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Extraction of reliable molecular information from diffusion NMR spectroscopy: hydrodynamic volume or molecular mass?

Francesco Zaccaria,*[a] Cristiano Zuccaccia,[a] Roberta Cipullo[b] and Alceo Macchioni[a]

Abstract: Measuring accurate translational self-diffusion coefficients (D_t) by NMR with modern spectrometers has become rather routine. On the contrary, the derivation of reliable molecular information therefrom still remains a non-trivial task. In this paper, two established approaches to estimate molecular size in terms of hydrodynamic volume (V_H) or molecular weight (MW) are compared. Ad-hoc designed experiments allow the critical aspects of their application to be explored, by translating relatively complex theoretical principles in practical take-home messages. For instance, comparing the D_t of three isosteric Cp_2MCl_2 complexes (Cp = cyclopentadienyl, M = Ti, Zr, Hf), having significantly different molecular mass, provided an empirical demonstration that V_H is the critical molecular property affecting D_t. This central concept served to clarify the assumptions behind the derivation of $D_t = f(MW)$ power laws from the Stokes-Einstein equation. Some pitfalls in establishing Log(D_t) vs Log(MW) linear correlations for a set of species have been highlighted by further investigations on selected examples. The effectiveness of the Stokes-Einstein equation itself in describing aggregation or polymerization of differently shaped species has been explored by comparing *e.g.* a ball-shaped silsesquioxane cage with its cigar-like dimeric form, or styrene with polystyrene macromolecules.

Introduction

NMR spectroscopy is the technique of choice for molecular structural characterization in solution. Along with ‘classical’ multinuclear and multidimensional experiments, diffusion NMR has emerged as a powerful tool to investigate molecular interactions in solution and determine molecular size.[1] The latter subjects are of considerable interest for chemists, especially to explore host-guest interactions,[1a, 1g, 2] supramolecular aggregations,[1b-e] and polymerization processes.[3]

Due to the advances in hardware and software development, the determination of accurate translational self-diffusion coefficients (D_t) by typical pulsed gradient spin echo (PGSE)[2a, 4] or diffusion ordered NMR spectroscopy (DOSY)[2b, 5] experiments has become a routine exercise also for non-expert NMR users. On the other hand, deriving reliable and quantitative molecular information from D_t is a delicate task, as the correlation between the latter and molecular size is not always straightforward.[1f]

The master law in this context is the Stokes-Einstein (SE) equation,[6] which allows the hydrodynamic radius (r_H) of the diffusing species to be derived from its D_t. In its original formulation, the SE equation is valid only for colloidal particles moving with uniform velocity in a fluid continuum (*i.e.* dense spherical shaped species being significantly larger than the solvent molecule). As these conditions do not apply to most of the systems of interest, the SE equation has been modified by introducing two numerical factors accounting for the shape of the studied molecule (f_s), and the ratio between the r_H of the analyte and that of the solvent (c),[1f] as reported in Equation (1):

$$D_t = \frac{k_B T}{c f_S \pi \eta r_H} \qquad (1)$$

where k_B is the Boltzmann constant, T the absolute temperature and η the fluid viscosity. Here, r_H represents the radius of a hypothetical hard sphere that diffuses with the same D_t of the particle under examination.

An alternative approach to obtain molecular size information from diffusion NMR is based on the correlation between D_t and molecular weight (MW) [Equation (2)]:

$$D_t = K \cdot MW^{\alpha} \qquad (2)$$

where K and α are constants typical of the studied molecule at a given temperature and in a specific solvent. This power law is actually a derivation of Equation (1), as first proposed by Polson in 1950.[7] This can be shown by considering the simplest case of a dense spherical particle; r_H can be expressed in terms of hydrodynamic volume (V_H), which in turn is given by the ratio between MW, the density of the species (ρ) and the Avogadro’s number (N_A) [Equation (3)]:[8]

$$r_H = \sqrt[3]{\frac{3}{4\pi} V_H} = \sqrt[3]{\frac{3}{4\pi}\frac{MW}{\rho N_A}} \qquad (3)$$

$$D_t = \frac{k_B T}{c f_S \pi \eta \sqrt[3]{\frac{3}{4\pi}\frac{MW}{\rho N_A}}} = \left(\frac{k_B T}{c f_S \pi \eta \sqrt[3]{\frac{3}{4\pi \rho N_A}}}\right) MW^{-1/3} \qquad (4)$$

