Diffusion NMR Spectroscopy in Supramolecular and Combinatorial Chemistry: An Old Parameter—New Insights

Yoram Cohen,* Liat Avram, and Limor Frish

Keywords:
- combinatorial chemistry · host–guest chemistry · molecular recognition · NMR spectroscopy · supramolecular chemistry

Dedicated to Professor Mordecai Rabinovitz on the occasion of his 70th birthday
Intermolecular interactions in solution play an important role in molecular recognition, which lies at the heart of supramolecular and combinatorial chemistry. Diffusion NMR spectroscopy gives information over such interactions and has become the method of choice for simultaneously measuring diffusion coefficients of multicomponent systems. The diffusion coefficient reflects the effective size and shape of a molecular species. Applications of this technique include the estimation of association constants and mapping the intermolecular interactions in multicomponent systems as well as investigating aggregation, ion pairing, encapsulation, and the size and structure of labile systems. Diffusion NMR spectroscopy can also be used to virtually separate mixtures and screen for specific ligands of different receptors, and may assist in finding lead compounds.

1. Introduction

1.1. Measuring High-Resolution Diffusion by NMR Spectroscopy

Over the last decade, pulsed field-gradient (PFG) NMR spectroscopy has become the method of choice for measuring diffusion in solutions in both chemical and biological systems. In principle, the diffusion coefficient of a certain molecular species under given conditions (for example, solvent and temperature) depends on its “effective” molecular weight, size, and shape. Therefore, it is evident that diffusion can be used to map intermolecular interactions that play an important role in both chemistry and biology in solution and which lie at the heart of molecular recognition, a process which is essential to supramolecular and combinatorial chemistry. Nevertheless, chemists working in these fields have only recently started to use diffusion NMR spectroscopy to study their systems.

The fact that molecular diffusion can be measured by NMR methods was realised in the early days of NMR spectroscopy. The most practical pulse sequence for measuring diffusion coefficients by NMR spectroscopy was introduced by Stejskal and Tanner in 1965, long before the advent of 2D NMR spectroscopy, which is currently routinely used by chemists worldwide. Indeed, diffusion NMR measurements have increasingly been used since 1965, and most of these applications in solution up until 1987 were reviewed by Stilbs and Kärger.

The last decade has witnessed an explosion in the utilization of gradients in all areas of NMR spectroscopy, ranging from coherence selection in high-resolution NMR spectroscopy to magnetic resonance imaging (MRI). Indeed, the use of diffusion MRI of the central nervous systems (CNS) has, in particular, increased considerably over the last decade. This is partially a result of the surprising efficacy of diffusion-weighted MRI in the early diagnosis of a stroke and the opportunities that diffusion tensor imaging (DTI) provides in mapping the fiber tracts in the CNS.

In view of the above, it may seem quite surprising that the application of diffusion NMR spectroscopy as a tool for studying molecular interactions in the context of supramolecular and combinatorial chemistry only began being implemented over the last few years. One reason for this is probably the fact that gradient sets, which are needed for the pulsed gradient spin echo (PGSE) experiment used to measure diffusion by NMR spectroscopy, were not commercially available until recently. However, with the advent of high-resolution gradient-enhanced spectroscopy and the technological improvement in gradient performance, mainly because of the development of MRI, such gradient sets became commercially available and are currently conventional accessories of standard modern high-resolution NMR spectrometers. These gradient-containing high-resolution probes provide a means to simultaneously determine the diffusion coefficient for the entire set of signals in a high-resolution spectrum with high sensitivity and accuracy. It should be noted that diffusion NMR spectroscopy, as will be demonstrated herein, provides a means for studying diffusion in systems in equilibrium where no concentration gradients exist. In addition, new pulse sequences and methodologies, some of which will be briefly discussed herein, were developed, thus enabling modern NMR spectrometers to routinely perform simple and complex NMR diffusion experiments.

1.2. Applications of Diffusion NMR Spectroscopy

Diffusion NMR measurements are used in many different fields ranging from the medical sciences to material sciences. Recently, with the advent of high-resolution...
gradient enhanced spectroscopy, some general reviews dealing with the theoretical and practical aspects of gradient NMR spectroscopy have been published.\textsuperscript{[11,17]} In addition, more-specific reviews on diffusion in polymers,\textsuperscript{[16b]} zeolites and porous systems,\textsuperscript{[16a,18]} surfactants,\textsuperscript{[19]} and liquid crystals and membranes\textsuperscript{[20]} have also been published. Since diffusion NMR spectroscopy is a totally non-invasive technique it is particularly suited to studying molecular dynamics and translational diffusion, and hence structural details in biological and physiological systems. Indeed, the application of diffusion NMR spectroscopy to membrane transport was recently reviewed,\textsuperscript{[21]} and several reviews dealt with diffusion in restricted geometries.\textsuperscript{[17]} q-Space diffusion NMR spectroscopy\textsuperscript{[22]} laid the foundations for the utilization of such experiments to obtain structural information and compartment size. In addition, because of the non-invasiveness of the technique and the fact that the current technology is suitable for studying compartments of only a few microns in size, q-space diffusion MR was recently used to study biological systems.\textsuperscript{[23–25]} Very recently, we expanded this approach to q-space MRI of the CNS.\textsuperscript{[26,27]} Callaghan and others have used diffusion NMR spectroscopy and MRI to study complex fluids.\textsuperscript{[28]} Van As and co-workers, for example, used these techniques to study flow in porous materials used for chromatography.\textsuperscript{[29]}

