Diffusion NMR of molecular cages and capsules

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In the last decade diffusion NMR and diffusion ordered spectroscopy (DOSY) have become important analytical tools for the characterization of supramolecular systems in solution. Diffusion NMR can be used to glean information on the (effective) size and shape of molecular species, as well as to probe inter-molecular interactions and can be used to estimate the association constant ($K_a$) of a complex. In addition, the diffusion coefficient, as obtained from diffusion NMR, is a much more intuitive parameter than the chemical shift for probing self-association, aggregation and inter-molecular interactions. The diffusion coefficient may be an even more important analytical parameter in systems in which the formed supramolecular entity has the same symmetry as its building units, when there is a large change in the molecular weight, where many molecular species are involved in the formation of the supramolecular systems, and when proton transfer may occur which, in turn, may affect the chemical shift. Some of the self-assembled molecular capsules and cages prepared in the last decade represent such supramolecular systems and in the present review, following a short introduction on diffusion NMR, we survey the contribution of diffusion NMR and DOSY in the field of molecular containers and capsules. We will first focus on the role played by diffusion NMR in the field of hydrogen bond driven self-assembled capsules. We then survey the contributions of diffusion NMR and DOSY to the study and characterization of metal–ligand cages and capsules. Finally, we describe a few recent applications of diffusion NMR in the field of hydrophobic, electrostatic and covalent containers.

1. Introduction

Molecular containers, cages and capsules have become a major theme in organic supramolecular chemistry in the last two decades.1–6 Their importance stems from the fact that molecular

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containers and capsules can, in fact, isolate certain molecules from the bulk i.e. from the outside of the containers. Molecular capsules and cages can thus be used as nanoreactors for catalysis and for the stabilization of reactive intermediates. Despite the fact that diffusion NMR experiments have been known for about 50 years,7,8 it was only in the mid-nineties that diffusion NMR made its entry into the field of organic supramolecular chemistry.9–12 This happened after conventional NMR instruments were equipped with reliable gradient systems for performing gradient enhanced multi-dimensional NMR.13,14 Since then diffusion NMR15 and diffusion ordered spectroscopy (DOSY)16 have been used to study mostly organometallic complexes,17 as well as supramolecular systems18 such as host–guest systems,9–12,20–25 helicates,26,27 grids,28 supramolecular polymers29–31 and more.32–35 However, the one field in supramolecular chemistry in which diffusion NMR seems to have contributed most is the field of hydrogen bond self-assembled molecular capsules.36–42 In this short review we will survey the applications of the diffusion NMR and DOSY in the study of cages and container molecules. We will do so after first describing, very briefly, some methodological and practical aspects concerning diffusion NMR. We decided to restrict ourselves to a short introduction on diffusion NMR since excellent reviews15–19 and monographs43,44 on the subject are available.

1.1. Measuring the diffusion coefficient \((D)\) using NMR

Diffusion NMR can be used to measure the translational diffusion coefficient \((D)\) which is the net result of the random walk originating from the thermally induced motion in solution in the absence of any chemical gradient. The basic NMR pulse sequences used for measuring diffusion were introduced in the sixties and seventies and include the pulsed gradient spin echo (PGSE) and the pulsed gradient stimulated echo (PGSTE) sequences (Fig. 1a and b, respectively).7,8 In the nineties the Johnson group introduced the longitudinal eddy current (LED) sequence45 and its bipolar (BP) version, i.e. the BPLED sequence,46 both of which are heavily used in DOSY46 packages of commercial NMR instruments (Fig. 1c and d, respectively).

All the above sequences rely on the fact that magnetic-field gradients can be used to label the position of NMR-active nuclei through their Larmor frequency \((\omega, \text{rad s}^{-1})\). Note that the Larmor frequency is given by eqn (1), where \(\gamma \text{ (rad s}^{-1} \text{ T}^{-1})\) is the gyromagnetic ratio, and \(B_0 (T)\) is the external magnetic field:

\[
\omega_0 = -\gamma B_0 \tag{1}
\]

From eqn (1) it follows that in the presence of (pulsed) field gradient along the \(z\)-direction (\(G_z\) in mT m\(^{-1}\)) in addition to the \(B_0, \omega\) becomes spatially dependent according to eqn (2):

\[
\omega_z = -\gamma (B_0 + G_z Z) \tag{2}
\]

From eqn (2) the logic of measuring diffusion using NMR becomes apparent. For simplicity let us first analyse the effect of the simple PGSE sequence, shown in Fig. 1a, on the magnetization of an ensemble of spins/molecules neglecting the effect of chemical shift, relaxation and spin–spin interactions. In the PGSE sequence,7 which is a Hahn spin-echo sequence47 in which two gradient pulses were embedded, the first \((\pi/2)_\text{G}\), pulse rotates the \(z\)-magnetization into the \(xy\)-plane and the first gradient pulse induces a phase dispersion. Then the \(\pi\), pulse rotates the magnetization in the \(xy\)-plane (this is in fact a phase inversion) and then the second gradient pulse with the same magnitude and length is applied. Here two scenarios can be envisioned: (1) the spins do not move during the time interval between the first and the second gradient pulses and hence full refocussing occurs and the full echo intensity is recovered, and (2) the spins do move in this time interval resulting in only partial refocussing of the phase shifts thus recovering only part of the echo intensity. Consequently, in the PGSE sequence the signal intensity, for spins exhibiting free (Gaussian) diffusion, at an echo time of \(2t\), generally is given by eqn (3),7,8

\[
I_{(2\pi,0)} = I_{(0,0)} \exp(-\gamma^2 G^2 \delta^2 (A - \delta/3)D) \tag{3}
\]

where \(I_{(0,0)}\) is the signal intensity immediately after the \(\pi/2\) pulse and \(I_{(2\pi,0)}\) is the signal intensity at an echo time of \(2\tau\) in the absence of \(G\). In eqn (3) all other parameters have the above meaning and \((A - \delta/3)\) is termed the diffusion time. Consequently, when repeating the PGSE experiment while keeping all parameters constant besides \(G\) for example, one can, in principle, extract the diffusion coefficient by plotting \(\ln(I_{(2\pi,0)}/I_{(2\pi,0)})\), generally abbreviated to \(\ln(U_{(0)})\) as a function of \(G^2\) as shown in eqn (4),