By substituting the r_H expression (3) in the SE Equation (1), Equation (4) is obtained where all the terms in brackets are constant for a given particle in a specific solvent and at a specific temperature, and therefore correspond to the K parameter in Equation (2). Analogous correlations between D_t and MW have been empirically derived for the specific case of synthetic polymers.[9]

Using Equation (2) in place of (1) has the advantage of providing molecular size information in terms of MW, which is a generally more intuitive molecular property than r_H. Furthermore, it does

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not require any explicit assumption on molecular shape, which is implicitly accounted for in the K and especially α parameter (extreme cases: $\alpha = -0.33$ for perfect spheres, $\alpha = -1$ for linear rigid rods).[8a, 10]

Equation (2) is typically employed in its logarithmic version [Equation (5)], providing a linear relationship between Log(D_t) and Log(MW). The latter has been often exploited to determine the MW of target species by using internal or external calibration curves.[5a, 8, 11]

$$Log(D_t) = \alpha \cdot Log(MW) + Log(K) \qquad (5)$$

However, caution is required since this apparently simple and powerful equation is only valid for a set of molecules under several assumptions, primarily that they have similar molecular density.[8b, 10a, 11c, 12]

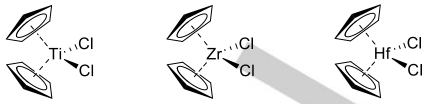
In this paper, the two approaches based on Equations (1) and (2) for extracting molecular information from diffusion NMR are compared. Selected and ad-hoc designed examples allow to highlight the potential pitfalls behind them, and provide suggestions for choosing the most appropriate method for accurately estimating molecular size in solution for each individual studied molecular system.

Results and Discussion

V_H and MW: what determines self-diffusion properties?

The first question that might arise from comparing Equation (1) and (2) concerns the molecular property that actually determines self-diffusion coefficients: is it V_H, MW or both of them? In order to clarify this point from an experimental point of view, it has been searched for molecules having the same V_H but significantly different MW. Good candidates have been found in the three bis-cyclopentadienyl complexes **Cp₂MCl₂** (Cp = cyclopentadienyl, M = Ti, Zr, Hf; Figure 1). Although the atomic radii of the metal centers are not exactly the same, these three homologous species are practically isosteric[13] and can be reasonably considered as having the same V_H; conversely, their MWs are significantly different (~50% increase going from **Cp_2TiCl_2**, 248.9 g/mol to **Cp_2HfCl_2**, 379.6 g/mol). The D_t for these three metallocenes in toluene-d_8 solutions at 298 K have been measured and found to be substantially the same, considering the 5% experimental uncertainty (Entries 1-3, Table 1).[1f, 14]

These results clearly indicate that the hydrodynamic volume, not the molecular mass, determines self-diffusion rates, as dictated by the SE equation. This information is crucial to avoid misconceptions in the manipulation of D_t (*vide infra*), but it is not necessarily obvious and sometimes overlooked. In this respect, it is worth emphasizing that, while the Equation (1) is a generalized correlation based on first principles, the derivation of Equation (2) depends on the shape and density of the species under examination, *i.e.* it is case-specific. Caution is therefore required when empirically extrapolating the K and α parameters for a set of different molecules. These considerations generally apply also to the other, more elaborated equations proposed to correlate D_t and MW,[8a, 10, 12, 15] which can be extremely useful and effective if correctly employed within their range of validity.



Cp_2TiCl_2 **Cp_2ZrCl_2** **Cp_2HfCl_2**

Figure 1. Bis-Cp complexes of the group IV metals.

Table 1. MW, measured D_t and estimated V_H of the species shown in Figures 1-2.

Entry	Name	MW	D_t	V_H
1	**Cp_2TiCl_2**	248.9	11.4±0.6	295±44
2	**Cp_2ZrCl_2**	292.3	11.7±0.6	280±42
3	**Cp_2HfCl_2**	379.6	11.5±0.6	291±43
4	**POSS(OH)₂**	891.6	5.3±0.3	1724±259
5	**POSS-Ti-POSS**	1828.1	4.0±0.2	3500±525
6	**MeAl(bht)₂**	480.7	7.0±0.4	847±127
7	**Al(bis-bht)Al**	945.4	4.7±0.2	2292±344

D_t measured performed in toluene-d_8 at 298K. 5% experimental error assumed on D_t,[1f, 14] implying 15% uncertainty on V_H and calculated MW. MW in g/mol, D_t values in 10^{10} m/s^2, V_H in Å^3.