In this Review the applications of high-resolution diffusion NMR spectroscopy in solution will be discussed, with special emphasis on applications in the fields of supramolecular and combinatorial chemistry. The Review will concentrate on the applications in these fields of chemistry rather than on an extensive description of the theory of diffusion NMR experiments which can be found in many of the recently published reviews.\textsuperscript{[9–11,16–21,28]} We shall include a brief description of diffusion in the context of NMR measurements (Section 2) and a basic description of the NMR methods used to measure diffusion, with emphasis mainly on the most simple and commonly ones used to study diffusion in isotropic solutions (Section 3). In the main body of this Review (Sections 4 and 5) we shall describe different applications of diffusion NMR spectroscopy to demonstrate, through selected literature examples, the potential of simple diffusion NMR measurements in supramolecular and combinatorial chemistry. The final section gives future prospects for diffusion NMR spectroscopy (Section 6).

2. Concepts of Molecular Diffusion

2.1. Translational Diffusion in Isotropic Systems—“Free Diffusion”

Translational diffusion is one of the most important modes of molecular transport.\textsuperscript{[30]} Self-diffusion is the random translational motion of ensembles of particles (molecules or ions) as a consequence of their thermal energy. In the case of self-diffusion, no (net) force acts on the molecular particles and, consequently, no net displacement is observed. In an isotropic homogeneous system the conditional probability \( P(r_0, r, t_d) \) of finding a molecule, which was initially at position \( r_0 \) at position \( r \) after a time \( t_d \) is given by Equation (1), where \( D \) is the self-diffusion coefficient. This equation shows that the volume occupied by a molecule, originally at position \( r_0 \) relative to an arbitrary reference position, in a nonrestricted system is a Gaussian distribution that broadens with the increase in the diffusion time \( t_d \) (Figure 1). Therefore, the mean displacement of a particle under these conditions in all three directions by random walk is zero. However, the self-diffusion root-mean-square displacement \( (\langle X^2 \rangle)_{1/2} \) in such systems is given by the Einstein equation [Eq. (2)], where \( n \) is 2, 4, or 6 for the cases of one-, two- and three-dimensional diffusion. From this equation it follows that the mean displacement for free diffusion increases linearly with the square root of the diffusion time.\textsuperscript{[31]}

In addition, it is well known that diffusion is closely related to molecular size, as seen from the Einstein–Smoluchowski equation [Eq. (3)]\textsuperscript{[21,30]} where \( k_b \) is the Boltzmann constant,

\[
D = \frac{k_b T}{f} = \frac{RT}{N_f}
\]

constant, \( T \) is the absolute temperature, \( f \) is the so-called hydrodynamic frictional coefficient, \( N \) is Avogadro’s number, and \( R \) is the gas constant. For a sphere in a continuous...
The medium of viscosity $\eta$, $f$ is given by the Stokes Equation [Eq. (4)]. In this equation, $r_s$ is the hydrodynamic radius, often called the Stokes’ radius. Combining Equations (3) and (4) leads to the familiar Stokes–Einstein equation [Eq. (5)]. It should be noted, however, that, different theories are needed to describe the hydrodynamic frictional coefficient $f$ for molecular species of different geometries.

Equation (5) indicates that, by measuring the self-diffusion coefficient of a given molecular species under controlled conditions, one may obtain information on its effective size or weight and, therefore, on the specific interactions of the species with its molecular environment. Thus, the diffusion coefficients are sensitive to structural properties of the observed molecular species such as weight, size, and shape, as well as binding phenomena, aggregation, and molecular interactions. In addition, there is no need for further interpretation of the diffusion coefficients, the values of which are directly related to the translational molecular displacement in the laboratory frame when diffusion occurs in a homogeneous medium that allows free and isotropic diffusion. However, in nonhomogeneous samples, where different modes of diffusion prevail, the extraction of diffusion coefficients from diffusion NMR experiments is a much more difficult task.

2.2. Other Modes of Diffusion: Restricted and Anisotropic Diffusion

As will be shown in Section 3, the spins are tagged at at least two time points in the different NMR methods used to study diffusion. Therefore, the signal decay in a diffusion NMR experiment depends on the mean displacement of the particles during a certain time, called the diffusion time. Figure 1 shows the displacement distribution profiles for different diffusion times for free and restricted diffusion (Figure 1 a and c), as well as the mean displacement as a function of the square root of the diffusion time for free isotropic diffusion and restricted diffusion (Figure 1 b and d). For the case of free diffusion, the mean displacement experienced by the diffusing molecular species increases linearly with the square root of the diffusion time, as expected from Equation (2). By plotting the mean displacement as a function of the square root of the diffusion time, a linear graph is obtained, the slope of which reflects the diffusion coefficient (Figure 1 b). In a system where there are barriers which prohibit free diffusion, a situation may be envisaged in which an increase in the diffusion time does not translate into an increase in the mean displacement of the diffusing species. In such a situation there is no longer a linear relationship between the mean displacement and the square root of the diffusion time as shown in Figure 1 d. Restricted diffusion prevails in this situation and only an apparent diffusion coefficient can be obtained. It is clear that such a restriction will occur when the diffusion time $t_d$ is larger than $l^2/2D$, where $l$ is the length of the compartment and $D$ the diffusion coefficient of the diffusing molecular species. This means that the extracted apparent diffusion coefficients in such systems may be affected by the diffusion time $t_d$ of the diffusion NMR experiment. Anisotropic diffusion may be
found in cases where the barriers which impose restriction are not uniformly distributed. Both restricted and anisotropic diffusion are extremely important phenomena in heterogeneous systems such as porous materials and biological systems and provide, under certain experimental conditions, a means for obtaining structural information on the investigated system. Since these phenomena are much less important in homogeneous solutions, we shall not elaborate any further on these modes of diffusion.