1.2. The key role of diffusion NMR in supramolecular chemistry

Diffusion NMR has been extensively used for the study of cage-like and container-like molecules and for imaging purposes,48,49 which is the result of the unique capacity to provide information on the internal dynamics of these molecules. In supramolecular chemistry, rapid changes in local environment such as induced for example by conformational changes can result in the modulation of the diffusion coefficient \((D)\). In the field of supramolecular chemistry, in fact, there are two types of diffusion NMR experiments: those used for the study of cages and container molecules and those used for the study of hydrogen bond self-assembled molecular capsules.36–42
where $b$ can be regarded as the diffusing weighting. Note that eqn (3) and (4), also known as the Stejskal–Tanner (ST) equation, are valid only for single coherence transitions. For discussion about the effect of pulse sequence used on the signal decay and hence on the ST equation see ref. 17c.

$$\ln \left( \frac{I(2\pi)}{I(0)} \right) = \ln \left( \frac{I(2\pi)}{I(2\pi)} \right) = -2G^2\delta^2(1 - \delta/3)D = bD \quad (4)$$

Clearly the PGSTE,\(^a \) as well as the LED\(^b\) and BPLED\(^c\) sequences, are more suitable than the PGSE sequence for measuring the diffusion coefficient of systems characterized by fast transverse relaxation (short $T_2$). The LED and BPLED both have an extra delay ($t_d$) which makes them less sensitive to eddy currents. Importantly, the BPLED sequence is even less sensitive to both eddy currents and background gradients which, in general, are less of a problem in high resolution NMR spectroscopy. However, both the LED and the BPLED sequences are more sensitive than the PGSE and PGSTE sequences to exchange effects (vide infra).

1.2. From the diffusion coefficient to effective hydrodynamic dimensions: size and shape

In the pursuit of information regarding the (effective) size, shape and then the molecular weight of molecular species, diffusion coefficients have been extensively used to calculate the hydrodynamic radius ($r_h$). The Einstein–Smoluchowski equation (eqn (5))\(^d\) provides the connection between the diffusion coefficient ($D$) and the so-called hydrodynamic friction factor ($f$) for infinitely diluted solutions. In this equation $k_B$ is the Boltzmann constant and $T$ is the absolute temperature. For $f$ equal to $6\pi\eta r_h$, one obtains the well-known Stokes–Einstein equation (eqn (6)) from which one can calculate the Stokes radius ($r_s$)\(^e\)\(^f\)\(^g\):

$$D = \frac{k_B T}{f} \quad (5)$$

$$D = \frac{k_B T}{6\pi\eta r_s} \quad (6)$$

In eqn (6) $\eta$ is the viscosity of solvent in which diffusion occurs and $r_s$ is the radius of the diffusing molecular species. However, it should be noted that this Stokes–Einstein Equation is valid only for hard spheres moving in a continuum fluid and neglecting all contributions from solvation and other specific interactions like ion-pair interactions for example. Clearly, the above approximations are valid for spherical nanoparticles. For many molecules and supramolecules, however, this hard sphere approximation may only be partially valid. In such cases one should use the modified Stokes–Einstein equation (eqn (7))\(^h\)\(^i\)\(^j\) which takes into account the deviations from the hard sphere approximation and the deviation from the continuum fluid approximation.

$$D = \frac{k_B T}{c\pi\eta r_s f_h} \quad (7)$$

In eqn (7), $f_h$ is the shape factor and is always greater than unity, while $C$ is the size factor and relates to the ratio of the size of the diffusing entity to the solvent in which diffusion occurs. The shape factor $f_h$ was computed for prolate and oblate ellipsoids,\(^k\) for ellipsoids having three different axes\(^l\) and for cylindrical species.\(^m\) It was found that for ellipsoids with semiaxis ratios higher than 3, a deviation of about 10% in $f_h$ is observed.\(^n\) For the size factor $C$, which is a measure of the continuous fluid approximation, the Wirtz micro-friction theory predicts a value close to 6 when the diffusing species have a radius of about 4 nm or more.\(^o\)\(^p\) In fact, it was found that $C$ is equal to 6 when the ratio between the solute and the solvent size is greater than 5. From a detailed study on the diffusion characteristics of double helical copper complexes, for which high-quality X-ray structures were obtained, it was found that $C$ changes from 5.2 to 5.7 when passing from $[\text{Cu}(\text{L}_1)_2]^+$ to $[\text{Cu}_2(\text{L}_3)_2]^{5+}$ (see Scheme 1).\(^q\) For this series it was found that $f_h$ changes from 1 to 1.14 when passing from $[\text{Cu}(\text{L}_1)_2]^+$ to $[\text{Cu}_2(\text{L}_3)_2]^{5+}$. Thus the product of $C \times f_h$ is equal to 6 only for $[\text{Cu}_2(\text{L}_3)_2]^{5+}$. However, for the other members of the series the $C \times f_h$ values were between ~5.2 to 6.5. These systems are clearly not hard spheres but the product of $C \times f_h$ deviated only little from 6 implying that the Stokes–Einstein equation (eqn (6)) may be a reasonable approximation for many supramolecular systems.\(^r\)

For many cages and molecular capsules which are relatively large molecules (compared to the solvent molecules) and in which the various axes are not very different, it appears that the simple Stokes–Einstein equation is a reasonable approximation. In addition it is also important to note that $r_s$ and $r_h$ extracted from eqn (6) and (7) represent the hydrodynamic sizes and as such are measures which are related to the nature of the solvent in which the diffusion occurs. In fact they represent the radius of a hypothetical hard sphere that diffuses with the same speed as...
the examined particle, and therefore possess some redundancy. What one can say is that the van der Waals radius ($r_{vdw}$) is the lower limit of $r$, and that $r_{vdw}$ is equal to $r_s$ only for small compact, rigid molecules with no voids. Otherwise $r_s$ should be larger than $r_{vdw}$. On the other hand the upper limit of $r_s$ is the crystallographic radius ($r_{cryst}$). For more details see ref. 51 and 52.