Strengths and limitations of the modified SE equation

Molecular size information in terms of r_H and V_H can be derived from D_t through the SE equation. As they are quite elusive molecular properties, relative rather than absolute hydrodynamic radii and volumes are considered in many cases; for instance, the level of aggregation of a supramolecular system in solution can be determined by dividing the V_H of the aggregate by that of the monomeric species (V_H^0).[1e, 14] In this respect, the main difficulties in the application of Equation (1) are the determination of the size c and shape f_s parameters of both aggregates and monomers.[16] While the first can be addressed by using suitable empirical equations,[1f] determining accurate f_s is more problematic, since it requires the shape of the particle to be accurately known.[17] As these pieces of information are often unavailable,[18] Equation (1) is frequently employed by simply assuming a spherical shape, *i.e.* $f_s = 1$. It has been shown that this represents a reasonable approximation in many cases.[1f] For instance, going from a perfectly spherical to a spheroidal particle having a semi-axis three times longer than the other, should lead to an increase of f_s from 1.0 to only 1.1; furthermore, the f_s factor is essentially normalized when considering V_H/V_H^0 ratios between species having comparable shapes.[1f]

Some examples can help familiarizing with these considerations. The two molecules shown in Figure 2a have been identified as a suitable introductory case. The silsesquioxane diol **POSS(OH)₂** has a cage-like structure that can be reasonably considered of spherical shape. By its reaction with half an equivalent of $Ti(O^iPr)_4$, **POSS-Ti-POSS** is obtained *via iso*-propanol elimination.[19] From a structural point of view, a single Ti atom bridging two 20-membered Si/O cages does not contribute significantly to the overall molecular volume. Therefore, comparing **POSS(OH)₂** with **POSS-Ti-POSS** can be seen as a representative example of a sphere-like particle having radius a vs a prolate spheroidal-like particle having semi-axis a and $b = 2 * a$. By applying Equation (1) with $f_s = 1$, the V_H of the latter

10.1002/chem.201900812

(3500 ± 525 Å^3) is found to be exactly twice bigger than that of the former (1724 ± 259 Å^3, Entries 4-5, Table 1). This demonstrates that the modified SE equation can be used within the spherical approximation to quantitatively evaluate POSS-dimerization even though it causes an evident change in the molecular shape. Analogous considerations apply to **MeAl(bht)$_2$** and its 'dimeric' form **Al(bis-bht)Al**, shown in Figure 2b. This couple of molecules represents an even more challenging example, as their structures deviate even more from a spherical shape. Nevertheless, assuming $f_s = 1$, a V_H/V_H^0 of ~2 has been found within experimental uncertainty for **Al(bis-bht)Al** (2292 ± 344 Å^3, Figure 2a) and **MeAl(bht)$_2$** (847 ± 127 Å^3), as expected.

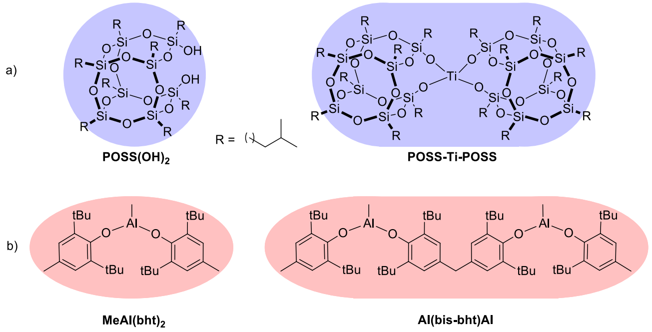


Figure 2. Structures of studied silsesquioxanes and Al-complexes.

While these encouraging examples indicate that some tolerance is acceptable when dealing with f_s, this has not to be considered as a *panacea*. In the generality of cases where more or less flexible and spheroidal molecules are considered, Equation (1) can be safely used within the spherical approximation to compare a monomeric species with its dimer, trimer or very short oligomers,[1e, 14, 20] even in the case of some biologically relevant peptides,[20d-e] as the associated variations in shape are usually small.[21] Caution is definitely required when considering longer polymers or higher aggregates, instead.

