

# Determining accurate molecular sizes in solution through NMR diffusion spectroscopy

Alceo Macchioni,\* Gianluca Ciancaleoni, Cristiano Zuccaccia and Daniele Zuccaccia

Received 21st September 2007

First published as an Advance Article on the web 11th October 2007

DOI: 10.1039/b615067p

This *tutorial review* deals with the methodological procedures that can be used to obtain accurate molecular sizes in solution from diffusion NMR spectroscopy. The critical aspects associated with the estimation of the size of molecules from the measured translational self-diffusion coefficient, using the Stokes–Einstein equation, are highlighted and discussed. From a theoretical point of view, it is shown how to take into account the size of the solute with respect to that of the solvent and its non-spherical shape using the appropriate correction factors in the frictional coefficient. From a practical point of view, the advantages of introducing an internal standard in the sample are presented. Initially, non-aggregating systems are considered in an attempt to clarify what hydrodynamic dimensions mean. Successively, aggregating systems are addressed showing how it is possible to understand the aggregation level and derive the thermodynamic parameters for some illustrative aggregation processes.

## 1.0 Introduction

Although diffusion NMR experiments have been known for a long time,<sup>1,2</sup> only in the last few years they have become a routine tool for evaluating the size of molecules in solution, mainly due to the implementation and commercialization of new NMR instrumentation having the default capability of generating pulsed-field-gradients along the direction of the magnetic field.

The physical observable that can be derived from diffusion NMR experiments is the translational self-diffusion coefficient ( $D_t$ ), *i.e.* the translational diffusion coefficient of a species in the absence of a chemical potential gradient. It is intuitive that

$D_t$  is related to the size of the diffusing molecules. The relationship was established a long time ago by combining the contributions of Stokes and Einstein.<sup>3</sup> According to the very famous Stokes–Einstein equation, the hydrodynamic dimension of a self-diffusing species can be accurately obtained from  $D_t$ , provided that colloidal particles are considered. For smaller particles, comparable to or only slightly larger than the solvent, the Stokes–Einstein equation would be not valid. Despite this, scientists frequently use it to obtain “qualitatively reasonable results”. Nevertheless, if quantitative information on the hydrodynamic dimensions is desired, the passage through the Stokes–Einstein equation becomes critical.

The main aim of this *tutorial review* is to show, discuss and, hopefully, suggest how to overcome the numerous problems associated with the accurate estimation of the dimension of small- or medium-sized molecules from  $D_t$  using the Stokes–Einstein equation. The first part of the review focuses on the manipulation of  $D_t$  data to obtain accurate hydrodynamic dimensions for non-aggregating molecular systems. In the second part, the difficulties connected with the investigation of aggregating systems are considered. In the third and final part, it is shown how the thermodynamic parameters of the aggregative processes can be derived from diffusion data.

Chemists have always had an intrinsic interest in the size of molecules but, in the last decades, that interest has increased due to the tremendous development of disciplines aimed at constructing nanometric molecular entities that have diversified and proper physical chemical properties through a bottom-up approach. Bottom-up approach means that the molecular complexity, and consequently the size, of the system is increased step by step; clearly, it would be highly desirable to have a tool for evaluating the accretion process. Herein, it is shown that diffusion NMR experiments are a good way to evaluate aggregation and obtain accurate hydrodynamic dimensions in solution provided that size- and shape-correcting factors are considered.

---

Dipartimento di Chimica, Via Elce di Sotto, 8 – 06123 Perugia, Italy.  
E-mail: [alceo@unipg.it](mailto:alceo@unipg.it); Fax: +39 075 5855598; Tel: +39 075 5855579

---



Alceo Macchioni

*Alceo Macchioni obtained his “Laurea” and PhD in Chemistry from the University of Perugia. After a two-year postdoctoral stay at the ETH of Zürich (Switzerland), in the groups of Professors Pregosin and Venanzi, he returned to Italy as a Researcher at the University of Perugia. In 2002 he was appointed Associate Professor in General and Inorganic Chemistry at the same University. In 2002 he received the “Raffaello Nasini” prize from the*

*Inorganic Division of the Italian Chemical Society. His research interests include the investigation of intermolecular metallorganic adducts in solution to understand how weak interactions alter their reactivity and structure.*

## 2.0 An easy access to $D_t$ by NMR

Several NMR methods based on the spin-echo building block have been developed for determining  $D_t$ . The aim of this review is not to discuss which are the underlying pulse NMR sequences nor to explain why it is possible to derive  $D_t$  through diffusion NMR. Excellent reviews have already been presented on these aspects.<sup>4–9</sup> Herein we want to discuss diffusion NMR experiments from a pragmatic point of view in order to show what information can be obtained and how reliable it is. Although most of the examples of this review concern organometallic compounds (our field of research), the methodology herein illustrated should have a general validity and could be applied to all chemical systems including supramolecular<sup>8</sup> and biomolecular<sup>9</sup> ones that have been deeply investigated through diffusion NMR spectroscopy.

First of all, diffusion experiments are simple to carry out and usually afford quick results if nuclei of reasonably high receptivity and rather concentrated samples are investigated. For instance, the most commonly used PGSE (Pulsed field Gradient Spin-Echo) NMR experiments require recording  $n$  monodimensional spectra as the strength of the pulsed-field-gradient ( $G$ ) along the  $z$  axis is varied. Plotting  $\log(I/I_0)$  versus  $G^2$ , where  $I$  and  $I_0$  are, respectively, the intensity of a resonance in the presence and in the absence of the pulsed-field-gradient, a straight line is obtained that has a slope that is directly proportional to  $D_t$ . Calibration with a substance with a known  $D_t$  (usually water) leads to the determination of the proportionality constant between  $D_t$  and the slope from which the  $D_t$  can be obtained for the studied sample. Decays of the resonances as a function of  $G$  and the related trends of  $\log(I/I_0)$  versus  $G^2$  for a solution of TMSS [tetrakis(trimethylsilyl)silane] in  $\text{CD}_2\text{Cl}_2$ , containing some amount of water, are illustrated in Fig. 1. Looking at the slope of the straight lines or at the rate of decay of the resonances, it is clear that  $D_t$  is inversely dependent on the size of the molecules.

If  $^1\text{H}$ - or  $^{19}\text{F}$ -PGSE experiments are carried out on samples with a concentration around 0.1–100 mM the total acquisition time can vary from 10 min to several hours on a standard NMR machine depending on the desired precision. In addition, the individual  $D_t$  values for all species in a mixture can be evaluated by a single diffusion experiment preferably when at least a resonance of each component is well-separated in the NMR spectrum. All these considerations indicate that  $D_t$  can be readily determined from diffusion NMR experiments.

## 3.0 The Stokes–Einstein equation: from $D_t$ to $r_H$

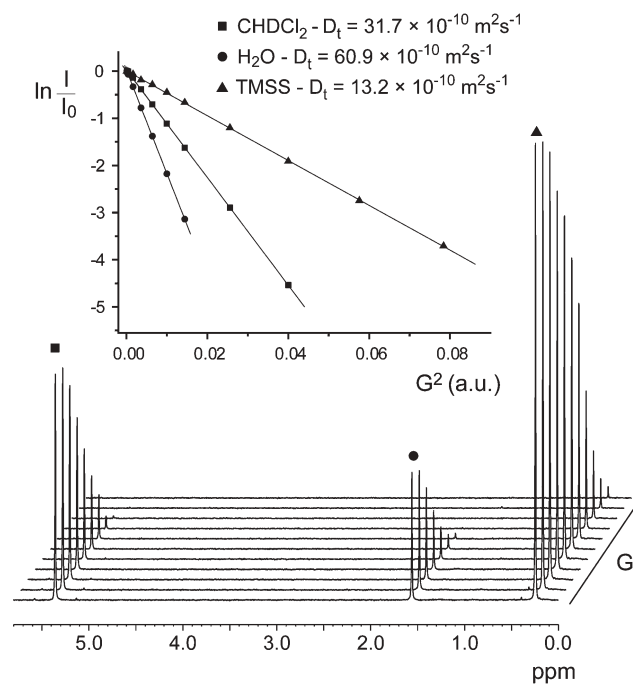
The Stokes–Einstein eqn (1) relates  $D_t$  to a structural property of the diffusing particle, *i.e.* the hydrodynamic radius ( $r_H$ ):