Scheme 1 shows the $r_s$ (or as in ref. 27 $r_{sph}$) and the $r_{eq}$ values obtained from eqn (8), for a series of copper helicates.

$$r_{eq} = \sqrt[3]{\frac{3MV}{4\pi NA}}$$  \hspace{1cm} (8)

In eqn (8) $V$ is the partial specific volume of the species, $M$ is its molecular weight and $N_A$ is the Avogadro constant. The data presented in Scheme 1 show that despite the fact that many of the helicates are clearly not spherical, the differences between the $r_{sph}$ and $r_{eq}$ values for this series of compounds, calculated from the diffusion data using the Stokes–Einstein equation and from the X-ray structures of the complexes, respectively, are in the order of 10–15%.27

### 1.3. From the diffusion coefficient to association constants ($K_a$s)

The diffusion coefficient is a much more intuitive measure for inter-molecular interactions than the widely exploited chemical shift. In the case of fast exchange between the free and the bound forms of a guest in host–guest systems diffusion measurements can, in principle, be used for evaluating the $K_a$ of such systems through diffusion NMR titrations, as described earlier in the diffusion NMR literature.58 The rationale is the same as when using chemical shift titrations to extract the $K_a$ of a complex. For the simple case of 1 : 1 complexes one can calculate the bond fraction ($X$) from eqn (9).9,12,24,25

$$D_{obs}^{\text{Guest}} = XD_{\text{bound}}^{\text{Guest}} + (1 - X)D_{\text{free}}^{\text{Guest}}$$  \hspace{1cm} (9)

Since generally the guest is much smaller than the host, $D_{\text{bound}}^{\text{Guest}}$ should not be very different from $D_{\text{host}}^{\text{Free}}$, i.e. the diffusion coefficient of the host. A better approximation for $D_{\text{bound}}^{\text{Guest}}$ would in fact be $D_{\text{host}}^{\text{Bound}}$, i.e. the diffusion coefficient of the host in the host–guest complex. These approximations, however, are more valid as the difference in sizes between the host and a guest is larger. So, in principle, one can obtain an acceptable estimate for an association constant from a single point diffusion NMR measurement. Fielding has shown that when the ratio of the molecular weight between the host and the guest is about 10, $D_{\text{host}}^{\text{Free}}$ is not very different from $D_{\text{bound}}^{\text{Guest}}$.24 However, when this ratio is about 3 there is a significant difference between the two values and a full titration is needed to obtain reasonable estimation of the $K_a$ of the system. Using diffusion NMR to extract association constants of complexes is especially appealing when proton transfer, which could dramatically affect the chemical shifts of the molecular partners, may occur.9,12 This was observed when (CH$_3$)$_2$N+H$_3$Cl was added to the solution of cryptands. There, proton transfer from the guest to the nitrogen of the cryptand host was detected.9 However, it is clearly beneficial to perform diffusion measurements for solutions with different host/guest ratios in the same way as done when chemical shift titrations are used.24,59,25 In addition, for accurate estimation of the $K_a$ of a host–guest system, it is important to measure the dependency of the diffusion coefficient of each component of the host–guest or the supramolecular system to verify that each of the components are not self-aggregating. It should be noted, however, that for systems with self-aggregation energy higher than 5–10 kcal mol$^{-1}$ this is more difficult to perform since it will require measuring diffusion of samples with very low concentration where the signal to noise ratio is low.18 The use of diffusion NMR to study association constants has been reviewed previously,25,60 and a more detailed description on how to use diffusion NMR data to extract $K_a$ of complexes of different stoichiometry can be found in ref. 52.

### 2. Diffusion NMR of molecular capsules and molecular containers

In the present review we survey the use of diffusion NMR and DOSY for studying different types of molecular capsules and cages which are herein classified according to the main non-covalent interactions used to construct those molecular containers.

#### 2.1. Hydrogen bond-based molecular capsules: from dimers to higher aggregates

Hydrogen-bond self-assembled molecular capsules were first reported by the Rebek group.61,62 The turning point in the field of such molecular capsules was the seminal paper by Shimizu and Rebek,53 who demonstrated that simple molecules like

![Scheme 2](https://example.com/scheme2.png)

calix[4]arenes substituted at their wider rim by four ary lurea residues, like the one prepared also by Böhmer (1 in Scheme 2),64,65 do form, spontaneously, dimers in non-polar organic solvents. Since then hydrogen bond dimers based on different systems (see compounds 2,66 3,67 and 468 in Scheme 2) have been prepared and thoroughly investigated.1,2,69,70

The first diffusion NMR study on calix[n]arenes was reported as early as 1995 by the Biali and Cohen10 groups and the first report on the use of diffusion NMR to probe and corroborate molecular encapsulation was reported by Frish et al. and dealt with dimeric capsules of the tetraureacalix[4]arene derivative 1.36 Encapsulated guests must be significantly smaller than the capsules in which they are encapsulated; however, they should diffuse as a single entity with the hosting capsule. Consequently, one should expect a dramatic change, in fact a dramatic decrease, in the diffusion coefficient of the guest upon encapsulation. These values should be the same as those of the hosting capsules in the case of slow exchange. Therefore it is not surprising that diffusion NMR was suggested as a reliable, flexible and easy to use tool for probing/corroborating guest encapsulation.36