The case of linear polymers is particularly problematic. For instance, the D_t of styrene (19.8 * 10^{-10} m/s^2) and of a commercial monodisperse 10 kDa polystyrene sample (PS; 1.43 * 10^{-10} m/s^2) have been measured under analogous conditions. keeping into account their different *c* parameter. Nevertheless, the estimated V_H/V_H^0 suggests a polymerization degree (P_n) of ~640, clearly overestimating the independently determined value of ~97. This discrepancy is likely due to the fact that the f_s of a single styrene unit is significantly different from that of a linear polystyrene macromolecule and, in particular, the latter deviates noticeably from the spherical approximation. Furthermore, it is known that the self-diffusion of polymers in dilute solutions exhibits some peculiarities with respect to typical small compounds, as occasional interactions between macromolecules lead to an increase of their frictional coefficient (*i.e.* a decrease of D_t) with respect to the solvent-dominated diffusion limit.[9c, 9d, 22] This makes the physical definition of V_H even more complex to grasp in the case of polymers.

The above considerations do not necessarily imply that only limited ranges of polymerization/aggregation can be analysed, as the modified SE equation can still work when considering large non-linear systems. For instance, ion pairs in non-polar solvents likely form 3D spheroidal aggregates rather than 2D linear systems; the spherical approximation might still hold in this case. As a matter of fact, methods based on Equation (1) are well-established to study ion pair aggregation.[1c, 1d, 4, 23] Likewise, other common applications concern the study of *e.g.* dendrimers,[24] and self-assembled supramolecular systems.[25]

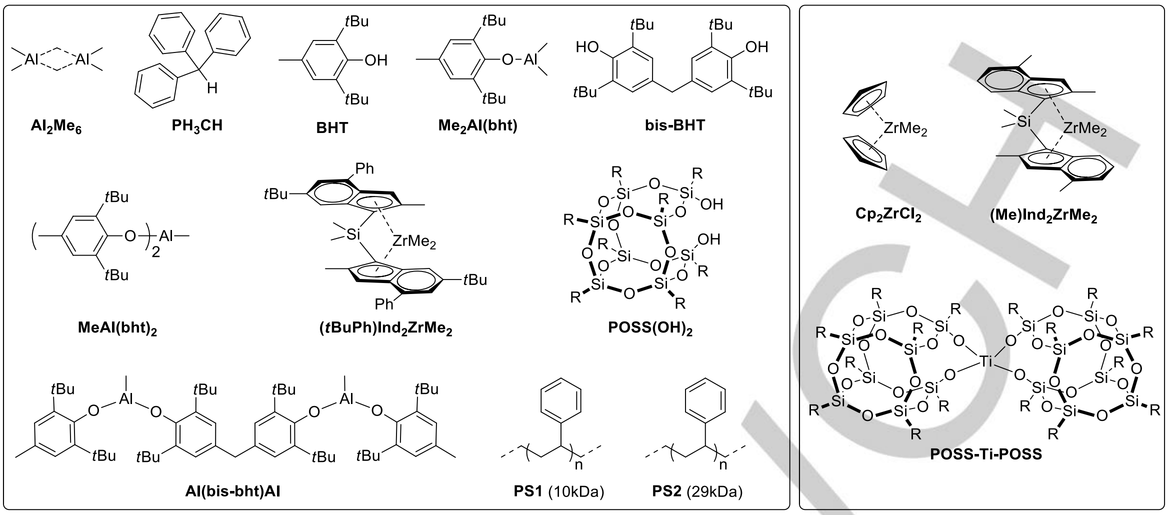
Finally, a peculiar and often tricky case to consider is that of hollow molecules.[1a-b] Based on the example of Figure 1, it is reasonable to conclude that species with empty internal cavities will self-diffuse at the same rate of analogous densely filled particles having the same dimensions.[26] Therefore, determining the number of elemental units in *e.g.* a hollow organometallic cluster from the V_H/V_H^0 ratio faces an additional hurdle, as not all the molecular volume (*i.e.* V_H) actually contains an elemental unit (*i.e.* V_H^0). This problem has been occasionally addressed by estimating some empirical correction factors for V_H that account for the internal cavities of the studied molecules, as reported for instance in the case of methylaluminoxane.[27]

Strengths and limitations of the MW power law

In principle, the application of Equation (2) and (5) for the determination of MW is rather simple. The D_t of a number of reference species have to be measured under the same experimental conditions to build up a Log(D_t) vs Log(MW) calibration curve; then, the MW of an unknown species can be estimated from its D_t (or *viceversa*) by interpolating or extrapolating it in this calibration curve.