### 3. NMR Methods for Measuring Diffusion

In recent years diffusion NMR methods have replaced the traditional way of measuring self-diffusion coefficients with radioactive tracers, since NMR methods are easier to perform, are totally non-invasive, and allow simultaneous determination of diffusion coefficients in multicomponent systems. In the next section we shall outline the effect of magnetic field gradients on the measured NMR signal. Thereafter, we shall outline some of the most useful NMR methods for measuring diffusion in solution.

#### 3.1. The Modified Spin-Echo Experiment: The Pulse Gradients

The basis for diffusion measurements is the fact that magnetic field gradients can be used indirectly to label the position of NMR-active nuclei through their Larmor frequency. This is done by applying an external gradient of the magnetic field, which is described by Equation (6), where $i, j, k$ are the unit vectors in the $x, y, z$ directions, respectively. Thus, the total external magnetic field at position $r$ is given by Equation (7). Spins precess with an angular frequency according to Equation (8). The acquired phase angle depends linearly on both $B(r)$ and the duration of the gradient $\delta$.

$$ G = \frac{\partial B_z}{\partial x} i + \frac{\partial B_z}{\partial y} j + \frac{\partial B_z}{\partial z} k \quad (6) $$

and $\hat{k}$ are the unit vectors in the $x, y, z$ directions, respectively. Thus, the total external magnetic field at position $r$ is given by Equation (7).

$$ B(r) = B_0 + G r \quad (7) $$

frequency according to Equation (8). The acquired phase angle depends linearly on both $B(r)$ and the duration of the gradient $\delta$.

$$ \omega(t) = -\gamma G r \quad (8) $$

In the following, we assume that only a $z$ gradient is present; hence, the gradient produces the position-dependent phase angle $\Phi(z)$ [Eq. (9)]. From these equations it is clear that the magnetic field gradient can be used to label the $z$ position of the spins.

$$ \Phi(z) = -\gamma G z \delta \quad (9) $$

The most common approach to measuring diffusion is to use the pulsed gradient spin echo (PGSE) NMR technique, which is a modification of the Hahn spin echo pulse sequence. In this sequence, two identical gradient pulses are inserted, one into each period $t$ of the spin-echo sequence (Figure 2). The PGSE sequence and a schematic representation of its effect on the magnetization of an ensemble of spins are shown in Figure 2. The net magnetization at the beginning of the experiment is oriented along the $z$-axis, which means that the ensemble of spins are in thermal equilibrium. A $90^\circ$ radiofrequency (RF) pulse is then applied, and, as a consequence, the magnetization rotates from the $z$-axis to the $x$-$y$ plane. A pulse gradient of duration $\delta$ and magnitude $G$ is then applied at a time point $t_1$. As a result, each spin experiences a phase shift according to Equation (10) at the end of the first period $r$, just before application of the $180^\circ$ RF pulse. The left term is the phase shift arising from the static magnetic field, and the term on the right is the phase shift arising from the applied magnetic gradient pulse.

The next step is the application of a $180^\circ$ RF pulse, which causes the reversal of the sign of the processing and the sign of the phase angle as depicted in Figure 2. At this point, two different scenarios can be considered. In the first scenario (Figure 2b) the spins do not undergo any translational motion along the $z$-axis, that is, there is no diffusion during the time interval. In this case, the phase shift of each spin after the first period $r$ is equal in magnitude to the phase shift of each spin.

![Figure 2](image-url)
after the second period \( t \). Hence, the effects of the two pulsed gradients cancel out and all the spins refocus. In this case, a maximum echo signal is obtained. In the presence of diffusion (the second scenario, Figure 2c), however, the phase shift of each spin after the first period \( t \) is different in magnitude from the phase shift of each spin after the second period \( t \). This effect occurs since, in the presence of diffusion, each species is located in a different position along the \( z \)-axis at times \( t_1 \) and \( t_1 + \Delta \), and hence each species is situated in a different magnetic field [Eq. (7)]. Therefore, the spins precess with altered angular frequencies in these different periods of time [Eq. (8)]. Thus, the phase angle fans out (at least partially) in the presence of diffusion, and the echo signal is consequently smaller. It can be intuitively deduced that larger diffusion is reflected by poorer refocusing of the spins and, consequently, by a smaller echo signal.