Fig. 2 shows, for example, the signal decays as a function of $G$, the diffusion coefficient ($D$) and the $r_s$ extracted from the diffusion data of the peaks of the free and the encapsulated benzene in the dimer of 1 and of one representative peak of 1. This data clearly shows that the encapsulated benzene and the dimer have exactly the same diffusion coefficient, indicating that they indeed diffuse as a single molecular entity.36

Fig. 3 shows the DOSY spectrum of the dimer of 1 in the presence of ferrocene (5) and the cobaltocenium cation (6), demonstrating how simple it is to determine that only 6 is encapsulated in the dimer of 1 using such a diffusion NMR spectrum.38 Clearly only the peak at about 2.8 ppm, observed when the cobaltocenium cation is added to the solution of 1, has the same diffusion coefficient as that of the dimer. The other peaks at 4.06 and 5.61 ppm have much higher diffusion coefficients and represent non-encapsulated 5 and 6, respectively. In addition, Frish et al. capitalized on the large difference in the molecular weight and hence in the diffusion coefficients of DMSO and 1. They used diffusion NMR titrations to show that, in C$_6$D$_6$, about four DMSO molecules are needed to disrupt the dimer of 1, i.e. an average of about one DMSO molecule for each urea group of 1.36 In such dimers, a much higher affinity towards the cavity of 1 of the tropylium cation as compared to benzene and of the cobaltocenium cation as compared to ferrocene was found.38 These findings demonstrate the importance of the π–cation interaction in determining the affinity towards the cavity of such dimers.38 More recently, the Paek group has demonstrated, with the aid of 2D-NOESY and 2D-DOSY NMR techniques, that the resorcin[4]arene-based tetrakis(N-hydantoinylamido)-cavitand 7 forms dimers by encapsulating two molecules of CH$_2$OSO$_3$ in C$_2$D$_2$Cl$_4$.71

![Fig. 2](image-url) (a) The structure and the schematic representation of molecule 1, and (b) signal decay as a function of $G$ for free benzene, encapsulated benzene and a representative peak of 1, in a 80% C$_6$H$_6$: 20% C$_6$D$_6$ solution of 1 along with the diffusion coefficient, $M_w$, and Stokes radius ($r_s$) of these species.36 Adapted from ref. 36, copyright of the Royal Society of Chemistry.

![Fig. 3](image-url) (a) Schematic representation of the reaction of 5 and 6 with 1, and (b) the DOSY spectrum (400 MHz, 298 K) of the C$_2$D$_2$Cl$_2$ solution of 1 in the presence of 5 and 6.38 Adapted with permission from ref. 19b, copyright © 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.
Böhmer and co-workers, who used tetraureacalix[4]arenes as scaffolds for the preparation, by self-assembly, of molecules with peculiar and complex topologies,\textsuperscript{72,73} have used diffusion NMR to determine the structures of some of the systems shown in Fig. 4.\textsuperscript{74} In this study from the diffusion coefficients it was concluded that the $r_h$ of the tetramers (10–11)$_4$ is, as expected, larger than that of the dimer 12–13$_2$. There $r_h$ of 2.6 nm and 2.1 nm were computed for (10–11)$_4$ and 12–13$_2$, respectively.\textsuperscript{74} Ballester, when introducing the dimeric capsules formed by tetraureacalix[4]pyrroles (4, in Scheme 2) used DOSY to support the formation of such dimers in analogy to the results previously described for the dimers of 1.\textsuperscript{68}

However, perhaps the most important demonstration for the added value of diffusion NMR in the context of molecular capsules relates to the hexameric capsules of resorcin[4]arenes (14) and pyrogallol[4]arenes (15) (Scheme 3). In a seminal paper, the Atwood group presented the fantastic solid-state structure of the hexamers of 14a, which was found to be a [14a$_6$(H$_2$O)$_8$]$_n$-type capsule.\textsuperscript{75} Subsequently, Rebek and his co-workers were able to show that, in the presence of suitable guests, the hexamers of 14c, the lipophilic analogue of 14a, can be observed in solution.\textsuperscript{76,77} However, it was only diffusion NMR that unequivocally demonstrated that indeed the hexameric capsules are the resting state of resorcin[4]arenes such as 14b and 14c, and pyrogallol[4]arenes such as 15a and 15b (Scheme 3) in non-polar organic solvents.\textsuperscript{39–42} Interestingly, because of the huge difference in the molecular weight of the hexamer of 14c (6768 Daltons) and H$_2$O (18 Daltons), diffusion was very instrumental in underpinning the number of water molecules which are part of the hexameric structure in solution, and it was concluded that [14$_6$(H$_2$O)$_8$]$_n$-type capsules prevail also in solution as in the solid state.\textsuperscript{40,42} The diffusion coefficient of the water molecules was found to be nearly twice that of the hexamer of 14c when the water:14c ratio was 8.4:6, and exactly the same when this ratio was 7.2:6.\textsuperscript{40} From these results, the fact that only one peak of water is observed in the $^1$H NMR spectra of the chloroform solution of the hexamers of 14b or 14c, the fact that the diffusion coefficients of the water peak depend on the amount of water in the solution, and the finding that the encapsulated chloroform molecules have the same diffusion coefficients as the hexamers, the full mapping of the interactions that prevail in solution was conceived as shown in Scheme 3b.\textsuperscript{39,40} In contrast, diffusion NMR also demonstrated, unequivocally, that water molecules are not part of the structure of the hexamer of 15a and 15b in organic solvents,\textsuperscript{41,42} as found in the solid state.\textsuperscript{78} Self-sorting and in Lehn’s words “the ability of self to recognize self” has become an important theme in supramolecular chemistry.\textsuperscript{79,80} Since both 14 and 15 form hexameric capsules and since diffusion NMR was found to be such an efficient means to probe the hexameric structure of these systems, the Cohen group has used diffusion NMR to...
demonstrate that the self-assembly of resorcin[4]arenes and pyrogallol[4]arenes proceeds with self-sorting affording only homo-hexamers. However, when different resorcin[4]arenes or different pyrogallol[4]arenes were mixed together, hetero-hexamers were formed demonstrating that within the families of compounds no self-sorting is observed in contrast to the case when resorcin[4]arenes were mixed with pyrogallol[4]arenes. Several years later the Rebek group, in a series of papers, obtained the same results albeit at lower concentrations using FRET spectroscopy, which required a more laborious synthetic effort to prepare the donor and the acceptor derivatives of each family of compounds.

