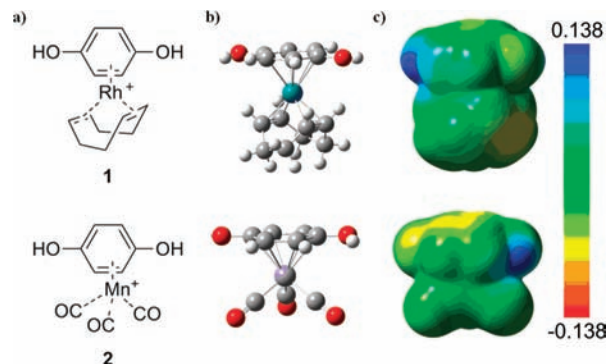

Figure 3. Molecular structures of complex **1** and **2** (a), stick-ball models from crystal structures (b), and electrostatic potential mapping [B3LYP/6-31+G(d)] (c).

We recently reported a DOSY NMR method using a microtube within a standard NMR tube to physically separate DOSY references from analytes. With the separation of the annular volume and capillary volume, we can focus only on

Table 1. D-FW Analysis of ^{1}H DOSY Data of References

entry	compd	fw (g mol^{-1})	10^{-10} D (m^2 s^{-1})	fw* (g mol^{-1})	% error
1	*t*-BuOH	74.122	7.487	72	2.5
2	Tris	121.14	6.255	125	−3.9
3	PIPES	302.37	4.733	298	1.3

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the chemical shift and formula weight distributions of the references, without regard to their chemical reactivity toward analytes. We adopted this method with our chosen DOSY references to identify two complexes **1** and **2** (Figure 3) in D_2O. According to our previous studies, the influences of three system parameters (viscosity, concentration, and density) can be overcome by using very low concentration so the density and viscosity for the annular and capillary volumes approach those of the pure solvent.[3]

The crystal structure of complex **1** has been reported. Its solution state structure was discussed.[4] The 1,5-cyclooctadiene (COD) was reported to bind to rhodium more tightly than hydroquinone. It is possible for hydroquinone to be replaced by water, as seen by its chemical shift change.

The ^1H NMR spectrum of the complex dissolved in D_2O exhibited two sets of chemical shifts from COD (4.35 ppm, 4.04 ppm) and hydroquinone (6.79 ppm, 6.46 ppm). Complex **1** was placed in the capillary volume and the references in the annular volume. The ^1H DOSY NMR spectrum agreed well with the expected diffusion order of compounds in water (Figure 4). From fast to slow, the diffusion order is *t*-butanol, Tris,
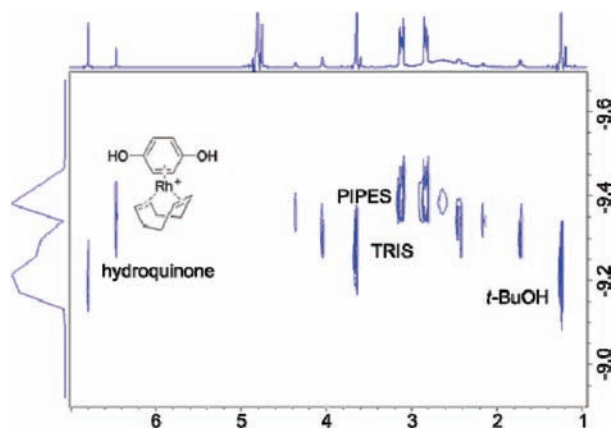

Figure 4. ^1H DOSY spectrum of **1** with references.

PIPES, and complex **1** (cation fw 321.2 g mol^{-1}). Diffusion coefficients generated by curve fitting of signal attenuation were used in D-FW analysis. The predicted formula weights were in good agreement with calculated values, with an r^2 value of 0.998, as shown in Figure 5. This shows excellent correlation of the chosen internal references. The predicted fw deviations of the references were all within 3% and the predicted fw of complex **1** was 300 g mol^{-1}, a 7% difference from its calculated value (Table 2). It was also noted that the other hydroquinone

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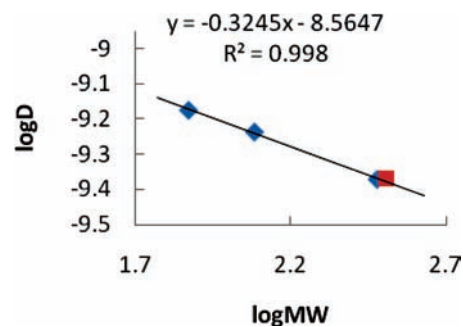
Figure 5. D-FW analysis of ^1H DOSY data of **1** with references.

Table 2. D-FW Analysis of ^1H DOSY Data of **1** with References

entry	compd	fw (g mol^{-1})	$10^{-10} D$ (m^2 s^{-1})	fw* (g mol^{-1})	% error
1	*t*-BuOH	74.12	0.6692	75	−1
2	Tris	121.14	0.5806	117	3
3	PIPES	302.37	0.4254	305	−1
4	**1**	321.20	0.4280	300	7

signal ($\delta = 6.79$ ppm) fell within the fw region between 74 g mol^{-1} (*t*-BuOH) and 121 g mol^{-1} (Tris), showing that it was free in solution. Additionally, the other COD signal ($\delta = 4.04$ ppm) was calculated to be 225 g mol^{-1}, a 6.6% difference from a rhodium with a COD. Hence, it is reasonable that complex **1** was partially decomposed in D_2O.