From these equations, it is apparent that the stronger and longer the phase of the pulsed gradients are, the poorer the refocusing of the spins and the smaller the recovered echo signal for diffusing spins. In addition, it is clear that the larger the \( \Delta \) value is (the duration between the pulsed gradients) the smaller the echo intensity will be. Thus, without presenting the complete mathematical manipulations, it is clear that the signal intensity should be described by Equation (11), where

\[
I_{(2,2)} = I_{(0,0)} \exp \left( \frac{2\pi}{T_z} \right) f(\delta, G, \Delta, D) = I_{(2,0)} f(\delta, G, \Delta, D)
\]

and Tanner have shown that the signal intensity for a single free-diffusing component is described in the case of rectangular pulse gradients by Equation (13),\(^{31}\) and gives Equation (14), where \( \gamma \) is the gyromagnetic ratio, \( G \) is the pulsed gradient strength, \( \Delta \) is the time separation between the pulsed-gradients, \( \delta \) is the duration of the pulse, and \( D \) is the diffusion coefficient. The product \( \gamma^2 G^2 \delta^2 (\Delta - \delta/3) \) is termed the \( b \) value. Thus, a plot of \( \ln(I_{(2,0)}/I_{(2,0)}) \) versus the \( b \) values for an isotropic solution should give a straight line, the slope of which is equal to \(-D\). In principle, any of the parameters \( \delta \), \( \Delta \), and \( G \) can be increased during the experiment to obtain increased signal attenuation. However, technical factors and the relaxation characteristics of the sample may limit the choice. The term \( (\Delta - \delta/3) \) is generally referred to as the diffusion time.\(^{30b}\)

Figure 3 shows the results for a PGSE experiment in which the strength of the gradient pulse was incremented from 0 to approximately 30 \( \text{G cm}^{-1} \) in ten steps while \( \delta \) and \( \Delta \) were kept constant. Figure 3a shows the signal attenuation of a small molecule, the diffusion coefficient of which is 1.81 \( \text{cm}^2 \text{s}^{-1} \), while Figure 3b shows the signal attenuation of another molecule having a diffusion coefficient of 0.33 \( \text{cm}^2 \text{s}^{-1} \). This figure shows that there is a more pronounced attenuation of the signal intensity for the fast diffusing species as reflected by the steeper slope of the graph of \( \ln(I_{(2,0)}/I_{(2,0)}) \) versus the \( b \) values. It should be noted that \( \ln(I_{(2,0)}/I_{(2,0)}) \) is generally abbreviated as \( \ln(I_{0}) \).

### 3.2. The Stimulated Echo Diffusion Sequence

The standard stimulated echo (STE) diffusion experiment is shown in Figure 4a.\(^{32} \) This sequence contains three 90° pulses; the echo after the third RF pulse was named by Hahn\(^{40} \) as the “stimulated echo”. The signal intensity of the STE diffusion experiment with rectangular pulse gradients is thus given by Equation (15).\(^{32} \) From this equation it is clear that the effects of relaxation and diffusion can again be separated in the signal decay. It was shown by performing the diffusion experiments with constant time intervals that the normalized signal decay in the STE diffusion experiments has the same dependency as the PGSE experiment.\(^{34,32} \) Comparison of Equation (15) with the expression for the signal attachment.

\[
I_{(\tau_a, \tau_1 + \tau_2)} = I_{(0,0)}/2 \exp \left( -\frac{2\pi}{T_z} \right) \left( \frac{T_\alpha}{T_\gamma} \right)
\]

\[
\exp(-\gamma^2 G^2 \delta^2 (\Delta - \delta/3) D) = I_{(\tau_a, \tau_1 + \tau_2)} \exp(-\gamma^2 G^2 \delta^2 (\Delta - \delta/3) D)
\]
The DOSY Technique

DOSY provides a means for “virtual separation” of compounds, by providing a 2D map in which one axis is the chemical shift while the other is that of the diffusion coefficient. It is based on the longitudinal eddy-current intensity of the PGSE [Eq. (13)] reveals two differences between the two equations: the reduction of the amplitude by a factor of two and—more importantly in the context of diffusion—the relaxation attenuation of the stimulated echo is dependent on $T_1$ during most of the diffusion interval (that is, during the time interval between the second and the third 90° pulses). This observation implies that one can use a STE diffusion sequence to obtain diffusion spectra of systems characterized by short $T_2$ times. This is an advantage since, in many systems, $T_1$ is significantly longer than $T_2$. The STE diffusion sequence allows an increase of the diffusion weighting by “paying” in the diffusion sequence an increase of the diffusion while the other is that of the diffusion compounds, by providing a 2D map in which one axis is the perpendicular axis (Figure 5). While the chemical shift information is obtained by fast Fourier transformation (FFT) of the time domain data, the diffusion information is obtained by an inverse Laplace transformation (ILT) of the signal decay data (Figure 6). The goal of the DOSY experiment is to separate species spectroscopically (not physically) present in a mixture of compounds. In this sense, the use of the DOSY experiment is reminiscent of the physical separation of compounds by chromatography. Thus, DOSY is also termed “NMR chromatography”. Figure 5 illustrates this concept. Each horizontal line represents a distinct diffusion coefficient.

3.3.1. The Basic LED and BPLED Experiments

An important requirement of the DOSY technique is the ability to discriminate between diffusion coefficients even when signals for similar-sized molecules overlap. To accomplish this goal it is essential to minimize spectral distortions that may result from eddy currents, which are induced from the gradient pulses and may, consequently, generate spectral distortions. The best way to avoid the effects of eddy currents is to prevent their formation in the first place. However, in spite of the best efforts, they may still be significant, especially when strong gradient pulses are used with short delays. Therefore, the LED sequence, which is a modification of the stimulated echo sequence shown in Figure 4a, was introduced (Figure 4b).