Using DOSY the Cohen group also demonstrated that lipophilic octahydroxypyridine[4]arenes (16), first synthesized by the Mattay group and claimed to form monomers and dimers in chloroform solution, also form hexameric capsules in non-polar organic solvents. In the latter case the more robust hexamer of 14c was used as an internal reference. Interestingly, in this system DOSY enabled us to identify the hexamers, the dimers and the monomer of 16 after adding 3 μl of trifluoro-acetic acid (TFA) to the chloroform solution of 14c and 16 as shown in Fig. 5. After addition of 3 μl of TFA the aggregates of 16 were disrupted while the hexamer of 14c, the internal reference, remained intact (Fig. 5b).

Diffusion NMR was also used to show that, in contrast to what was known from the solid-state study when tetraethyl ammonium salts (Et₄N⁺) are added to 14c, in non-polar organic solvents, hexamers are formed which encapsulate several Et₄N⁺ molecules. In that case the covalent dimer, prepared by Parisi and colleagues, was used as an internal reference, to unequivocally show that the systems formed are indeed hexamers which encapsulate several Et₄N⁺ molecules and not dimers. More recently, diffusion NMR was instrumental in showing that both trialkylamines and tetraalkyl ammonium cations interact with hexamers of 14c, both from the inside and from the outside. Diffusion NMR also enabled us to follow the disintegration of the different non-covalent interactions in the chloroform solutions of
the hexamers of 14c and trialkylamines upon addition of methanol, which disrupts the hydrogen bonds, as shown in Fig. 6.90 For example, adding up to 40 equivalents of methanol does not disrupt the hexamer of 14c, and triocetylamine (17) is still encapsulated in the hexamer of 14c. However, the external interaction of 17 with the hexamers of 14c is disrupted (Fig. 6a II). Addition of up to 80 equivalents of methanol disrupts the hexamer of 14c, and different lower aggregates of 14c to which 17 is still attached prevail in the solution (Fig. 6a III). An excess of methanol drives the equilibrium towards isolated monomers of 14c and 17 (Fig. 6a IV).

The interaction of 14c with the β-anomer of methyl-α-glucopyranoside (18) and glutaric acid (19) and other hydroxyl containing systems was investigated extensively by the Aoyama group.90,91 They concluded that 14c forms a dimeric complex with 18 and a 1:1 complex with 19, however, diffusion NMR showed that the formed systems are hexamers encapsulating three molecules of 18 and six molecules of 19, respectively.92 Here again to substantiate the results, a covalent dimer whose molecular weight is 2398 g mol⁻¹ i.e. slightly larger than that of the dimer of 14c (2208 g mol⁻¹), was used as an internal reference and indeed was found to have a significantly higher diffusion coefficient than the one observed for the hexamers of 14c.92

As it became clear that water molecules play a crucial role in the self-assembly of resorcin[4]arene hexamers,10 Ugono and Holman studied, in the solid state, the interaction of alcohols with such hexamers.93 More recently the Mattay group has claimed, based on diffusion NMR, that monomers, dimers and hexamers of 14c are formed when different alcohols are used.94 Subsequently Slovak and Cohen studied in much detail the interactions of different alcohols with the hexamers of 14c.95,96 Indeed it was found that very bulky alcohols such as 2-octyl-1-dodecanol (20) and 1-octadecanol (21) do not interact with the hexamers of 14c while other, less bulky alcohols, for example 1-butanol (22), 1-hexanol (23), 1-octanol (24), 2-ethyl-1-butanol (25), 2-ethyl-1-hexanol (26), are either encapsulated in the hexamers of 14c or are part of the structure of the hexamers.95,96 Interestingly, by using the LED or the BPLED sequences to study the diffusion characteristics of such systems, sequences which are routinely used in DOSY experiments and which are more sensitive to NMR exchange effects, it was possible to distinguish between alcohols which are simply encapsulated in these hexamers and those which are part of the hexameric structure.95,96 Indeed it was found that some alcohols are able to replace the water molecules in the [14c₆(H₂O)₆]-type hexamers but addition of water may reverse the situation.95,96

Both water and alcohols, which are part of the structure of the hexamers of 14c, gave the expected results when their diffusion characteristics were examined using the PGSE and PGTSE pulse sequences.95–97 However, when the diffusion of water and alcohols in the solution of the hexamers of 14b and 14c was monitored using the LED and the BPLED sequences, peculiar diffusion behaviour, at least in the first instance, was recorded.40,95–97 In these cases the signal decay as a function of $G^2$ was found to be non mono-exponential and the deviation from the mono-exponential behaviour was found to be dependent on the $t_e$ values of the LED and BPLED sequences (see Fig. 1c and d).95–97 These deviations from the mono-exponential decay became less apparent when the $t_e$ became shorter. For the water molecules in the solution of the resorcin[4]arene hexamers, for which only one peak is observed in the $^1$H NMR spectrum, increasing the $t_e$ results in an increase in the population of the slow diffusing component for which the diffusion coefficient approaches that of the hexamers.