$$D_t = \frac{kT}{6\pi\eta r_H} \quad (1)$$

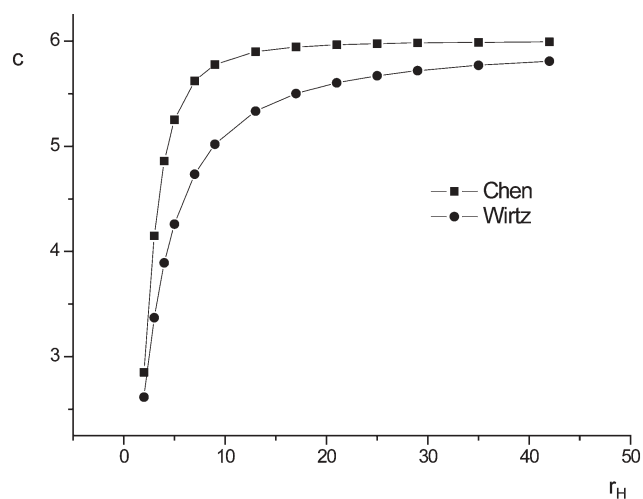
In eqn (1),  $k$  is the Boltzman constant,  $T$  is the absolute temperature and  $\eta$  is the fluid viscosity. Setting aside the definition of  $r_H$  only for a while, it is important to recall that the Stokes–Einstein equation was derived by assuming that a spherical particle of colloidal dimension (much higher than that of the solvent) moves with uniform velocity in a fluid

continuum. In spite of this, eqn (1) is used more and more frequently to derive  $r_H$  from the  $D_t$  of non-spherical molecules of comparable or slightly larger size than the solvent. In principle, eqn (1) is not more valid in such cases; consequently the question is: does the derived  $r_H$  have any meaning? In addition, is it possible to modify eqn (1) in order to make it valid for non-spherical small molecules? Initially, it was proposed that for small molecules the denominator of eqn (1) (frictional coefficient) could be equal to  $4\pi\eta r_H$  (slip-boundary condition) instead of  $6\pi\eta r_H$  (stick-boundary condition).<sup>3</sup> Nevertheless, such an approach that attempts to maintain part of the Stokes model has been criticized and it has been suggested to use a numerical factor  $c$  instead of “6” or “4” depending on the ratio between the radius of solvent ( $r_{\text{solv}}$ )<sup>10</sup> and  $r_H$ . An expression of factor  $c$  as a function of  $r_{\text{solv}}/r_H$  was derived by Wirtz<sup>11</sup> according to a microfrictional theory and semi-empirically improved by Chen.<sup>12</sup> Typical trends of  $c$  as a function of  $r_H$  are reported in Fig. 2 for methylene chloride ( $r_{\text{solv}} = 2.49 \text{ \AA}$ ). Both trends tend to 6 as  $r_H$  increases. While the expression derived by Chen predicts a  $c$  value equal to 6 already when  $r_H \approx 15 \text{ \AA}$ , according to Wirtz the  $c$  value is close to 6 only when the  $r_H$  values are greater than  $40 \text{ \AA}$ . Hence, even considering Chen’s expression, the classical Stokes–Einstein eqn (1) can be used when the radius of the molecules is at least five times larger than that of the solvent. It is also interesting to note that for medium-sized molecules, having a radius ranging from 3 to 6  $\text{\AA}$ , the value of  $c$  differs significantly both from 4 and 6. Thus, the proper  $c$  factor must be introduced in the analysis of diffusion data for most of organic and inorganic molecules.

Another correction factor ( $f_s$ ) must be included in the denominator of eqn (3) when the shape of the molecule is being



**Fig. 1** Dependence of the resonance intensities on  $G$  and trends of  $\log(I/I_0)$  versus  $G^2$  (a.u. = arbitrary units) for a solution of TMSS and  $\text{H}_2\text{O}$  in  $\text{CD}_2\text{Cl}_2$ .



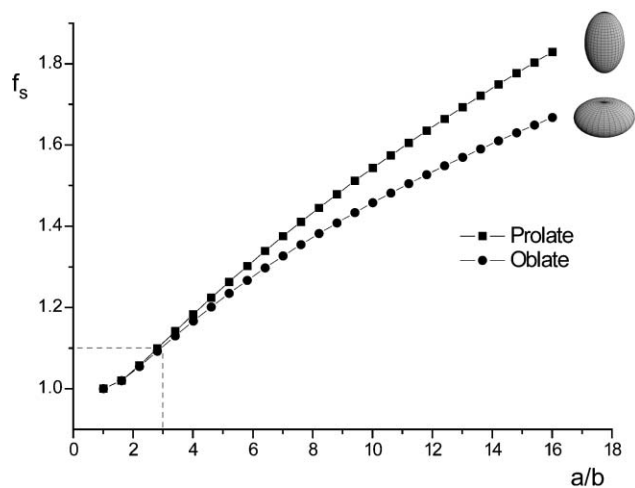
**Fig. 2** Dependence of the  $c$  size factor on  $r_H$  according to the expressions of Wirtz and Chen taking methylene chloride as solvent.

considered.<sup>13</sup> For prolate (cigar-like) or oblate (disc-like) ellipsoid molecules, the dependence of parameter  $f_s$  on the ratio between the minor ( $b$ ) and major ( $a$ ) semi-axis of the ellipsoid was developed by Perrin about seventy years ago.<sup>14</sup> The resulting modified Stokes–Einstein equation that takes into account both the relative solute/solvent size and the shape of the molecules is:

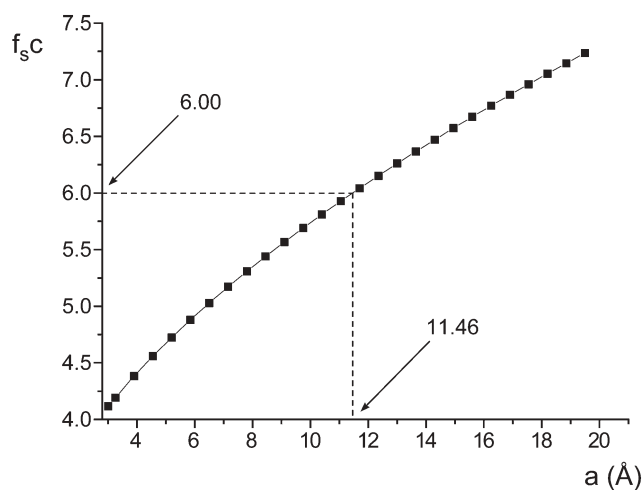
$$D_t = \frac{kT}{c(r_{\text{solv}}, r_H) f_s(a, b) \pi \eta r_H} \quad (2)$$

The dependence of  $f_s$  on  $a/b$  is illustrated in Fig. 3 for both prolate and oblate molecules. When the ratio between the semi-axes is greater than three, the error will be higher than 10% (Fig. 3) if the non-spherical shape of the diffusing molecule is ignored.

When both  $c$  and  $f_s$  correcting factors have to be applied to the Stokes–Einstein equation, *i.e.* when medium- or small-sized ( $c < 6$ ) non-spherical molecules ( $f_s > 1$ ) are considered, partial or even total compensation can occur with the  $cf_s$  product



**Fig. 3** Dependence of the  $f_s$  shape factor according to the Perrin's equations for prolate and oblate molecules.



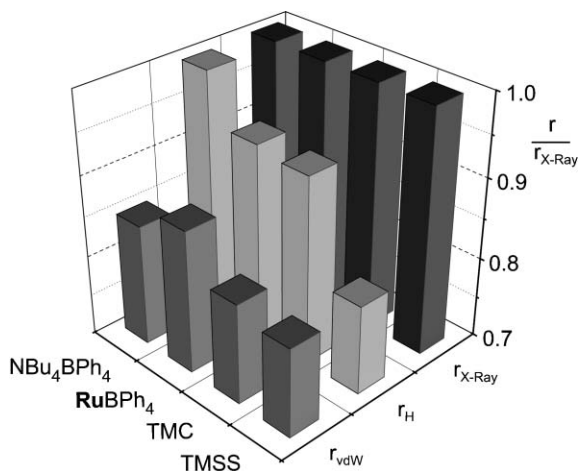
**Fig. 4** Dependence of the  $f_s c$  factor on the semi-axis  $a$  of a prolate ellipsoid having the other semi-axis  $b$  equal to 3.0 Å taking methylene chloride as solvent.

approaching 6. This is shown in Fig. 4 where the  $cf_s$  trend for a prolate ellipsoid molecule as a function of the major semi-axis  $a$  is illustrated for the Chen expression of  $c(r_{\text{solv}}, r_H)$  with methylene chloride as solvent and  $b$  equal to 3 Å.

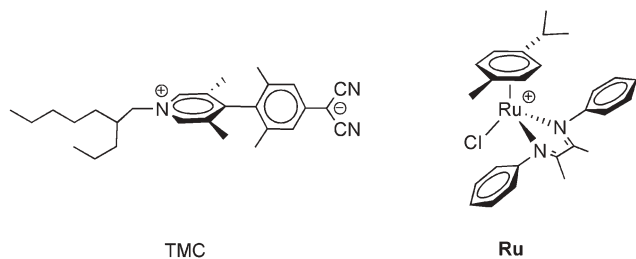
Thus, for a prolate ellipsoid molecule with  $a = 11.46$  Å and  $b = 3.0$  Å, the  $f_s$  and  $c$  factors are perfectly balanced and the classical Stokes–Einstein equation appears to be valid but this is due to the compensation of the two errors.