As shown in Figure 4, the difference between these two sequences is the addition of two 90° pulses and a delay $\tau$ at the end of the stimulated echo sequence. As a result of the fourth 90° pulse, the magnetization is stored in the longitudinal direction while the eddy currents decay. After the eddy current settling period $\tau$, the magnetization is recalled with an additional 90° pulse and an acquisition takes place (Figure 4b).

The bipolar LED (BPLED) sequence, which is a modification of the LED sequence, is shown in Figure 4c. In this sequence, each gradient pulse in the LED sequence is replaced by two pulses of different polarity separated by a 180° pulse. There are two advantages to using the BPLED over the LED experiment: Firstly, eddy currents are reduced to a minimum and, secondly, the effective gradient output is doubled. Thus, this sequence is useful in cases where large gradients are required to measure relatively low diffusion coefficients. For these reasons BPLED is the sequence of choice at present for many DOSY experiments. The DOSY sequence was also coupled into other methods such as INEPT and DEPT.

3.3.2. The 2D DOSY Technique

The diffusion experiments presented above can be processed and displayed as a 2D matrix with chemical shifts plotted along one axis and diffusion coefficients plotted along the perpendicular axis (Figure 5). While the chemical shift information is obtained by fast Fourier transformation (FFT) of the time domain data, the diffusion information is obtained by an inverse Laplace transformation (ILT) of the signal decay data (Figure 6). The goal of the DOSY experiment is to separate species spectroscopically (not physically) present in a mixture of compounds. In this sense, the use of the DOSY experiment is reminiscent of the physical separation of compounds by chromatography. Thus, DOSY is also termed “NMR chromatography”. Figure 5 illustrates this concept. Each horizontal line represents a distinct diffusion coefficient.
and, hence, all the peaks on this horizontal line correlate with signals in the chemical shifts dimension, and should be attributed to a specific molecular species.

At a certain frequency, where a continuum of diffusion coefficients should be considered, the data sets $I(s)$, which describe the attenuation of this signal, should be described as

$$I(s) = \int_0^\infty a(\lambda) \exp(-\lambda s) d\lambda.$$  \hspace{1cm} (16)

From Equation (16) it can be seen that $I(s)$ is the Laplace transform of $a(\lambda)$ and that $a(\lambda)$ is the Laplace spectrum of the diffusion coefficients. Thus, the desired spectrum $a(\lambda)$ is the inverse Laplace transform (ILT) of the decay function $I(s)$.

It should be noted that a perfect transform produces the Laplace transform of delta functions and the inverse transformation should therefore exist. The required situation is that a unique transformation will exist as in the case of the FT shown in Figure 6a. However, there is no perfect transformation and the dotted lines in Figure 6b are the practical results which depict this statement. Hence, when running a DOSY experiment it is preferable that the diffusion coefficients differ as much as possible from one another and the standard errors in the diffusion coefficients be as marginal as possible.

3.3.3. The 3D DOSY Technique

In 3D DOSY experiments a diffusion coordinate is added to the conventional 2D map. Like the conventional 2D applications, these experiments reduce the probability of spectral overlap by spreading the NMR signals of the same species over an entire 2D plane rather than along a single axis, while spreading the different species on a third axis on the basis of their diffusion coefficients. Indeed, in the first 3D DOSY experiment which was a DOSY-NOESY sequence, the overlapping peaks from a DNA duplex and a dinucleotide were resolved.

Figure 7a shows the pulse sequence for a DOSY-COSY experiment. This pulse sequence is constructed by linking the BPLED and the COSY sequences, with an eddy current delay $t$, introduced between the BPLED and COSY sequences. Other 3D DOSY sequences, for example, DOSY-TOCSY and DOSY-HMQC, have been reported following the same rationale. Figure 7b shows a schematic representation of the results of a 3D DOSY sequence, and depicts the ability of this sequence to “virtually separate”
different components. The various compounds are spectroscopically separated according to their different diffusion coefficients. Thus, the planes at different altitudes on the diffusion axis of the cube represents the 2D spectrum of a different species. Figure 7b shows two schematic COSY spectra of compounds A and B, which clearly differ in their diffusion coefficients. It should be kept in mind that a 3D DOSY experiment is a time-consuming method, as are all 3D NMR methods. Indeed this is one of the major disadvantages of the 3D DOSY method.

4. Applications of NMR Diffusion Measurements in Supramolecular Chemistry

4.1. Molecular Interactions

Molecular interactions, which are so essential in supramolecular and combinatorial chemistry, have been studied by many different spectroscopic methods.[40] Despite the major role played by NMR spectroscopy in these fields, diffusion NMR spectroscopy, which we believe has great potential in assisting the characterization of such systems, is still not fully exploited. However, an increasing number of applications have demonstrated this potential in recent years. Some of these applications will be outlined below in a way that emphasizes the chemical information that can be obtained from such measurements.

4.1.1 Binding and Association Constants

The association constant $K_a$ is a valuable measure that quantifies molecular interactions. In recent decades, tens of thousands of such constants have been determined by many different methods.[40,41] Despite the fact that NMR spectroscopy has become an important tool for studying association constants over the last two decades, diffusion NMR spectroscopy was not even mentioned as a possible option for studying host–guest systems in many of these reviews.[40u,42] Only a few very recent reviews devoted a short paragraph to the use of diffusion NMR spectroscopy for determining association constants.[43]

It was suggested many years ago that diffusion can be used to determine association constants.[44] The first example of such an application on a system that can be classified as an organic host–guest system was reported by Stilbs et al., who pioneered diffusion NMR spectroscopy of chemical systems by using a home-built gradient system.[45] In 1983 they measured the $K_a$ values of different alcohols with $\alpha$- and $\beta$-cyclodextrins ($\alpha$-CD (1) and $\beta$-CD (2)) in D$_2$O.[46] The experimental errors for the determined association constants were relatively high in some cases,[46] However, the advancement of gradient technology has made such measurements much more accurate and reliable today.