![Fig. 6](image-url) (a) Schematic mapping of the non-covalent interactions that prevail in a solution of the hexamers of 14c and triocetylamine (17) and their disruption upon adding methanol, and (b) the diffusion coefficients associated with the molecular species that prevail in the solution at each stage.89
For the alcohols, however, which are part of the structure of the hexamers of 14c and where two sets of peaks are observed, increasing the $t_e$, which should have no effect on the diffusion measurements, results in an increase in the population of the fast diffusing component, for which diffusion at long $t_e$ approaches the diffusion coefficient of the free alcohols (see Fig. 7a–c). These results demonstrate that the LED and BPLED sequences (see Fig. 1c and d), often used in DOSY, are sensitive to exchange effects which develop during the extra delay i.e. the $t_e$ of these sequences, which may indeed complicate the interpretation of diffusion NMR data collected with these sequences. However, as demonstrated above, if one is aware of this effect one can use it to gain even further information on the structure and, more importantly, on the dynamics of a supramolecular system. Of course such results need to be supported with NOE, NOESY or EXSY NMR experiments.

2.2. Metal-based molecular containers and capsules

Some of the most spectacular self-assembled molecular cages prepared in the last decades have utilized metal–ligand interactions to form molecular containers. Such metal-based molecular containers, of which many are soluble in aqueous solutions, were shown to catalyse different reactions. However, since such systems are less labile compared to hydrogen bond capsules for example, and the structures of many of these systems were amenable to X-ray crystallography, diffusion NMR was mainly used to corroborate their structures. Importantly, in some studies diffusion NMR was used to determine the structure of supramolecular metal–ligand cages for which crystal structures were not obtainable. For example, Stang has used PGSE experiments to support the formation of his spectacular platinum dodocahedra formed from 30 metal complexes and 20 organic ligands. Fujita and co-workers have used diffusion NMR to determine, inter alia, the structures of several metal–ligand containers obtained by the reaction of the different bi-pyridyl ligands with Pd to obtain M$_{12}$L$_{24}$-type cages. In these studies a good correlation was obtained between the $r_e$ derived from the diffusion data and the radii estimated from either the crystal structure as in the case of

![Fig. 7](image-url)
Pd$_{12}$$L_{24}$, or from calculations as for Pd$_{12}$$L_{24}$ where L was 28 and 29 (Fig. 8).

More recently, Fujita and co-workers have used DOSY to corroborate the formation of the giant 72-component Pd$_{24}$$L_{48}$ spherical cage prepared by the reaction of Pd$^{2+}$ with the sulphur analogue of 27, i.e. 30. In this study the $r_s$ extracted from the DOSY experiments for the Pd$_{24}$$L_{48}$ spherical cage was found to be about 5.0 nm, which is much higher than the 3.4 nm found for the Pd$_{12}$$L_{24}$ complex. In the latter case a combination of DOSY and mass spectrometry was used to clearly demonstrate the different complexes that prevail in solution when different ratios of 27 : 30 are reacted with the Pd$^{2+}$ salt. In this study the reference solution was that of a 1 : 1 mixture of Pd$_{12}$$L_{24}$ and Pd$_{24}$$L_{48}$ prepared separately (see Fig. 9c).

Interestingly, although not stated, it seems that in these studies the simple Stokes–Einstein equation, i.e. eqn (6), was used to calculate the $r_s$ which was found to correlate well to the radii estimated from other methods. This occurred probably due to the fact that the hard sphere approximation and the continuum fluid approximation are both acceptable or even good approximations for such large spherical systems, and despite the fact that these systems, being highly charged, are probably also significantly solvated.

The Raymond group has used diffusion NMR to study ion association, in water, to their anionic [Ga$_4$$L_6$$]^ {12-}$ cage (when L is 1,5-bis(2,3-dihydrobenzeneamido)naphthalene (31)). They found that the more lipophilic cation Pr$_4$$N^+$ has higher affinity compared to Et$_4$$N^+$ towards the cavity of the cage. Interestingly, they could show by using diffusion NMR that these ions interact with the gallium cages from the exterior of the cage as well, as found by Slovak and Cohen for the resorcin[4]arene hexamers hosting trialkylamines or tetra-ammonium salts. Evidently, this exterior interaction was easier to probe in this highly charged multi units system because of the large difference between the diffusion coefficients of the “free” cations and the [Ga$_4$$L_6$$]^ {12-}$ cage. Here again, the cations interacting with the [Ga$_4$$L_6$$]^ {12-}$ cage from the exterior were found to be in fast exchange with cations in the bulk. By monitoring the diffusion coefficient of Et$_4$$N^+$ in the presence of 100-fold excess of KCl the much stronger exterior interaction of Et$_4$$N^+$ with the cage was apparent, demonstrating that in addition to Coulomb attractions, which, if any, should be stronger for K$, some additional attractive forces such as cation–π or/and the van der Waals force have to be involved.

Recently, the de Mendoza group have used DOSY to corroborate the formation of their polyhedral cages obtained from calix[4]arene and calix[5]arene carboxylates with uranyl salts but there, according to the authors, diffusion was not used quantitatively.