Let us now suppose that  $D_t$  was measured for a big, spherical molecule as well as a small, ellipsoidal molecule and that an  $r_H$  value was derived through eqn (1) or (2), respectively, in the latter case correctly taking into account the  $c$  and  $f_s$  factors. What does  $r_H$  represent? By definition, hydrodynamic sizes are related to the nature of the solvent (hydro) and the dynamic of the diffusional motion. In eqn (1) and (2),  $r_H$  is the radius of a hypothetical hard sphere that diffuses with the same speed as the particle under examination.<sup>15</sup> This definition causes some visualization problems because the real molecular system under investigation could be a non-spherical, possibly solvated, molecule with cavities or inlets. By saying that the derived  $r_H$  is just the radius of a hypothetical sphere that diffuses with the same speed does not allow its size in solution to be completely understood. Is there any relationship between  $r_H$  and other kinds of radii such as van der Waals radius ( $r_{\text{vdW}}$ ), the crystallographic radius ( $r_{\text{X-Ray}}$ ), the radius of gyration ( $r_g$ ) and the radius obtained from the standard partial molar volume ( $r_M$ )?

What starts to become clear is that  $r_{\text{vdW}}$  is the lower limit of  $r_H$  and that  $r_H$  is equal to  $r_{\text{vdW}}$  only for compact molecules without any inlet. Otherwise,  $r_H$  is greater than  $r_{\text{vdW}}$  and is always greater than  $r_g$  that, in turn, is smaller than  $r_{\text{vdW}}$ . The upper limit of  $r_H$  is frequently  $r_{\text{X-Ray}}$  that can be deduced from X-Ray by simply dividing the volume of the crystallographic cell by the number of contained molecules, assuming the latter has a spherical shape. In Fig. 5 a graphical comparison between  $r_{\text{vdW}}$ ,  $r_H$  (in  $\text{CD}_2\text{Cl}_2$ ) and  $r_{\text{X-Ray}}$  is reported for  $\text{NBu}_4\text{BPh}_4$ , a twisted molecular chromophore (TMC),<sup>16</sup>  $\text{RuBPh}_4$ <sup>17</sup> and  $\text{TMSS}$ <sup>18</sup> (Scheme 1).



**Fig. 5** Different types of radii normalized to  $r_{X\text{-Ray}}$  for selected molecules ( $r_H$  were determined in  $\text{CD}_2\text{Cl}_2$ ).



**Scheme 1**

It can be noted that while the regular, almost spherical, molecule TMSS has  $r_H \approx r_{vdW} < r_{X\text{-Ray}}$  ( $r_H/r_{X\text{-Ray}} = 0.81$ ),  $\text{NBu}_4\text{BPh}_4$  that is elongated and indented has  $r_H \approx r_{X\text{-Ray}} > r_{vdW}$  ( $r_{vdW}/r_{X\text{-Ray}} = 0.85$ ). The other two molecules show  $r_H$  values between  $r_{vdW}$  and  $r_{X\text{-Ray}}$  with the elongated TMC having  $r_H$  closer to  $r_{X\text{-Ray}}$  while for  $\text{RuBPh}_4$   $r_H$  is almost halfway between  $r_{vdW}$  and  $r_{X\text{-Ray}}$ . Although not many direct comparisons have been made, it appears that a reasonably good agreement is obtained between  $r_H$  and the radius derived from the measurements of standard partial molar volume ( $r_M$ ). For instance,  $r_M$  for  $\text{NBu}_4\text{BPh}_4$  is  $5.95 \text{ \AA}$  which is in excellent agreement with  $r_H = 5.97 \text{ \AA}$  obtained from diffusion measurements. This is, in a certain sense, reasonable because the standard partial volume ( $V_M$ ) is the limiting value of the partial molar volume of the solute as the concentration of the solute approaches infinite dilution in the solvent.<sup>19</sup> Consequently,  $V_M$  defines the volume of the solution occupied by the solute that is critically dependent on the solute–solvent interactions. The influence of the latter on the translational motion of the solute together with its size and shape is the information that is included in  $D_t$  that leads to the determination of  $V_H$ .

#### 4.0 Manipulating $D_t$ data

Whether eqn (1) or (2) is used for manipulating  $D_t$  data, there are always some practical problems associated with the calibration of the pulsed-field gradients and correct evaluation of the temperature and fluid viscosity.<sup>20</sup> These can be readily

overcome by using an internal standard that can be the solvent itself or, more frequently, TMS (tetramethylsilane) or TMSS.

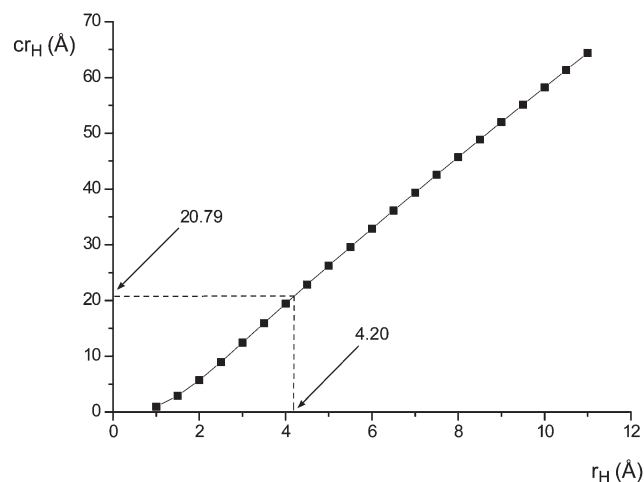
An internal standard must satisfy the following criteria. It must have at least one resonance falling in a free region of the NMR spectrum. It must be soluble in the most commonly used solvents, thermally stable, as chemically inert as possible and it must not self-aggregate. Furthermore, it is necessary that the internal standard does not undergo any specific interaction with the solvent or, better still, it would be desirable that it interacts with the solvent in the same way as the solute.

Once a proper internal standard has been selected, a preliminary diffusion measurement must be carried out on the internal standard alone, in the solvent of interest, while carefully controlling the strength of the pulsed-field gradient, the temperature and the viscosity. The latter is usually assumed to be equal to that of the pure solvent since dilute solutions of the neutral internal standard are considered. The  $cf_s r_H$  product for the internal standard is derived from the obtained  $D_t$  value.<sup>20</sup> In the case of a spherical internal standard ( $f_s = 1$ ),  $c$  and  $r_H$  can be evaluated once  $r_{\text{solV}}$  is known by preferably using the relationship developed by Chen. For instance, the trend  $cr_H$  versus  $r_H$  for  $\text{CD}_2\text{Cl}_2$  according to the Chen equation is shown in Fig. 6 together with the experimentally measured  $cr_H$  for TMSS. The value of  $r_H$  can be graphically derived from which  $c$  is obtained.

Measuring  $cf_s r_H$  for the internal standard greatly simplifies the determination of  $r_H$  for other samples. Being in the same solution, the internal standard experiences the same temperature, viscosity and eventual erroneous calibration of the pulsed-field gradients consequently the ratio of the  $D_t$  values of the standard (st) and the sample (sa) is not affected by such parameters. The ratio between the  $cf_s r_H$  factors is:

$$\frac{D_t^{\text{sa}}}{D_t^{\text{st}}} = \frac{c^{\text{st}} f_s^{\text{st}} r_H^{\text{st}}}{c^{\text{sa}} f_s^{\text{sa}} r_H^{\text{sa}}} \quad (3)$$

The  $D_t$  ratio is measured, while  $c^{\text{st}} f_s^{\text{st}} r_H^{\text{st}}$  for the standard is known. Therefore  $c^{\text{sa}} f_s^{\text{sa}} r_H^{\text{sa}}$  is obtained from eqn (3). If the sample under investigation is spherical ( $f_s^{\text{sa}} = 1$ ),  $c^{\text{sa}}$  and  $r_H^{\text{sa}}$  can be derived as described above for the internal standard. If



**Fig. 6** Dependence of  $cr_H$  on  $r_H$  for  $\text{CD}_2\text{Cl}_2$  according to the Chen equation. The example of a measurement for TMSS is shown.

the sample is prolate or oblate, the situation is much more complicated because the product depends on the  $a$  and  $b$  variables (expressing  $r_H$  as a function of  $a$  and  $b$ ). One of these variables should be known in order to determine the other.

## 5.0 Investigating non-covalent supramolecular systems through diffusion NMR

The considerations in previous paragraphs indicate that the hydrodynamic size of covalently bonded non-aggregating molecules can be accurately determined by diffusion NMR experiments provided that the relative solute/solvent dimensions and the shape of the solute have been correctly taken into account. This is, in itself, a precious information obtainable by diffusion NMR measurements. At the same time, it is propaedeutic to the investigation of non-covalent supramolecular adducts, *i.e.* molecular systems whose units are held together by electrostatic interactions, hydrogen bonds,  $\pi$ - $\pi$  stacking and C-H stacking interactions, *etc.* The marked increase of interest in biomolecular sciences and nanomaterials, whose structure and functionality critically depend on non-covalent interactions, makes the possibility of investigating them particularly attractive.