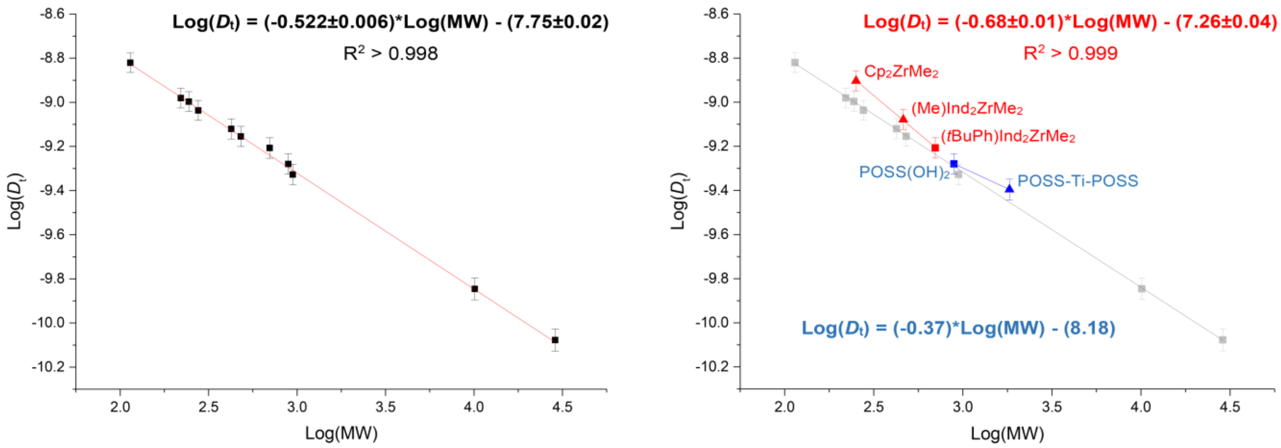
Among others,[5a, 8, 10a, 11a-c, 15a, 25b, 28] a typical, successful case of application is the determination of the average MW of linear synthetic polymers, for which a plethora of examples is available in the literature.[3a, 9a, 15b, 29] This approach relies on the assumption of a monomodal MW distribution,[30] although advanced methods to determine polydispersity by diffusion NMR have been proposed.[9a, 31] The application of Equation (2) or (5) is especially advantageous in this case. The calibration curve is usually built by using known samples of the same kind of polymer of the analyte, having different and independently determined MWs (*e.g.* by gel permeation chromatography or mass spectrometry). Within a certain range of P_n,[32] all the contributions to D_t that are especially difficult to estimate for polymers (*i.e.* in terms of shape, density, intramolecular interactions affecting the friction factor) can be assumed to be constant for all these species, and are empirically accounted for in the fitted K and α parameters. Thus, as the MW of the analyte is determined with respect to its shorter or longer homologues (not to the monomer), the aforementioned problems related to size estimation of polymers are circumvented, as all the critical factors are normalized.

The choice of the reference species is indeed crucial to obtain a reliable MW estimation; the calibration set has to be sufficiently large and compatible with the analyte in terms of molecular shape and composition (and therefore molecular density).[10a, 11c] Concerning the latter, the contribution of 'heavy' atoms like metals or halides, as well as of molecular cavities, has to be carefully accounted for, as it will likely impact molecular density significantly. Likewise, also comparing hydrogenated and deuterated species can be problematic.[11c] In this respect, it is



a) b)

Figure 3. Set of molecules used to build up the calibration curve of Figure 4a (a), and species used as stress test (b). R = iso-butyl.



a) b)

Figure 4. Log(D_t) vs Log(MW) plot obtained for the set of molecules of Figure 3a (a), and comparison with analogous plots obtained using the stress test molecules of Figure 3b (b). □ = molecules in the calibration set (Figure 3a); Δ = molecules used as stress test (see Figure 3b and main text). Fitted equations for the points in red and blue reported for semi-quantitative comparison.

Table 2. MW and experimental D_t of the species in Figure 3, and calculated MW using the calibration curve of Figure 4a.