In fact, since NMR diffusion coefficients can be directly observed by NMR spectroscopy, it can be used in a very similar way as chemical shifts to determine the stoichiometry and association constants of complexes.[43,44,46,47] For the simple case of a 1:1 stoichiometric host–guest complex (HG) formed between a host (H) and a guest (G), $K_a$ is determined by Equation (17), where [H], [G], and [HG] are the concentrations of the host, guest, and host–guest complex formed at equilibrium, respectively. In the case of slow exchange on the NMR time scale, the association constant can be determined by simple integration of the peaks of a solution of known concentrations. In these cases, diffusion measurements can only be used to probe the association between the different molecular species, however, numerical values of $K_a$ cannot be obtained. However, in the case of fast exchange, the numerical value of the association constant can be determined.

The rationale behind the extraction of the bound fraction from diffusion NMR measurement is simple.[43,44,46,47] The host and guest have their own diffusion coefficients in the free state that reflect their molecular weight and shape. However, when a complex is formed and the host and guest are tightly bound, they should have the same diffusion coefficient since they diffuse as a single molecular entity.[44,46,47] In the case of a weak or negligible association, the diffusion coefficients of the host and the guest will remain unchanged. For any other case, assuming fast exchange on the NMR time scale, the observed (measured) diffusion coefficient ($D_{obs}$) is a weighted average of the free and bound diffusion coefficients ($D_{free}$ and $D_{bound}$, respectively) and can, therefore, be used to calculate the bound fraction $X$, as shown in Equation (18), in the same way that chemical shifts are used. Therefore, in principle, the same graphical and curve-fitting methodologies used to obtain $K_a$ values from changes in chemical shifts in titration experiments[41] can be used to obtain association constants from diffusion NMR measurements. The most important difference between the two methods is that in many cases a complete titration to find $D_{bound}$ for the guest is not a necessity with the former method. This is true in cases where there is a large difference between the molecular weight of the host and the guest (usually the guest has a significantly lower molecular weight) and, hence, one can predict, a priori, that the $D_{bound}$ value of the guest will be very similar to the $D_{free}$ value of the much larger host.[47]

One of the first examples of the determination of an association constant in the context of supramolecular chemis-
try by diffusion NMR spectroscopy using a conventional high-resolution NMR probe on a commercial instrument was the determination of the association constants of methylammonium chloride with [18]crown-6 (4) and [2.2.2]cryptand (5) in water and methanol.\cite{47} Figure 8 shows the signal decay of 4 and methylammonium chloride in the absence and presence of 4 in methanol as a function of the gradient strength (G).

This plot demonstrates that the signal attenuation of methylammonium chloride as a function of G in the presence of 4 is significantly smaller because of the formation of a complex with 4. Here, the diffusion coefficients were extracted using the Stejskal–Tanner equation \[\text{Eq. (14)}\]. Figure 9 shows the normalized signal decays of 4, methylammonium chloride, and their 1:1 mixture in CD$_3$OD as a function of G$^2$. The diffusion coefficients of these species and their 1:1 mixture, as well as the extracted association constants determined from the changes in the intensities of the tetramethylammonium signal in the presence and absence of 4, are means ± standard deviation. m = macrocycle.

In principle, the determination of association constants using diffusion NMR measurements has advantages and limitations arising from the fact that this is an NMR-based method.\cite{43} Therefore, on the one hand, this method is less prone to misinterpretation because of minor impurities than methods based on UV and fluorescence, for example.\cite{48} On the other hand, diffusion NMR spectroscopy is only suitable for measuring, in a direct way, association constants in the range of $10^{-5}$–$10^{-1}$ M$^{-1}$. However, one of the main advantages of using diffusion NMR measurements to extract association constants, as compared with other NMR-based techniques such as chemical-shift titrations, is the elimination of one of the main possible sources of error in such techniques, namely, confusing acid–base chemistry with binding processes.\cite{47} It should be noted, however, that in contrast to chemical shifts, proton transfer has only a marginal effect on the measured

**Table 1: Diffusion coefficients $D$ and logarithm of the association constants $K_a$ for the methylammonium chloride complex of [18]crown-6 (4) and [2.2.2]cryptand (5) in D$_2$O and CD$_3$OD at various temperatures.**\cite{47}