Due to the small amount of energy associated with non-covalent interactions, equilibria between the supramolecules and their components are usually established in solution. The interconversion rate is often fast compared to the chemical shift NMR time-scale and consequently a single set of resonances is observed. This implies that the average  $D_t$  value, obtained from diffusion NMR experiments, contains information about the level of aggregation and is, consequently, associated with the thermodynamic parameters of equilibria. Before considering how such information can be extracted, it is important to understand the implications related to the investigation of aggregating systems in terms of treating  $D_t$  data. Starting with the simplifications, as supramolecular adducts have usually a large average size, the importance of using the correction factor  $c$  decreases. As shown in Fig. 2,  $c$  already approaches 6 when the  $r_H$  value is 15 Å, according to Chen. The two most important complications are knowing  $r_H$  for the elemental aggregating units and the shape of the supramolecular systems.

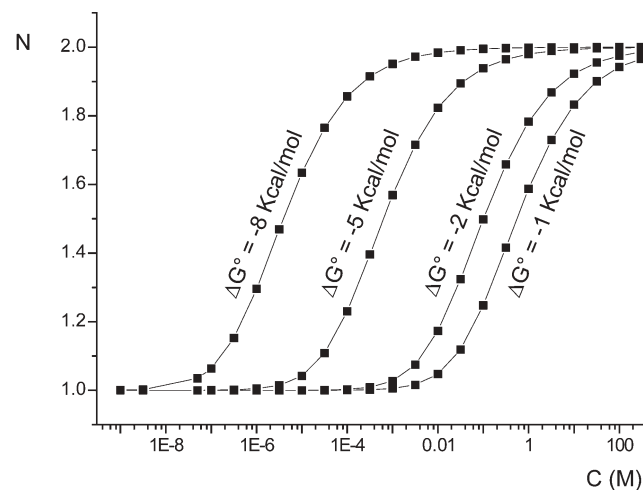
### 5.1 Hydrodynamic size of the elemental building block(s)

The determination of the hydrodynamic size of the elemental building blocks is not an easy task. Supposing that  $D_t$  has been determined for a spherical supramolecular system formed by the self-aggregation of  $N$  molecular units and that  $r_H$  has been derived using eqn (2); the hydrodynamic volume ( $V_H$ ) of the supramolecular system can be easily evaluated from  $r_H$  since we have supposed a spherical supramolecule. In order to understand how many units constitute the supramolecule it is necessary to know the hydrodynamic volume of the single unit ( $V_H^0$ ). The aggregation number ( $N$ )<sup>21</sup> is the parameter usually used to quantify the degree of aggregation and it is defined simply as the ratio  $V_H/V_H^0$ .  $N$  represents the average number of units constituting the supramolecule. Both  $V_H^0$  and, consequently,  $N$  could be determined if  $r_H^0$  were known. As

discussed before,  $r_H$  can differ markedly from both  $r_{vdW}$  and  $r_{X-Ray}$  that, instead, could be easily derived from theoretical calculations and/or diffraction studies;  $r_H$  is usually higher than  $r_{vdW}$  and lower than  $r_{X-Ray}$  but its exact value is difficult to predict. This implies that  $r_H^0$  has to be measured under experimental conditions in which the supramolecule completely disaggregates. The softest way to obtain the disaggregation is by diluting the system; or else the solvent can be changed and the concentration can be reduced in the assumption that  $r_H^0$  does not depend on the solvent, as usually observed for medium-sized molecules.<sup>19</sup> Finally, if by changing the solvent the system does not completely disaggregate a “chemical intervention”, aimed at eliminating the source of aggregation, becomes necessary. Care must be taken not to alter the shape and size of the elemental building block too much.

**5.1.1 Dilution and energetic of the aggregation.** The possibility of disaggregating a supramolecular system by dilution critically depends on the  $\Delta G^0$  of its formation reaction from the elemental units. This can be appreciated considering the simplest case of two units ( $2A$ ) that aggregate forming a dimer ( $A_2$ ). For such a system  $N$  can assume values between 1 and 2 and can be theoretically derived.<sup>22</sup> Trends of  $N$ , as a function of the concentration  $C$  are reported in Fig. 7 for different values of  $\Delta G^0$ . It can be noted that a concentration of  $10^{-4}$  M is necessary to disaggregate the two  $A$  units if  $\Delta G^0$  is  $-2$  kcal mol<sup>-1</sup>. A  $\Delta G^0$  value of  $-5$  kcal mol<sup>-1</sup> requires a dilution down to  $10^{-6}$  M, while  $-8$  kcal mol<sup>-1</sup> requires more than a  $10^{-8}$  M dilution.

The results concerning the self-association of the highest generations of DAB-dendr-[NH(O)COCH<sub>2</sub>CH<sub>2</sub>OC(O)C<sub>5</sub>H<sub>4</sub>Rh(NBD)]<sub>*n*</sub> dendrimers [ $n = 32$  (**Rh-Dab32**) and 64 (**Rh-Dab64**)] in CD<sub>2</sub>Cl<sub>2</sub> are in perfect agreement with the considerations just discussed.<sup>23</sup> For **Rh-Dab32**,  $V_H$  decreases on decreasing  $C$  reaching a plateau at  $3 \times 10^{-5}$  M that corresponds to a  $V_H$  value of *ca.* 40 500 Å<sup>3</sup>, which is reasonably related to the complete disaggregation of megamers



**Fig. 7**  $N$  as a function of the concentration ( $C$ ) for the equilibrium  $2A = A_2$  for different values of  $\Delta G^0$  at 298 K. A logarithm scale is used for the abscissa.

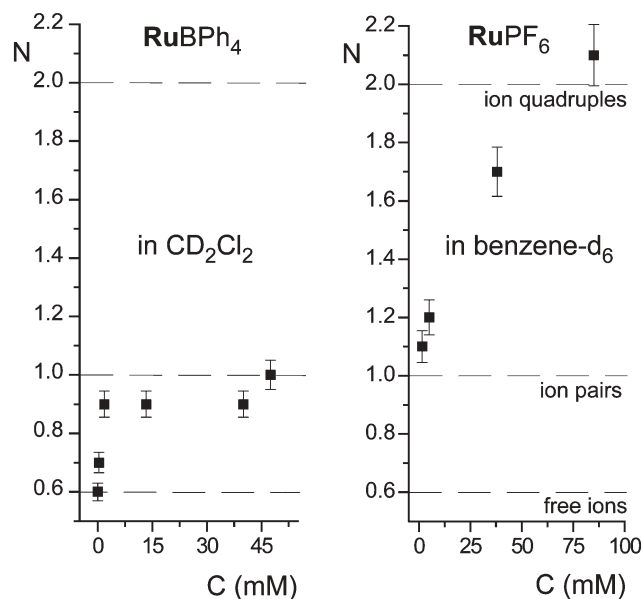
to single dendrimers. Taking the latter as  $V_H^0$ , the aggregation numbers ( $N$ ) and the equilibrium constant can be calculated using the model  $A/A_2$  seen above.  $K$  amounts to  $530 \pm 150 \text{ M}^{-1}$  ( $\Delta G_{296}^0 = -3.7 \text{ kcal mol}^{-1}$ ).

From a practical point of view, it is important to note that the lowest concentration that can be investigated through diffusion measurements, and at which reliable results can be obtained, falls in the  $10^{-4}$ – $10^{-5} \text{ M}$  range, when working on highly receptive nuclei such as  $^1\text{H}$  and  $^{19}\text{F}$ , using standard instrumentation. Thus, it appears difficult to obtain  $r_H^0$  for supramolecular systems whose units have an interaction energy higher than  $5 \text{ kcal mol}^{-1}$  when measuring the dependence of  $D_t$  on the concentration. The situation may positively change if diffusion measurements are carried out with NMR instruments equipped with a cryoprobe that allows the sensitivity to be increased.

**5.1.2 Changing solvent.** When  $-\Delta G^0$  for the formation of  $A_2$  from  $2A$  is higher than  $5 \text{ kcal mol}^{-1}$ , in a particular solvent, disaggregation can be obtained by changing the solvent. The selection of the proper solvent clearly depends on the nature of the interactions between the building blocks. Supramolecular assemblies held together by hydrophobic interactions are better transformed into the elemental building blocks in less polar and solute-like solvents. Instead, the interaction energy of hydrogen bonded supramolecules and ion pairs is usually substantially reduced in polar and protic solvents. For example, **RuX** salts are mainly present as ion quadruples in benzene- $d_6$ , having a relative permittivity ( $\epsilon_r$ ) of 2.24 at  $20^\circ\text{C}$ , at high concentration, (the case of **RuPF<sub>6</sub>** is shown in Fig. 8).<sup>17</sup> Dilution down to  $0.1 \text{ mM}$  causes dissociation of ion quadruples into ion pairs since the interaction of two ion pairs is small ( $\Delta G_{296}^0 = -2.5 \text{ kcal mol}^{-1}$ , *vide infra*) while it does not lead to the dissociation of ion pairs into free ions. It should be noted that the latter process occurs in  $\text{CD}_2\text{Cl}_2$  which has a much higher  $\epsilon_r$  (8.93), as shown in Fig. 8.