Entry	Name	MW	D_t	Log(MW)	Log(D_t)	calc. MW
1	**Al_2Me_6**	114.2	15.1±0.8	2.06	-8.82	112±16
2	**BHT**	220.4	10.5±0.5	2.34	-8.98	227±33
3	**Ph_3CH**	244.3	10.1±0.5	2.39	-9.00	243±36
4	**$Me_2Al(bht)$**	276.4	9.2±0.5	2.44	-9.04	290±43
5	**bis-BHT**	424.7	7.6±0.4	2.63	-9.12	420±62
6	**$MeAl(bht)_2$**	480.7	7.0±0.4	2.68	-9.15	488±72
7	**(*t*BuPh)Ind_2ZrMe_2**	700.2	6.21±0.4	2.84	-9.21	615±91
8	**POSS**	891.6	5.3±0.3	2.95	-9.28	847±125
9	**Al(bis-bht)Al**	945.4	4.7±0.2	2.98	-9.33	1047±154
10	**PS 1**	10110	1.4±0.1	4.00	-9.85	10300±1515
11	**PS 2**	28770	0.84±0.04	4.46	-10.08	28593±4027
12	**Cp_2ZrMe_2**	251.5	12.5±0.4	2.40	-8.90	161±24
13	**(Me)Ind_2ZrMe_2**	463.3	8.3±0.5	2.66	-9.08	349±51
14	**POSS-Ti-POSS**	1828.1	4.0±0.2	3.26	-9.40	1415±208

Data ordered according to increasing MW. D_t measured in toluene-d_8 at 298K. 5% experimental error assumed on D_t,[1f, 14] implying 15% uncertainty on V_H and calculated MW. D_t values in 10^{10} m/s^2, MW in g/mol.

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instructive to recall the example reported in Figure 1 (see also Entries 1-3, Table 1). A single atom is replaced going from **Cp_2TiCl_2** to **Cp_2ZrCl_2** and **Cp_2HfCl_2**, and this results in a significant variation of molecular density. This implies that, in order to obtain reliable molecular information, the MWs of these three complexes should be estimated separately by using three specific calibration curves for titanocenes, zirconocenes and hafnocenes, respectively.

Obviously, some flexibility can be acceptable in the selection of the reference species, depending on the type of problem that has to be addressed. In some case, it has been shown that chemically useful information can be obtained also from correlations established among a quite diversified set of molecules,[8a, 11a, 12, 15a] but to what extent can this be done? The answer to this question largely depends on the specificity of the case study. However, a general, important point to make is that, even if a good linear correlation is obtained for certain group of molecules, it does not imply that the resulting plot can be used as a reliable calibration curve.

For instance, the species reported in Figure 3a have been selected to construct the Log(D_t) vs Log(MW) curve shown in Figure 4a. An excellent linear fitting has been obtained ($R^2 > 0.998$), despite the various nature of the reference molecules spanning a very wide range of MW (Entries 1-11, Table 2). Within a reasonable degree of approximation, only the subgroup including the small organic molecules (*e.g.* **Ph_3CH**, **BHT**, **bis-BHT**) and the Al-complexes (*e.g.* **$Me_2Al(bht)$**, **$MeAl(bht)_2$**, **Al(bis-bht)Al**) can be assumed to have comparable shape and density, since variations in molecular shape are not dramatic (*vide supra*) and ρ should not be affected considerably by the presence of 'light' metal centers like the aluminium ones. Conversely, the same considerations hardly apply to the other species in the calibration set.

To gain further insight in this correlation, zirconocenes **Cp_2ZrMe_2** (251.5 g/mol) and **$(Me)Ind_2ZrMe_2$** (463.3 g/mol), and **POSS-Ti-POSS** (1828.1 g/mol) in Figure 3b can be considered as suitable stress tests for this calibration curve, as they are analogous to the reference species **$(t$BuPh$)Ind_2ZrMe_2$** and **$POSS(OH)_2$**.[33] The estimated MW for these three analytes are found to be 161 ± 24, 349 ± 51 and 1415 ± 208 g/mol, respectively, differing significantly from the expected values (Entries 12-14, Table 2). A graphical analysis of results can help rationalizing these observations. Figure 4b compares the calibration curve of Figure 4a (squared points) with the Log(D_t) vs Log(MW) plots obtained for the aforementioned three dimethyl zirconocenes (red points) and two silsesquioxanes (blue). As they are established among homologous species, the latter two linear correlations are definitely more reliable than the former, although the limited number of experimental points provide only semi-quantitative information. By analysing the graph in Figure 4b, it can be concluded that **$(t$BuPh$)Ind_2ZrMe_2$** and **$POSS(OH)_2$** are found to correlate with the other species in Figure 3a just accidentally, because it happens that they lie at the intersections between the curve of Figure 4a and those specific for zirconocenes and silsesquioxanes, respectively. Analogous considerations likely apply to the two PS polymers, the D_t of which cannot be directly compared to those of small molecules, as discussed in the previous paragraphs.