| Substance | Solvent | T/K | $D_n$ \[10^{-5}$ cm$^2$ s$^{-1}$\] | $D_{	ext{macro}}$ \[10^{-5}$ cm$^2$ s$^{-1}$\] | $\lg K_a$ | \[
\begin{array}{lllll}
4 + \text{MeNH}_3^+ & D_2O & 298 & 0.55 ± 0.01 & 1.23 ± 0.02 & 0.67 \\
4 & D_2O & 298 & 0.56 ± 0.01 & - & - \\
\text{MeNH}_3^+ & D_2O & 298 & - & 1.36 ± 0.01 & - \\
4 + \text{MeNH}_3^+ & CD_3OD & 298 & 1.34 ± 0.01 & 1.37 ± 0.02 & 3.69 \\
4 & CD_3OD & 298 & 1.35 ± 0.02 & - & - \\
\text{MeNH}_3^+ & CD_3OD & 298 & - & 1.70 ± 0.01 & - \\
5 + \text{MeNH}_3^+ & D_2O & 298 & 0.46 ± 0.02 & 0.95 ± 0.04 & 1.53 \\
5 & D_2O & 298 & 0.45 ± 0.04 & - & - \\
\text{MeNH}_3^+ & D_2O & 298 & - & 1.38 ± 0.01 & - \\
5 + \text{MeNH}_3^+ & D_2O & 277 & 0.21 ± 0.01 & 0.59 ± 0.01 & 1.67 \\
5 & D_2O & 277 & 0.20 ± 0.01 & - & - \\
\text{MeNH}_3^+ & D_2O & 277 & - & 0.72 ± 0.01 & - \\
5 + \text{MeNH}_3^+ & CD_3OD & 298 & 1.14 ± 0.03 & 1.14 ± 0.03 & > 4 \\
\text{MeNH}_3^+ & CD_3OD & 298 & - & 1.64 ± 0.01 & - \\
5 + \text{MeNH}_3^+ & CD_3OD & 213 & 0.28 ± 0.01 & 0.29 ± 0.01 & > 4 \\
\text{MeNH}_3^+ & CD_3OD & 213 & - & 0.42 ± 0.06 & - \\
\end{array}
\]

[a] All experiments were performed three times and the reported values are means ± standard deviation. m = macrocycle.
diffusion coefficient.\[^{[47]}\] This property enabled simple extraction of the association constant between methylammonium chloride and 5, where partial protonation of 5 occurred as expected.

Since the diffusion of a certain molecular species depends on its effective molecular size, which should change with any intermolecular interaction, it is clear that diffusion coefficients are intuitively related to aggregation and intermolecular interactions. This means that only in diffusion measurements can the observable parameters of the bound guest be predicted a priori, thus reducing the need for a complete titration. The main limitation of the diffusion coefficient \(D\) as a parameter for the determination of the association constant is in systems where the interacting species happen to have similar diffusion coefficients in the free state, thus making the method much less sensitive or even impractical.

Particularly interesting host–guest systems are those in which the guest itself can act as the host for yet another smaller guest. Early examples of such systems are the macrocycle complexes with \(\gamma\)-CD (3) introduced by Vögtle and Muller as early as 1979.\[^{[49]}\] These systems, subsequently analyzed by X-ray crystallography,\[^{[50,51]}\] were recently studied by electrodeometry of the association constant between methylammonium chloride and \(\gamma\)-CD and that the pH value had practically no effect on the extracted association constants, thus suggesting that hydrogen bonding is not a dominant factor governing the association constant in these host–guest systems.\[^{[52]}\] It was also found in these complexes that hydrophobic interactions, which are the major driving force of many of the complexes formed from cycloextrins and organic systems in water, are not the major factor responsible for complexation.\[^{[52,53]}\] In these systems, the changes in the chemical shifts were rather small and both the cation and the \(\gamma\)-CD before and after addition of lithium acetate (LiOAc) as an example. Figure 10 shows the normalized signal decay of 6 in the absence and presence of \(\gamma\)-CD and calculated association constants, are given in Table 2. Interestingly, it was found that the presence of alkali metal salts decreased the association between the macrocycles and the \(\gamma\)-CD and that the pH value had practically no effect on the extracted association constants.

![Figure 10](image)

**Figure 10.** Normalized signal decay as a function of the gradient strength squared \((G^2)\) in \(D_2O\). \(6\): 6 (+ 3), 6 (+ LiOAc + 3), \(\nabla\): 6 (+ LiOAc), 3 (+ LiOAc + 6), 3 (+ 6). All measurements were performed at 298 K and \(pD = 7.6\). Adapted with permission from Ref. [52].