Another interesting example of solvent effect on the extent of aggregation has been reported by Nakahara and co-workers studying cholesterol in low-polarity organic solvents that mimic the membrane bilayer hydrophobic core.<sup>24</sup> They found that cholesterol forms aggregates in chloroform and cyclohexane but it does not in 1-octanol. The aggregation process is driven by the hydrogen bonding.

**5.1.3 Chemical turn off of the aggregation process.** It can happen that the interaction energy between the self-assembly units is so high that, even if the solvent is changed and the lowest possible concentration is used, the disaggregation process is incomplete. This situation can also be difficult to recognize. In fact, obtaining the same  $D_t$  from measurements at different concentrations may give the illusion that this could be the value of the elemental building block. An easy tool to check if the system is still aggregated is to compare the obtained  $V_H$  with the van der Waals ( $V_{\text{vdW}}$ ) and, above all, the crystallographic volume ( $V_{\text{X-Ray}}$ ). If the determined  $V_H$  is higher than  $V_{\text{X-Ray}}$  this probably means that the total dissociation of the supramolecules has not been reached. In such a case, derivatives of the building block unit can be synthesized by trying to maintain as much as possible their size



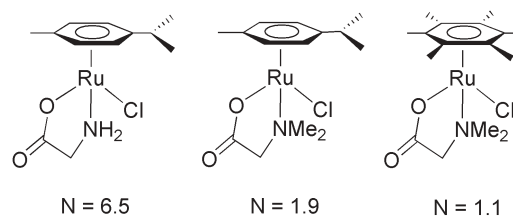
**Fig. 8** Trends of  $N$  versus  $C$  for **RuBPh<sub>4</sub>** in  $\text{CD}_2\text{Cl}_2$  (left) and **RuPF<sub>6</sub>** in benzene- $d_6$  (right).

and shape but, at the same time, attempting to turn off the aggregation motif.

This procedure has been used to investigate the self-aggregation tendency of  $[\text{RuCl}(\text{AA})(\text{Arene})]$  complexes (AA = Amino-Acidate, Scheme 2).<sup>25</sup> They have shown a marked tendency to self-aggregate in solution through a cooperative action of different weak interactions. Nevertheless, a key role is played by the N–H functionality of AA that allowed the establishment of intermolecular H-bonds.

When N–H is present, dimers (or higher aggregates) are always predominant even at the lowest concentration investigated in all solvents. Complex  $[\text{RuCl}(\text{gly})(\text{cymene})]$  in  $\text{CDCl}_3$  had a  $N$  value equal to 6.5 at *ca.*  $2 \text{ mM}$ . The substitution of the N–H functionalities with N–Me ones strongly reduces, but does not completely eliminate, the aggregation tendency. As indicated above,  $[\text{RuCl}(\text{Me}_2\text{-gly})(\text{cymene})]$  in  $\text{CDCl}_3$  at the same concentration is mainly present as dimers ( $N = 1.9$ ). When cymene is replaced by hexamethylbenzene the tendency to aggregate is completely annulled ( $N = 1.1$ ). In agreement with the previous considerations regarding the hydrodynamic radius, for  $[\text{RuCl}(\text{AA})(\text{Arene})]$  complexes, having a spherical shape without inlets,  $r_{\text{vdW}} \approx r_H^0$ .

Another example where chemical modification of the system (using different counterions) leads to a complete disaggregation involves ion pairing of **RuX** salts. This is shown in Fig. 9



**Scheme 2**

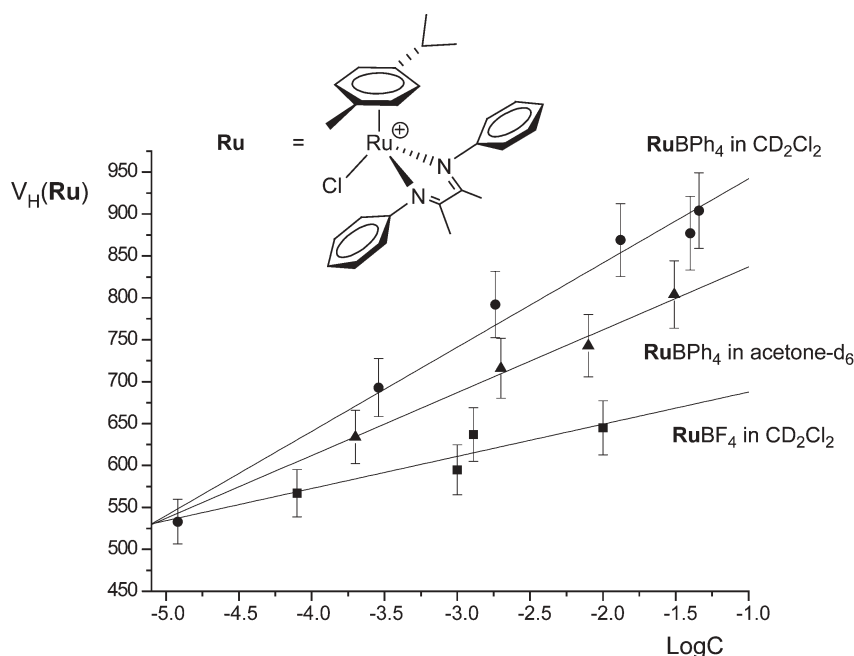


Fig. 9 Trends of  $V_H^+$  versus  $\log C$  ( $C$  in M) for  $\text{RuX}$  salts.

where  $V_H^+$  is reported as a function of concentration for different counterions and solvents.  $\text{RuBPh}_4$  and  $\text{RuBARF}$  show a higher  $V_H^+$  at low concentration, since their ion pairs are bigger than that of  $\text{RuBF}_4$ , but they tend to the volume of the free cation  $V_H^{+0}$  more rapidly as the concentration decreases. Interestingly, all trends illustrated in Fig. 9, including that of  $\text{RuBPh}_4$  in acetone, converge into a single point, within the experimental error, corresponding to the  $V_H^{+0}$  of the cation.

Recently, a new methodology for rapidly determining  $V_H^{+0}$  and  $V_H^{-0}$  was proposed by our group. It is based on combining conductometry and diffusion NMR spectroscopy.<sup>26</sup> The basic idea is simple. The equilibrium constant of ion pairing ( $K_{\text{IP}}$ ) and the dissociation degree ( $\alpha$ ) of the ion pairs (corrected for the activity coefficients) are evaluated by conductometric measurements. The average hydrodynamic volume for the cation and the anion,  $V_H^+$  and  $V_H^-$ , respectively, are derived from PGSE NMR measurements. By mathematical manipulations of the data<sup>26</sup> the  $V_H^{+0}$  and  $V_H^{-0}$  values that are obtained are in remarkable agreement with those derived by diffusion NMR experiments alone.

## 5.2 Shape of the supramolecular aggregates

The other critical parameter to consider in the formation of supramolecular adducts is their shape. According to what was stated in paragraph 3.0, the molecular ellipticity ( $a/b$ ) must be higher than 3 so that  $f_s$  is relevant. Thus, when spheroidal elemental building blocks without particularly directional aggregating motifs form supramolecular adducts,  $f_s$  may be considered equal to 1. This happens, for instance, when ion pairs aggregate to ion quadruples or, as mentioned before,  $[\text{RuCl}(\text{AA})(\text{Arene})]$  complexes undergo extensive aggregation. It should be mentioned that the shape factor  $f_s$  cannot be neglected in the aggregation of elongated units.

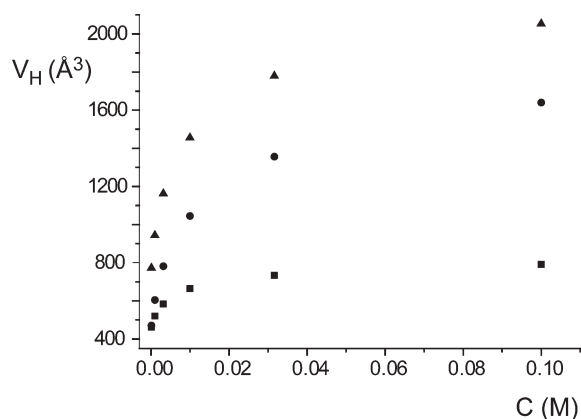
Considering the simplest case of the dimerization of a flat molecule, it makes a substantial difference whether the dimers are formed by head-to-tail or stack interactions of two monomers, with respect to the  $a/b$  ratio (Scheme 3). In the former case, the ellipticity is strongly increased, while in the latter it is decreased.