In a recent interesting study the Kato and Fujita groups showed that their M$_4$$L_{24}$ cages in which one of the ligands is

Fig. 8 (a) Schematic representation of the formation of the 36-component metal–ligand supramolecular spheres, (b) the ligands used to construct these molecular spheres along with the X-ray and calculated structures of the formed spherical complexes, and (c) DOSY spectra of the spherical complexes in DMSO$_d$$^6$ along with their diffusion coefficients and Stokes radii extracted from X-ray or calculations ($R_C$, $R_R$, respectively). Adapted and reproduced with permission from ref. 102, copyright © 2004 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Fig. 9 (a) and (b) The structures of ligands 27 and 30, the preparation and the X-ray structures of the spherical cages of Pd$_{12}$$L_{24}$ and Pd$_{24}$$L_{48}$ along with the $M_{eq}$, their diffusion coefficients and their $r_s$, and the DOSY spectra (500 MHz, DMSO$_d$$^6$) of (c) a 1 : 1 mixture of Pd$_{12}$$L_{24}$ and Pd$_{24}$$L_{48}$ prepared separately, (d) a Pd$^{2+}$ solution of a 3 : 7 mixture of 30, and (e) a Pd$^{2+}$ solution of a 2 : 8 mixture of 30 showing the different complexes formed. Adapted and reproduced with permission from ref. 103, copyright of American Association of Advancement of Science (AAAS).
covalently linked to ubiquitin, a well-known globular protein which is often used as a model system, can be prepared.\textsuperscript{106} Although the authors in this study have used analytical ultracentrifugation (AUC) and X-ray crystallography, much of the evidence for the formation of these large cages came from DOSY experiments, some of which are presented in Fig. 10. The authors prepared, \textit{inter alia}, ligands 32 and 33 from which, by reaction with Pd\textsuperscript{2+}, they prepared the Pd\textsubscript{12}32\textsubscript{23}33 and the Pd\textsubscript{12}32\textsubscript{24} cages (Fig. 10).\textsuperscript{106}

Fig. 10 shows that only one diffusion coefficient is observed for the cage postulated to encapsulate the ubiquitin (Fig. 10c) and this diffusion coefficient is, as expected, smaller than the diffusion coefficient of ubiquitin under the same experimental conditions (Fig. 10b). The authors also measured, as references, the diffusion coefficient of the cage that does not include the ubiquitin moiety \textit{i.e.} Pd\textsubscript{12}32\textsubscript{24}, both in the absence and in the presence of ubiquitin, as shown in Fig. 10d and e, respectively. Clearly, when ubiquitin is added to the solution of cage Pd\textsubscript{12}32\textsubscript{24}, two distinguishable diffusion coefficients are observed (Fig. 10e), which are very similar to those of ubiquitin and Pd\textsubscript{12}32\textsubscript{24} presented in Fig. 10b and d. The AUC results, the partial crystallographic information and the DOSY results provide convincing evidence that a capsule that encapsulates the ubiquitin is indeed formed when ligands 33 and 32 are mixed in a 1:23 ratio with Pd\textsuperscript{2+}.\textsuperscript{106} Very recently the Lützen group used DOSY experiments to determine the structures of two enantiopure organometallic cages of
dimeric capsules based on the octaacid derivatives such as

to assist the structural determination in solution of their

has used diffusion NMR and DOSY as an additional tool

smaller M$_{6}$

Fig. 12b.111,112

wise would have been very difficult to obtain as shown in

interesting insights into these systems, insights which other-

and

affords a M$_{12}$

In this study the DOSY spectra of each complex were collected

In that study the chemical shifts gave

that prevail in solution are monomeric. Increasing the alkane

For example, Gibb and co-workers were able to demon-

M$_{6}$L$_{12}$ and M$_{12}$L$_{24}$
types with ligands 34 and 35, which were reacted with Pd$^{2+}$ (Fig. 11a).107

D$_{0}$ = 0.60·10$^{-6}$ cm$^2$s$^{-1}$

M$_w$ = 8.019 gr mol$^{-1}$

$r_1$ = 13.0±15.1 Å

$r_{\text{calcd}}$ = 10.5±16.2 Å

D$_{0}$ = 0.33·10$^{-6}$ cm$^2$s$^{-1}$

M$_w$ = 16.037 gr mol$^{-1}$

$r_1$ = 27.6 Å

$r_{\text{calcd}}$ = 26.7 Å

Fig. 11 (a) The structure of ligands 34 and 35. (b) the DFT-calculated structures of the [Pd$_6$L$_{12}$]$^{2+}$ and [Pd$_{50}$L$_{24}$]$^{24+}$ along with (c) their DOSY spectra in DMSO$_{dc}$, their diffusion coefficients and their $M_w$, $r_1$ and $r_{\text{calcd}}$.107

MOM stands for methoxymethyl. Adapted with permission from ref. 107, copyright © 2011 of American Chemical Society.

$M_6$12-type cage, 35 affords a M$_{12}$L$_{24}$-type cage (Fig. 11b). Interestingly, a better agreement between the $r_s$ extracted from diffusion data and the $r_{\text{calcd}}$ extracted from the DFT-calculated model was found for the M$_{12}$L$_{24}$-type cage compared to the M$_6$L$_{12}$ type cage.107 This may well be due to the fact that the hard sphere approximation better describes the M$_{12}$L$_{24}$ cage than the M$_6$L$_{12}$ cage as shown in Fig. 11b.107 In addition, the continuum fluid approximation should also be more appropriate for the large M$_{12}$L$_{24}$-type cages than in the case of the smaller M$_6$L$_{12}$ cages.

2.3. Container molecules, molecular cages and capsules based on hydrophobic, electrostatic and covalent interactions

Containers molecules based on hydrophobic interactions in

water, like the ones developed by the Gibb group, represent intriguing examples of enzyme mimics.108 The Gibb group has used diffusion NMR and DOSY as an additional tool to assist the structural determination in solution of their dimeric capsules based on the octaacid derivatives such as compounds 36 and 37 presented in Fig. 12a.109,110 However, it is only more recently that diffusion NMR has provided some interesting insights into these systems, insights which otherwise would have been very difficult to obtain as shown in Fig. 12b.111,112