Conclusions

Although diffusion NMR techniques are powerful tools to investigate molecular size in solution, some caution is required when quantitative information is desired. In this paper, the performances of two established protocols to estimate V_H or MW of target molecules from their D_t have been compared, highlighting their strengths and limitations. Practical examples have allowed to recall the basic principles describing translational self-diffusion of molecules in solution, the most important being that particles with same dimensions diffuse at the same rate independently from their mass. Starting from this crucial observation, the analysis of ad hoc selected case studies has allowed to translate more elaborate theoretical concepts in simpler prescriptions for routine applications.

The take-home messages can be summarized saying that deriving r_H and V_H, by using the SE equation, is preferable when molecules with non-uniform molecular density are investigated. In many cases, this approach can be highly simplified by assuming a spherical shape of the studied species; the resulting V_H/V_H^0 ratios are generally reliable parameters to quantitatively estimate dimerization or trimerization processes, and higher aggregation or polymerization degrees provided that the molecular shape of the monomer is reasonably retained. The determination of MW from D_t using Equations (2) or (5) is instead preferable when known homologues of the analyte can be used as reference compounds, and it is especially convenient for species deviating significantly from the spherical approximation and/or having peculiar factors determining D_t (*e.g.* linear macromolecules).

Experimental Section

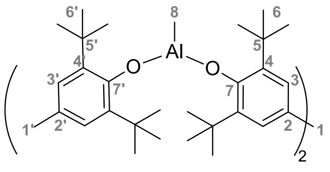
Manipulations of air-sensitive compounds were performed under rigorous exclusion of oxygen and moisture in flame-dried Schlenk-type glassware interfaced to a high-vacuum line ($< 10^{-5}$ Torr), or in a nitrogen-filled MBraun glovebox (<0.5 ppm O_2). Molecular sieves (4Å, MS) were activated for 24 h at ca. 200-230 °C under dynamic vacuum. Toluene-d_8 was freeze-pump-thaw degassed on a high-vacuum line, dried over Na/K alloy, vacuum-transferred to a dry storage-tube with a PTFE valve and stored over activated MS in the glovebox. Bis-cyclopentadienyl complexes, triphenylmethane, POSS, trimethylaluminum, styrene, BHT and bis-BHT were purchased from Sigma-Aldrich, while $Ti(O^iPr)_4$ from Abcr and PS1-PS2 from Agilent Technologies, and used without further purifications. **$Me_2Al(bht)$**,[34] **$MeAl(bht)_2$**,[34] **$(Me)Ind_2ZrMe_2$**,[35] **$(t$BuPh$)Ind_2ZrMe_2$**[36] and **POSS-Ti-POSS**[19] were synthesized according to previously established procedures.

NMR experiments were performed using a Bruker Avance III HD 400 instrument equipped with a smartprobe (400 MHz for ^1H) with a z gradient coil. ^1H NMR spectra were referenced to the residual protons of the deuterated solvent used; ^{13}C NMR spectra were referenced internally to the D-coupled ^{13}C resonances of the NMR solvent. To describe the multiplicity of the signals, the following abbreviations are used: s, singlet. DOSY and PGSE NMR measurements were performed at 298K in toluene-d_8 by using the standard double-stimulated echo pulse sequence without spinning. The shape of the gradients was rectangular, their duration (δ) was 4 ms, and their strength (G) was varied during the experiments. All the spectra were acquired using 32K points, between 16 and 128 scans, a spectral width of 6400 Hz, an acquisition time of 0.5 s and a relaxation delay of 1 s per transient. The DOSY spectra were acquired using 128 or 256 increments and processed by means of the Bruker *Dynamics Center* software package (version 2.5.5), using a line broadening of 1.0 Hz in the direct dimension. The DOSY maps were obtained by using the Inverse Laplace Transform routine and choosing

128 or 256 points in the vertical dimension. Toluene-d_8 was used as internal standard to account for temperature and viscosity fluctuations; its D_t was calibrated using an external sample of HDO in D_2O[37] under the same exact conditions. D_t data were treated as described in the literature in order to derive the hydrodynamic dimensions.[1f, 14] In the cases where the aliphatic resonance of some species overlap with that of toluene-d_8, self-diffusion coefficients were calculated with respect to that of Ph_3CH. The D_t of Ph_3CH in toluene-d_8 was separately calibrated using the diffusion coefficient of residual solvent resonance in a dilute solution (0.1 mM). The obtained Stejskal-Tanner[38] plots are reported in the Supporting Information.