Let us assume that the monomer is prolate with  $a = 12 \text{ \AA}$  and  $b = 3 \text{ \AA}$  and it forms head-to-tail dimers. Assuming also that the dimerization equilibrium constant at a certain temperature  $T$  is known (set to  $K = 250 \text{ M}^{-1}$ ), the  $V_H$  versus  $C$  trend can be easily estimated since the average values (weighted by the molar fraction) of  $V_H$  can be obtained for each value of  $C$  from the known equilibrium concentration of the monomer and dimer. The trend is reported in Fig. 10.

Consistent with having imposed a head-to-tail dimerization,  $V_H$  at high concentration tends to  $2V_H^0$ . The denominator of eqn (2),  $f_s c r_H$ , can also be estimated since  $r_H$  is deduced from  $V_H$ , while  $c$  and  $f_s$  can be obtained from the average of the values related to monomer and dimer weighted by the molar fraction. As stated above,  $f_s c r_H$  is what is determined by diffusion measurements. It is instructive to treat these data hypothesizing either stack or spherical aggregation. The trends are reported in Fig. 10.  $V_H$  values at high concentration become unrealistic if aggregation cannot proceed beyond dimerization since they overcome  $2V_H^0$ . From the numerical point of view,  $V_H$  values can be readily explained since both stacked- and spherical-dimers have lower  $f_s$  values than that of



Scheme 3



**Fig. 10**  $V_H$  versus  $C$  trend for the head-to-tail dimerization of a prolate molecule ( $a = 12 \text{ \AA}$ ,  $b = 3 \text{ \AA}$ ) (■) and hypothetical trends that would be obtained from the same  $D_t$  data if the shape of the dimer were assumed to derive from stacking (●) or to be spherical (▲).  $K$  was arbitrarily fixed to  $250 \text{ M}^{-1}$  ( $\Delta G_{296}^0 = -3.2 \text{ kcal mol}^{-1}$ ).

the head-to-tail dimer. Consequently, if  $f_s cr_H$  is the same,  $r_H$  for the former must be higher than for the latter.

This simple case illustrates that if the dimension and shape of a markedly prolate or oblate monomer is known and it only affords dimers, the modality of dimerization can be determined by performing diffusion measurements at different concentrations and analyzing the trends. Unfortunately, the shape of the supramolecular adducts is only known *a priori* in a few cases<sup>27</sup> and the differences in shape of the different modalities of aggregation are not as pronounced as in the hypothetical case just discussed. In such cases, there is no rational criterion that allows the correct shape to be derived than chemical common sense.

### 5.3 Determining $K$ and $\Delta G^0$

The determined hydrodynamic dimensions of aggregating systems that undergo fast exchange in the chemical shift NMR time-scale, are average values that are weighted by the relative abundance of the species present in solution. Thus, in principle, they can be used to derive the equilibrium constant and the  $\Delta G^0$  of the aggregative process<sup>28</sup> if the activity coefficients of possible ionic species are taken into account. Three prototypical examples are reported below relative to the ions/ion pairs, ion pairs/ion quadruples and indefinite self-association of a single monomer.

**5.3.1 Ion pairing.** It is well-known that a  $C^+A^-$  salt in a slightly polar solvent undergoes the following ion pairing equilibrium:<sup>29</sup>



Ion pairing has been extensively studied from a qualitative point of view by diffusion NMR<sup>30</sup> but it is also possible to quantify it by obtaining thermodynamic parameters. In fact, the equilibrium concentration of all three species can be calculated by measuring  $V_H^+$  or  $V_H^-$ , if  $V_H^{+0}$  and  $V_H^{-0}$  are

known, since a third constraint (eqn (7)) is obtained in addition to those of mass and charge conservations:

$$[C^+A^-] + [C^+] = C_S \quad (5)$$

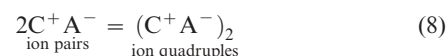
$$[C^+] = [A^-] \quad (6)$$

$$(V_H^+ - V_H^{+0})[C^+] + (V_H^- - V_H^{-0})[C^+A^-] = 0 \quad (7)$$

where  $C_S$  is the analytical concentration of the salt and  $V_H^{-0}$  is the hydrodynamic volume of the ion pair. The system of eqn (5)–(7) can be easily solved and the concentration of the species expressed as a function of  $V_H^{+0}$ ,  $V_H^{-0}$  and  $C_S$ .<sup>31</sup> A relationship analogous to 7 can be written when  $V_H^-$  is measured instead of  $V_H^+$ . Having determined the equilibrium concentration of all three species, the equilibrium constant for the formation of pairs ( $K_{IP}$ ) could be obtained if the activity coefficients ( $\gamma$ ) were known. While it can be reasonably assumed that the  $\gamma_{IP}$  of the neutral ion pair is equal to 1,  $\gamma_+$  and  $\gamma_-$  have to be calculated. According to the Debye–Hückel theory,<sup>32</sup> the mean activity coefficient ( $\gamma_{\pm}$ ) can be evaluated by the equation:  $\log \gamma_{\pm}^2 = -2A(\alpha C_S)^{1/2}$ , where  $\alpha$  is the dissociation degree and  $A$  is a constant that depends on  $\epsilon_R$  and  $T$  [for a univalent electrolyte  $A = 1.823 \times 10^6 / (\epsilon_R T)^{3/2}$ ].

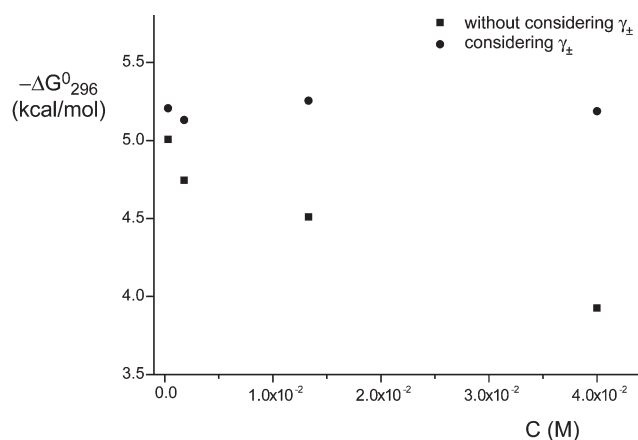
Recently,  $V_H^+$  and  $V_H^-$  were measured for **RuBPh<sub>4</sub>** in  $CD_2Cl_2$  over a rather wide concentration range (from  $3 \times 10^{-4}$  to  $4 \times 10^{-2}$ ) at 296 K.<sup>17</sup> By using the methodology just illustrated,  $K_{IP}$  and  $\Delta G^0$  can be determined for every single measurement. Clearly, they must assume the same values, within the experimental error implicit in the  $V_H$  measurement (*ca.* 10%), at each concentration.  $K_{IP}$  oscillates between  $6.4 \times 10^3$  and  $8.9 \times 10^3 \text{ M}^{-1}$  if the activity coefficients are taken into account; this is in very good agreement with the value obtained by conductometry ( $7.1 \times 10^3 \text{ M}^{-1}$ ).<sup>26</sup> The oscillation of  $K_{IP}$  values is consistent with a 10% error on  $V_H$ . The  $\Delta G^0$  values determined at different concentrations are depicted in Fig. 11 along with those that were obtained without considering the activity coefficients of the ions. The importance of considering  $\gamma_{\pm}$  is evident;  $\Delta G^0$  is reasonably constant and amounts to  $-5.2 \text{ kcal mol}^{-1}$ .

**5.3.2 Ion “quadrupling”.** Recent diffusion NMR studies have shown that ionic aggregates greater than ion pairs form when salts are dissolved in slightly polar solvents.<sup>17,33–35</sup> Clear indications about the formation of ion quadruples have been obtained in solvents with a relative permittivity equal to or lower than that of chloroform ( $\epsilon_R^{20^\circ C} = 4.81$ ). In organometallic chemistry the possibility of having ion quadruples instead of, or in addition to, ion pairs is of particular interest considering the numerous stoichiometric and catalytic reactions that are mediated by organometallic salts.<sup>36</sup>



Diffusion NMR experiments can reliably allow ion “quadrupling” to be evidenced if all the above-discussed precautions for treating the  $D_t$  data are taken. The average  $r_H$  and  $V_H$  derived from  $D_t$  can be used to evaluate the equilibrium constant for ion “quadrupling” ( $K_{IQ}$ ) and, consequently, the





**Fig. 11**  $-\Delta G^0$  for the association of **Ru** and  $\text{BPh}_4^-$  ions into ion pairs in  $\text{CD}_2\text{Cl}_2$  at 296 K.