For system 36 it was found that for short $n$-alkanes, mono-

meric host assemblies prevail in the solution. As the $n$-alkane

guest became longer and its hydrophobicity increased, the

equilibrium was found to shift from a monomeric towards a dimeric assembly. From C$_4$ to C$_{14}$-alkanes the major species in the solution was assigned, based on diffusion data, to be of dimeric nature. In system 37, however, this dependency was found to be even more complex.111 For compound 37, one observes first a decrease in the diffusion coefficient of the host when the guest is changed from C$_4$ to C$_8$, $n$-alkanes suggesting the formation of higher aggregates. However, an increase in the diffusion coefficient, i.e. a decrease in aggregate radii and volume, is observed when C$_5$–C$_8$ $n$-alkanes are used as guests suggesting that for octane the major species that prevail in solution are monomeric. Increasing the alkane length further from C$_8$ to C$_{14}$ results in a shift of the equilibrium towards dimeric species, which become the dominant species in the solution when C$_{12}$–C$_{14}$ $n$-alkanes are used as guests.111

Recently, Gan and Gibb demonstrated that compound 37, also called TEMOA (tetra-endomethyl octaacid), can form dimers, tetramers and hexamers in the presence of different guests.112 Gan and Gibb have used the Stokes–Einstein equation to calculate the $r$ of the different species in solution from which they calculated the hydrodynamic volume (HV) of the species that prevail in solution.112 In that study the chemical shifts gave no clue to the fact that host 37 affords dimers with C$_{14}$, tetramers with C$_{17}$, and hexamers with C$_{24}$-alkanes as shown in Fig. 13. For the dimers, tetramers and hexamers, hydrodynamic volumes (HVs) of 11.5, 22.8 and 48.9 nm$^3$, respectively, were computed based on the diffusion NMR data.112
Reinhoudt, Schrader, and Verboom reported on the formation of dimeric capsules based on electrostatic interactions in polar solvents having $C_{4v}$–$C_{4v}$ or $C_{4v}$–$D_{4h}$ symmetries, and more recently Kusukawa and co-workers have reported an electrostatic dimer having $C_{3v}$–$C_{3v}$ and $C_{3v}$–$D_{3h}$ symmetries. The water soluble electrostatic dimers were prepared from the flexible cationic unit (38) and from the substituted homocyclic-based phosphonate units (39a–c) (see Fig. 14).

Kusukawa et al. have used DOSY to corroborate the formation of these dimers and found reasonable agreement between the HV extracted from the diffusion data and the volume calculated based on the molecular models of each species ($V_{\text{calc}}$). To better resolve the diffusion peak of each species Kusukawa and his co-workers have used long diffusion times, i.e., 100–300 ms, in their DOSY measurements.

The first molecular container molecules that were prepared by Cram and his group were the carcerands which are, in fact, covalent container molecules. More recently, several intriguing covalent capsules were prepared by the Warmuth group using single-pot, dynamic multi-components synthesis. Since this multi-components synthesis could, in principle, result in aggregates of different sizes the Warmuth group have used, inter alia, diffusion NMR to determine the stoichiometry of the different aggregates formed and to estimate, in combination with chemical shift argumentations, whether a specific guest is encapsulated in the resulting capsules. For example, it was found that negatively charged guests such as ATP, dAMP and dGMP were not encapsulated in the water soluble hexacavitands shown in Fig. 15a. In such a case a decrease in the diffusion coefficients of the guests was found upon addition of 41, however, no change in the chemical shifts of these guests was observed. Therefore, an external interaction between 41 and these guests was invoked.

For other guests such as $p$-toluenesulfonic acid, for example, the decrease in the diffusion coefficient of the guests upon addition of 41 was accompanied by large changes in their chemical shifts and therefore the authors argued that these compounds are indeed encapsulated in those covalent capsules. The diffusion coefficients of the guests claimed to be encapsulated was significantly higher than the diffusion coefficient of 41 indicating fast exchange, on the NMR time scale, between the encapsulated and non-encapsulated guests. In addition, Warmuth and his co-workers also demonstrated that there is an acceptable correlation between the sizes extracted from DOSY data and the sizes extracted from the energy-minimized AMBER-structures of a series of hexacavitands as shown in Fig. 15b.
3. Concluding remarks

It has been about twenty years since it was demonstrated that gradient systems of conventional NMR spectrometers are suitable for high-resolution diffusion measurements of supramolecular systems in solution and fifteen years since the first application of diffusion NMR in the field of molecular capsules. Since then diffusion NMR became even more simple and reliable, due to the introduction of digital gradient systems and the implementation and commercialization of DOSY packages. With current technology high-resolution diffusion NMR provides a simple means to simultaneously determine the diffusion coefficients, in solution, of ensembles of peaks quite easily, thus allowing the addition of diffusion coefficients into the arsenal of NMR parameters used by chemists to characterize chemical systems in solutions. Since the diffusion coefficients of molecular species reflect their effective sizes and shapes, it is not surprising that it is an excellent tool for probing inter-molecular interactions. Diffusion coefficients can be used to study aggregation, ion-pairing, hydration and to evaluate association constants between different hosts and guests. Importantly, diffusion coefficients are not affected by proton transfer, while chemical shifts, often used to evaluate association constants of supramolecular complexes are. In addition, many capsules and cages are formed by (self)-aggregation and are found to have the same symmetry as their monomers. The encapsulated guests are generally much smaller than their hosting capsules, making diffusion NMR an excellent tool for probing encapsulation. DOSY can also be used for virtual separation of mixtures. Therefore it is not surprising that, in recent years, more research groups are using diffusion NMR and DOSY to characterize molecular capsules and cages in solution.

Until recently most routine applications of diffusion NMR and DOSY were performed on systems which are at thermodynamic equilibrium, mainly since diffusion NMR is a NMR method which requires relatively long acquisition times. However, with the advent of fast and ultra-fast diffusion NMR methodologies that enable measuring diffusion coefficients in a time-resolution of seconds, one may envision the use of diffusion NMR to follow the formation and the dynamics of different supramolecular systems in general, and molecular capsules in particular. Clearly, after a slow start twenty years ago we report that diffusion NMR is routinely used by supramolecular chemists in general and even more so by supramolecular chemists working in the field of capsules, cages and molecular containers, and we expect more new insights and surprises along the way.

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References