Synthesis of Al(bis-bht)Al. TMA (3 µL, 20 µmol) was added to a solution of BHT (4.4 mg, 20 µmol) in toluene-d_8 (1.3 mL). This reaction proceeds rapidly giving **$Me_2Al(bht)$**, along with minor amounts of **$MeAl(bht)_2$** and residual TMA.[34] Upon addition of bis-BHT (6.4 mg, 15 µmol), **$Me_2Al(bht)$** reacts practically quantitatively giving **Al(bis-bht)Al**, while **$Me_2Al(bht)$** remains unchanged and TMA forms small amounts of oligomeric Al-phenolate complexes. The minor side products do not represent a significant hurdle for accurate and complete NMR characterization of **Al(bis-bht)Al** , as well as for its analysis by DOSY NMR (accomplished by monitoring diagnostic signals being well separated from those of other species, *e.g.* CH_2 protons).

^1H NMR (400 MHz, toluene-d_8): 7.32 (s, H3), 7.15 (s, H3‘), 4.06 (s, H1), 2.32 (s, H1‘), 1.60 (s, H6), 1.59 (s, H6‘), -0.24 (s, H8) ppm. ^{13}C NMR (100 MHz, toluene-d_8): 152.2 (C7 and C7‘), 138.1 (C4 and C4‘), 131.9 (C2), 127.6 (C2‘), 125.8 (C3), 125.7 (C3‘), 41.7 (C1), 34.8 (C5 and C5‘), 31.6 (C6 and C6‘), 21.0 (C1‘), -9.5 (C8) ppm.



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Keywords: DOSY, PGSE, hydrodynamic volume, molecular weight, Stokes Einstein

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[17] a) For instance, the Perrin’s equation (ref. 17b) allows to derive accurate r_H for prolate (cigar-like) and oblate (disk-like) spheroidal particles, respectively (see ref. 17c-e for some recent examples), while more elaborated approaches are necessary for species having three different semiaxis (ref. 17f); b) F. Perrin, *J. Phys. Radium.*, **1936**, *7*, 1; c) Giuseppone, N.; Schmitt, J.-L.; Allouche, L.; Lehn, J.-M., *Angew. Chem. Int. Ed.* **2008**, *47*, 2235-2239; d) Wang, L.; Liu, R.; Gu, J.; Song, B.; Wang, H.; Jiang, X.; Zhang, K.; Han, X.; Hao, X.-Q.; Bai, S.; Wang, M.; Li, X.; Xu, B.; Li, X., *J. Am. Chem. Soc.* **2018**, *140*, 14087-14096; e) Zhang, Z.; Wang, H.; Wang, X.; Li, Y.; Song, B.; Bolarinwa, O.; Reese, R. A.; Zhang, T.; Wang, X.-Q.; Cai, J.; Xu, B.; Wang, M.; Liu, C.; Yang, H.-B.; Li, X., *J. Am. Chem. Soc.* **2017**, *139*, 8174-8185; f) P. H. Elworthy, *J. Chem. Soc.*, **1962**, 3718–3723.

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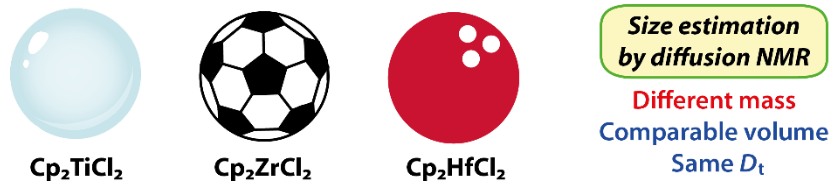

Diffusion NMR techniques can be used to determine the size of molecules in solution. Selected examples allow the basic principles underlying translational self-diffusion to be recalled and derive practical lessons for the accurate manipulation of self-diffusion coefficients (D_t).

Francesco Zaccaria, Cristiano Zuccaccia, Roberta Cipullo and Alceo Macchioni*

Page No. – Page No.

Extraction of reliable molecular information from diffusion NMR spectroscopy: hydrodynamic volume or molecular mass?