$\Delta G^0_{\text{IQ}}$ . Since solvents in which ion quadruples are present have low  $\epsilon_{\text{R}}$  values, it can be assumed that free ions can be neglected. The extraction of the thermodynamic data becomes rather simple. The underlying reasoning is similar to that given for ion pairing in which the mass conservation law and a constraint derived from  $V_{\text{H}^+}$  or  $V_{\text{H}^-}$  are used assuming that the activity coefficients of ion pairs and ion quadruples are equal to one. Alternatively, the  $N$  data at different  $C$  values can be fitted by the expression of  $N$  previously seen for the dimerization of  $A$  into  $A_2$  [assimilating  $C^+A^-$  to  $A$  and  $(C^+A^-)_2$  to  $A_2$ ]<sup>22</sup> or by using eqn (11) (as described in the following section for extensive self-association). The latter fitting procedure is preferable since ion pair association does not necessarily lead to ion quadruples; it can continue up to and beyond ion hexaples. The fact that only rarely aggregates higher than ion quadruples are observed mainly depends on their low solubility in solvents with little  $\epsilon_{\text{r}}$ . Thus, differently from the  $A/A_2$  equilibrium seen before (Fig. 7), the  $N$  versus  $C$  trends for ion “quadrupling” do not show a marked plateau when  $N$  approaches 2.

Despite the paucity of data reported in the literature, some  $K_{\text{IQ}}$  can be evaluated. For example, the tendency of **RuX** complexes to form ion quadruples as a function of the counterion  $X^-$  has been determined.<sup>17</sup> One of the most important results is that such a tendency increases as the counterion becomes less coordinating. This has been explained by considering that the least coordinating counterions lead to a higher dipolar moment that facilitates ion pair aggregation into ion quadruples. In agreement, for **RuBF<sub>4</sub>** and **RuPF<sub>6</sub>** that have small counterions that closely dock with the cation, the  $K_{\text{IQ}}$  is 40–100  $\text{M}^{-1}$  ( $-\Delta G^0_{\text{IQ}} = 2.0$ – $2.8 \text{ kcal mol}^{-1}$ ) in  $\text{CDCl}_3$ . In the same solvent  $K_{\text{IQ}}$  is equal to 200–700  $\text{M}^{-1}$  ( $-\Delta G^0_{\text{IQ}} = 3.1$ – $3.8 \text{ kcal mol}^{-1}$ ) for **RuBPh<sub>4</sub>**.

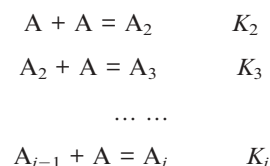
Metallocene catalysts for olefin polymerization are an important class of compounds for which the formation of ion quadruples has been demonstrated<sup>33–35</sup> but there is still some controversy about their possible role in catalytic processes. The latter are usually carried out in low polar solvents (benzene, toluene, isoparaffins, *etc.*) and it is reasonable that ion pairs associate by forming ion quadruples. The  $K_{\text{IQ}}$  and  $\Delta G^0_{\text{IQ}}$  values calculated from the PGSE data

reported for the aggregation in benzene- $d_6$  of some zirconocene compounds,<sup>34</sup> are graphically illustrated in Scheme 4.

It can be noted that  $K_{\text{IQ}}$  and  $\Delta G^0_{\text{IQ}}$  are not very sensitive to the variations in the compound and do not differ substantially from the analogous parameters derived for the **RuBPh<sub>4</sub>** complex. Having obtained the  $K_{\text{IQ}}$  values, the concentration of ion quadruples at catalytic concentration can be estimated.

Considering an average  $K_{\text{IQ}}$  of 1000  $\text{M}^{-1}$ , at  $C_{\text{s}} = 1 \times 10^{-7} \text{ M}$ , the concentration of ion quadruples should be  $10^{-11} \text{ M}$ , *i.e.* about four orders of magnitude lower than that of ion pairs. This indicates that ion quadruples can only be considered relevant in the catalytic process, in benzene, if they have a reactivity that is markedly higher than that of ion pairs.

**5.3.3 Extensive self-association of a monomeric unit.** The final example that is taken into account is the indefinite self-association of a monomer  $A$ :



Several models have been developed for treating the indefinite self-association process.<sup>22</sup> The simplest, and most commonly used, is the isodesmic model called Equal K (EK). It assumes that all equilibrium constants for the self-association are equal ( $K_2 = K_3 = \dots = K_i = K$ ). Under this assumption, the analytical concentration ( $C$ ) can be written as:

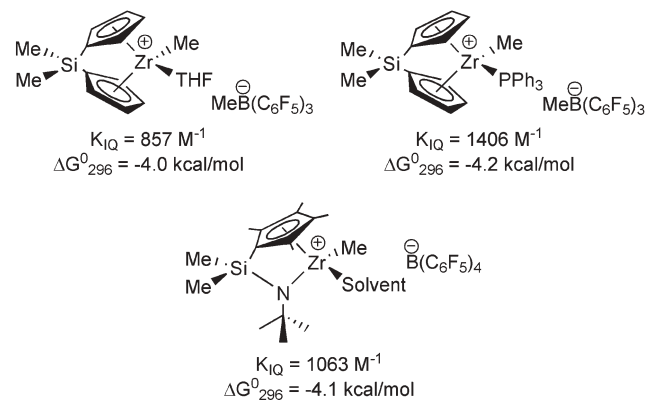
$$C = [A] + 2[A_2] + \dots + i[A_i] + \dots = [A]/(1 - K[A])^2 \quad (9)$$

while the total concentration of aggregates ( $C_{\text{A}}$ ) can be written as:

$$C_{\text{A}} = [A] + [A_2] + \dots + [A_i] + \dots = [A]/(1 - K[A]) \quad (10)$$

The aggregation number  $N$  can be expressed as the ratio  $C/C_{\text{A}}$ . We have recently shown that a simple, useful expression to derive  $K$  can be obtained by algebraic manipulations of eqn 9–10 leading to:<sup>37</sup>

$$N(N - 1) = KC \quad (11)$$



**Scheme 4**

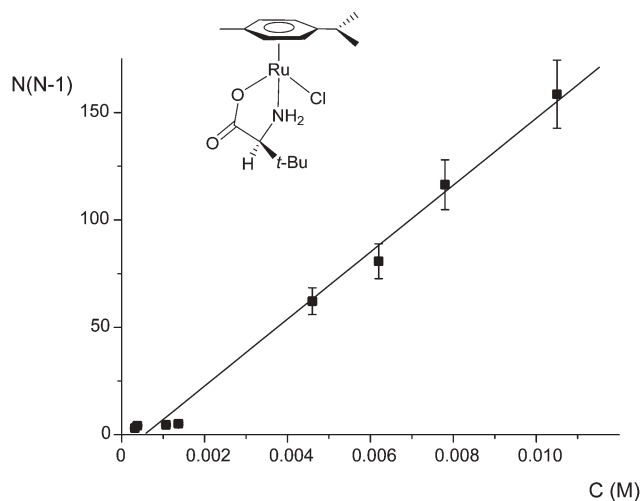
Plotting  $N(N - 1)$  data, derived from PGSE NMR measurements, versus  $C$  should generate a linear trend that can be fitted with eqn (11). The slope of the straight line obtained from the fitting affords  $K$  directly.

The method has been successfully used to investigate the self-association of [RuCl(AA)(Arene)] complexes. For example, the trend of  $N(N - 1)$  as a function of  $C$  in  $\text{CDCl}_3$  for [RuCl(*t*-leu)(cymene)] is reported in Fig. 12.

From the slope a  $K$  value of  $15\,600 \pm 1600 \text{ M}^{-1}$  was obtained with a  $\Delta G^0 = -5.7 \pm 0.6 \text{ kcal mol}^{-1}$ . These values are consistent with aggregates mainly held together by hydrogen bonding in  $\text{CDCl}_3$ . It is important to outline that in case of extensive aggregation ( $N > 3$ ) the interstitial space between the elemental units cannot be neglected.<sup>25</sup>

## 6.0 Conclusions

The main message of the present review is that the determination of accurate hydrodynamic dimensions of a molecular system in solution frequently necessitates diffusion NMR experiments as a function of the concentration. In fact,  $r_H$  determined from a single measurement can, in itself, be ambiguous even after having (i) overcome all experimental problems of correctly controlling the temperature, viscosity and calibration of pulsed-field gradient by using an internal standard and (ii) taken into account the relative solute/solvent dimensions and the shape of the solute by introducing the  $c$  and  $f_s$  factors, respectively. In the final analysis, the ambiguity derives from the fact that  $r_H$  frequently differs from other easily accessible radii ( $r_{\text{vdW}}$  and  $r_{\text{X-Ray}}$ ). It is only when  $r_H = r_{\text{vdW}}$ , that usually corresponds to compact molecules without inlets, or when  $r_H = r_M$ , that a single measurement directly affords the hydrodynamic dimension. Otherwise, the effect of concentration on  $r_H$  must be studied in order to determine if its deviation from  $r_{\text{vdW}}$  and  $r_{\text{X-Ray}}$  is “real” or due to self-aggregation. If the  $r_H$  value changes as the concentration changes, the limiting value at infinite dilution can be obtained corresponding to the “real”  $r_H$ . If  $r_H$  is invariant with the concentration it may be due to the absence of self-aggregation



**Fig. 12** Dependence of  $N(N - 1)$  on the concentration for the shown complex in  $\text{CDCl}_3$  at 296 K.

or self-aggregation with high interaction energy ( $-\Delta G^0 > 5 \text{ kcal mol}^{-1}$ , at room temperature). Chemical considerations usually allow realizing if the system has the possibility of undergoing strong self-aggregation (usually driven by electrostatic interactions such as hydrogen bonding and ion pairing). In this case, it is preferable to carry out measurements as a function of the concentration in another solvent properly chosen to facilitate disaggregation. As a final possibility, the system must be chemically modified to remove the causes of aggregation while being careful not to markedly alter its size and shape.