With the success on complex **1**, we continued to examine complex **2**. The crystal structure of complex **2** has also been reported.[5] Complex **2** was dissolved in D_2O and placed in the capillary volume. From ^1H NMR, two peaks are apparent for hydroquinone. It is possible that one is from free hydroquinone and the other is still bound to the tricarbonyl manganese. The references were added to the annular volume.

The ^1H DOSY NMR spectrum agreed well with the expected diffusion order of compounds in water. From fast to slow, the diffusion order is *tert*-butanol, Tris, complex **2** (cation fw 243.2 g mol^{-1}), and PIPES (Figure 6). The diffusion coefficients used in D-FW correlation analysis were generated by curve fitting of signal attenuation. The predicted formula weights were in good agreement with calculated values with an r^2 value of 0.959, as shown in Figure 7. This shows a good correlation of the references. The predicted fw of complex **2** was 241 g mol^{-1}, with only 1% difference from its expected value (Table 3). The other hydroquinone signal appeared between 74 g mol^{-1} (*t*-BuOH) and 121 g mol^{-1} (Tris), showing that it was free in solution. Similar to complex **1**, complex **2** was also partially decomposed in D_2O.

The above examples prove the value of DOSY NMR for structural characterization in the aqueous phase when coupled with D-FW correlation analysis. The successful application of new internal references expands the scope of the D-FW correlation method. As shown above, not only can this
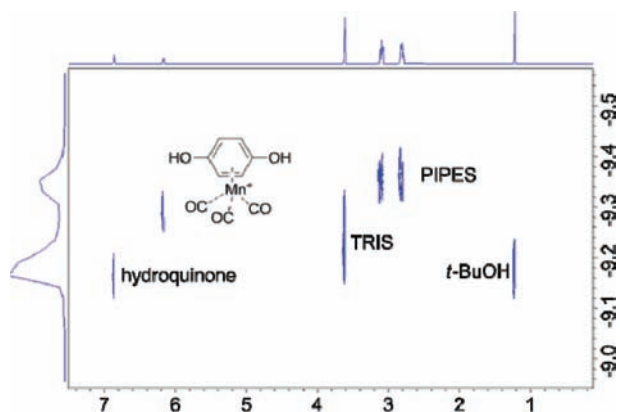
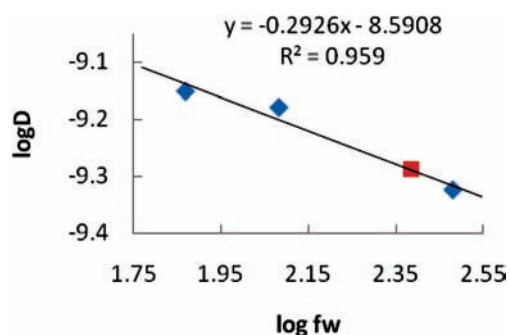**Figure 6.** ¹H DOSY spectrum of **2** with references.

Figure 7. D-FW analysis of ¹H DOSY data of **2** with references.

method be applied to complexes with previously established crystal structures, but also it can aid the study of unknown ligand binding competition. The excellent correlations and accurate formula weight predictions demonstrate the applicability of this method to water-soluble compounds.

An important consideration in the aqueous phase is that different compounds may have sufficiently different electronic distributions such that they diffuse unexpectedly in the extremely polar environment, rather than their diffusion being based solely on their size. Hence, for charged

Table 3. D-FW Analysis of ¹H DOSY Data of **2** with References

entry	compd	fw (g mol⁻¹)	$10^{-10} D$ (m² s⁻¹)	fw* (g mol⁻¹)	% error
1	*t*-BuOH	74.122	7.054	82	−11
2	Tris	121.14	6.619	102	15
3	PIPES	302.37	4.744	320	−6
4	**2**	243.20	5.156	241	1

compounds, one should consider the solvation structures formed by water molecules. Prior to this work, our D-FW correlation analyses were done in organic solvents, with dielectric constants of solvents ranging from 1.844 (*n*-pentane) to 20.6 (acetone), as compared to 79.7 for water.[6]

Rhodium complex **1** and manganese complex **2** were chosen as analytes due to their good solubility and relative stability in water. The electrostatic potential mapping [B3LYP/6-31+G(d)] of the X-ray crystal structures of complexes **1** and **2** demonstrate an even distribution of electron density (Figure 1).

These results describe development of new aqueous references for ¹H DOSY NMR and these are also shown to be successful in physically separated systems. This presents opportunities for the use of this method in green chemistry and biological applications. The limit of applicability to which we have tested this method presently is that it can only be applied to noncharged or lightly charged compounds. With more highly charged compounds, we suspect that nontrivial water solvation shell effects must be considered to avoid large errors in D-FW correlation analysis. This research is in progress.

Supporting Information Available: Full spectroscopic data for NMR experiments and diffusion data. This material is available free of charge via the Internet at http://pubs.acs.org.

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