The accurate evaluation of the hydrodynamic dimension of the molecular system derived from diffusion NMR spectroscopy provides, in itself, important structural information. It is also propaedeutic to deriving the thermodynamic parameters of the aggregation process. In fact, diffusion experiments for aggregating systems allow an average  $r_H$ , and consequently  $V_H$ , to be derived. Only the accurate knowing of the volume of the elemental building block(s) of the aggregation process can lead to reliably evaluate the equilibrium constant and, consequently,  $\Delta G^0$ .

## Acknowledgements

We thank the Ministero dell'Università e della Ricerca (MUR, Rome, Italy) and the Dow Chemical Company for support.

## References

- 1 E. L. Hahn, *Phys. Rev.*, 1950, **80**, 580.
  - 2 E. O. Stejskal and J. E. Tanner, *J. Chem. Phys.*, 1965, **42**, 288.
  - 3 J. T. Edward, *J. Chem. Educ.*, 1970, **47**, 261.
  - 4 P. Stilbs, *Prog. Nucl. Magn. Reson. Spectrosc.*, 1987, **19**, 1.
  - 5 W. S. Price, *Concepts Magn. Res.*, 1997, **9**, 299.
  - 6 W. S. Price, *Concepts Magn. Reson.*, 1998, **10**, 197.
  - 7 C. S. Johnson, Jr., *Prog. Nucl. Magn. Reson. Spectrosc.*, 1999, **34**, 203.
  - 8 Y. Cohen, L. Avram and L. Frish, *Angew. Chem., Int. Ed.*, 2005, **44**, 520.
  - 9 A. Dehner and H. Kessler, *ChemBioChem*, 2005, **6**, 1550.
  - 10  $r_{\text{solv}}$  is set equal to the van der Waals radius for all solvents except the protic ones (Ref. 20).
  - 11 A. Gierer and K. Z. Wirtz, *Z. Naturforsch., A: Astrophys., Phys., Phys. Chem.*, 1953, **8**, 522; A. Gierer and K. Z. Wirtz, *Z. Naturforsch., A: Astrophys., Phys., Phys. Chem.*, 1953, **8**, 532
- $$c = \frac{6}{\frac{3r_{\text{solv}}}{2r_H} + \frac{1}{1 + \frac{r_{\text{solv}}}{r_H}}}$$
- 12 H.-C. Chen and S.-H. Chen, *J. Phys. Chem.*, 1984, **88**, 5118
- $$c = \frac{6}{1 + 0.695 \left( \frac{r_{\text{solv}}}{r_H} \right)^{2.234}}$$
- 13 In this review only ellipsoidal shapes are taken into account. As far as the hydrodynamic theory of a cylinder moving in a viscous fluid is concerned see: G. Li and J. X. Tang, *Phys. Rev. E: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top.*, 2004, **69**, 061921 and references therein.
  - 14 F. Perrin, *J. Phys. Radium.*, 1936, **7**, 1. For a prolate ellipsoid:
 
$$f_s = \frac{\sqrt{1 - \left(\frac{b}{a}\right)^2}}{\left(\frac{b}{a}\right)^3 \ln \frac{1 + \sqrt{1 - \left(\frac{b}{a}\right)^2}}{\left(\frac{b}{a}\right)}}$$
 For an oblate ellipsoid:
 
$$f_s = \frac{\sqrt{\left(\frac{b}{a}\right)^2 - 1}}{\left(\frac{b}{a}\right)^2 \arctan \sqrt{\left(\frac{b}{a}\right)^2 - 1}}$$
  - 15 R. Piazza and V. Degiorgio, *J. Phys.: Condens. Matter*, 1993, **5**, B173.

- 16 H. Kang, A. Facchetti, H. Jiang, E. Cariati, S. Righetto, R. Ugo, C. Zuccaccia, A. Macchioni, C. L. Stern, Z. Liu, S.-T. Ho, E. C. Brown, M. A. Ratner and T. J. Marks, *J. Am. Chem. Soc.*, 2007, **129**, 3267.
- 17 D. Zuccaccia, G. Bellachioma, G. Cardaci, G. Ciancaleoni, C. Zuccaccia, E. Clot and A. Macchioni, *Organometallics*, 2007, **26**, 3930.
- 18 R. E. Dinnebier, W. A. Dollase, X. Helluy, J. Kümmerlen, A. Sebald, M. U. Schmidt, S. Pagola, P. W. Stephens and S. van Smaalen, *Acta Crystallogr., Sect. B*, 1999, **B55**, 1014.
- 19 Y. Marcus and G. Hefter, *Chem. Rev.*, 2004, **104**, 3405.
- 20 D. Zuccaccia and A. Macchioni, *Organometallics*, 2005, **24**, 3476 and references therein.
- 21 S. S. Pochapsky, H. Mo and T. Pochapsky, *J. Chem. Soc., Chem. Commun.*, 1995, 2513.
- 22 R. B. Martin, *Chem. Rev.*, 1996, **96**, 3043 and references therein. The dependence of  $N$  on  $C$  for a monomer/dimer equilibrium is:
- $$N = \frac{2C}{C + \frac{\sqrt{1+8KC}-1}{4K}}$$
- 23 D. Zuccaccia, L. Busetto, M. C. Cassani, A. Macchioni and R. Mazzoni, *Organometallics*, 2006, **25**, 2201.
- 24 C. Giordani, C. Wakai, E. Okamura, N. Matubayasi and M. Nakahara, *J. Phys. Chem. B*, 2006, **110**, 15205.
- 25 G. Ciancaleoni, I. Di Maio, D. Zuccaccia and A. Macchioni, *Organometallics*, 2007, **26**, 489.
- 26 G. Ciancaleoni, C. Zuccaccia, D. Zuccaccia and A. Macchioni, *Organometallics*, 2007, **26**, 3624.  $V_{\text{H}}^{+0}$  and  $V_{\text{H}}^{-0}$  are the unknown quantities of the following system of equations that can be obtained on the assumption that  $V_{\text{H}}^{\text{IP0}}$  is equal to the sum of  $V_{\text{H}}^{+0}$  and  $V_{\text{H}}^{-0}$ :
- $$\begin{cases} V_{\text{H}}^{+0} = \alpha V_{\text{H}}^{+0} + (1-\alpha) V_{\text{H}}^{\text{IP0}} \\ V_{\text{H}}^{-0} = \alpha V_{\text{H}}^{-0} + (1-\alpha) V_{\text{H}}^{\text{IP0}} \end{cases}$$
- 27 L. Allouche, A. Marquis and J.-M. Lehn, *Chem.–Eur. J.*, 2006, **12**, 7520.
- 28 C. Cabaleiro-Lago, M. Nilsson and O. Söderman, *Langmuir*, 2005, **21**, 11637.
- 29 Y. Marcus and G. Hefter, *Chem. Rev.*, 2006, **106**, 4585 and references therein.
- 30 P. S. Pregosin, *Prog. Nucl. Magn. Reson. Spectrosc.*, 2006, **49**, 261 and references therein.
- 31  $[C^+] = [A^-] = C_S \frac{V^{\text{IP0}} - V^{+or-}}{V^{\text{IP0}} - V^{+0} \text{ or } -}$  and  $[C^+ A^-] = C_S \frac{V^{+or-} - V^{+0} \text{ or } -}{V^{\text{IP0}} - V^{+0} \text{ or } -}$ .
- 32 P. W. Debye and E. Huckel, *Phys. Z.*, 1923, **24**, 185.
- 33 S. Beck, A. Geyer and H.-H. Brintzinger, *Chem. Commun.*, 1999, 2477.
- 34 C. Zuccaccia, N. G. Stahl, A. Macchioni, M.-C. Chen, J. A. Roberts and T. J. Marks, *J. Am. Chem. Soc.*, 2004, **126**, 1448.
- 35 F. Song, S. J. Lancaster, R. D. Cannon, M. Schormann, S. M. Humphrey, C. Zuccaccia, A. Macchioni and M. Bochmann, *Organometallics*, 2005, **24**, 1315.
- 36 A. Macchioni, *Chem. Rev.*, 2005, **105**, 2039 and references therein.
- 37 In fact,
- $$N(N-1) = \frac{C}{C_A} \left( \frac{C}{C_A} - 1 \right) = \frac{C}{C_A} \left( \frac{K[A]}{1-K[A]} \right) = C \left( \frac{1-K[A]}{[A]} \right) \left( \frac{K[A]}{1-K[A]} \right) = KC$$