<table>
<thead>
<tr>
<th>System</th>
<th>(D_1)</th>
<th>(D_m)</th>
<th>(D_{calc})</th>
<th>(K_a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(6 + 3 + \text{LiOAc})</td>
<td>0.27 ± 0.01</td>
<td>0.56 ± 0.01</td>
<td>0.86 ± 0.02</td>
<td>11</td>
</tr>
<tr>
<td>(6 + 3)</td>
<td>0.27 ± 0.01</td>
<td>0.48 ± 0.01</td>
<td>0.86 ± 0.02</td>
<td>187</td>
</tr>
<tr>
<td>(6)</td>
<td>--</td>
<td>0.68 ± 0.02</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>(3)</td>
<td>0.32 ± 0.02</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>(\text{LiOAc})</td>
<td>--</td>
<td>0.60 ± 0.01</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>(6 + \text{LiOAc})</td>
<td>--</td>
<td>0.60 ± 0.01</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>(7 + 3 + \text{LiOAc})</td>
<td>0.30 ± 0.01</td>
<td>0.53 ± 0.01</td>
<td>0.96 ± 0.01</td>
<td>19</td>
</tr>
<tr>
<td>(7 + 3)</td>
<td>0.29 ± 0.01</td>
<td>0.42 ± 0.01</td>
<td>0.96 ± 0.01</td>
<td>165</td>
</tr>
<tr>
<td>(7)</td>
<td>--</td>
<td>0.60 ± 0.01</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>(3)</td>
<td>0.32 ± 0.02</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>(\text{LiOAc})</td>
<td>--</td>
<td>0.60 ± 0.01</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>(6 + \text{LiOAc})</td>
<td>--</td>
<td>0.58 ± 0.01</td>
<td>0.96 ± 0.01</td>
<td>29</td>
</tr>
<tr>
<td>(8 + 3 + \text{LiOAc})</td>
<td>0.19 ± 0.02</td>
<td>0.36 ± 0.02</td>
<td>0.82 ± 0.01</td>
<td>21</td>
</tr>
<tr>
<td>(8 + 3)</td>
<td>0.19 ± 0.01</td>
<td>0.34 ± 0.02</td>
<td>--</td>
<td>69</td>
</tr>
<tr>
<td>(8)</td>
<td>--</td>
<td>0.41 ± 0.02</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>(3)</td>
<td>0.24 ± 0.02</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>(\text{LiOAc})</td>
<td>--</td>
<td>0.87 ± 0.02</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>(8 + \text{LiOAc})</td>
<td>--</td>
<td>0.39 ± 0.01</td>
<td>0.84 ± 0.01</td>
<td>10</td>
</tr>
</tbody>
</table>

\[a\] All experiments were performed three times and the reported values are averages ± standard deviation. \[b\] Systems with 6 and 7 were measured in a \(D_2O\) solution at \(pD = 7.6\) while the system with 8 was measured in \(D_6DMSO\). \(m = \) macrocycle.
and the diffusion coefficient, which changed uniformly for all signals, was found to be the most robust parameter for characterization of the association constant for these systems.

As previously stated, one of the main advantages of using diffusion coefficients to extract the association constants of host–guest systems, as compared with chemical shifts for example, is the fact that $D_{\text{bound}}$ for the guest is usually not very different from that of $D_{\text{free}}$ of the host since the host is much larger than the guest in most host–guest systems. This may, in some cases (but not all), eliminate the need for a complete titration to describe the binding curve. It is clear that the larger the difference in the size of the interacting molecules, the better this approximation, and Cameron and Fielding recently tested this assumption both theoretically and experimentally.[55] It was found that when $\beta$-CD ($M_r = 1135$) forms a complex with cyclohexylacetic acid (15, $M_r = 136$) with $K_a = 1800 \pm 100$ there is a small gradual reduction in the diffusion coefficient of $\beta$-CD upon addition of 15, but these changes were on the order of the error bars of the diffusion measurements. However, much more significant changes in the value of $D_{\text{bound}}$ were found following the addition of cholic acid (16, $M_r = 420$) as the guest, which implies that $D_{\text{complex}}$ differs from $D_{\text{bound}}$ and thus emphasizing the need for a complete titration, host and thus reinforcing the need for a complete titration, in CDCl$_3$ (5a)]. Vancomycin is an important antibiotic that acts by binding to the muropeptide precursors of the cell wall through the terminal D-alanyl-D-alanine sequence.[60] Recently, however, resistance toward this class of antibiotics has been reported and the need for synthetic analogues of vancomycin has emerged. Since 18, like vancomycin, contains several functional groups of different types, its interaction with the D-alanyl-D-alanine residue may be a superposition of electrostatic and/or hydrophobic interaction and may also involve $\pi$–hydrogen and dipole–dipole interactions or hydrogen bonds. Therefore, the association constant had to be determined in different solvents with guests having or lacking the D-alanyl-D-alanine residue (19–24). Diffusion NMR spectroscopy was used since NMR titration was difficult to apply and gave inconsistent results with relatively large errors. From the changes in the diffusion coefficients of a series of guests, some of which contain one or two alanine residues, upon addition of 18 in different solvents it was concluded that[56] in CDCl$_3$ 18 forms a complex of
moderate stability with lauric acid (19; \( \lg K_a = 2.7 \pm 0.2 \)) and stronger complexes with guests containing alanine residues (\( \lg K_a \approx 4.0 \)). Hydrogen-bond-competing solvents resulted in a decrease in the \( K_a \) values, with this decrease being more pronounced in complexes formed between 18 and guests 20, 23, and 24, which contain the alanine group, than in the complex of 18 with 19. The \( K_a \) values determined by diffusion NMR spectroscopy for the various guests in the different solvents are consistent with the fact that hydrogen bonds (amongst other factors) play a significant role in these complexes in chloroform. This study was the first direct quantitative evaluation of the binding constant of 18 with dipeptides, since it was not possible to determine the \( K_a \) values of these complexes by classical 1H NMR experiments. This result demonstrates that diffusion measurements may be superior to standard NMR techniques for determination of the association constants when complexation is associated with proton exchange.

A demonstration of the ability of a quick qualitative measurement of diffusion coefficients in determining the mutual interaction between different molecular species in solution is the study of the \( p \)-tert-butylcalix[4]arene–Cs–CH₃CN complex 25 in CDCl₃. The solid-state structure of this complex, in which a CH₃CN molecule was found to be coordinated to the cesium ion, was said to maintain its structure in solution based on its cesium NMR spectrum. The 1H NMR spectrum of the isolated crystals and a side view of the crystal structure of 25 are shown in Figure 12a and Figure 12b, respectively. Figure 12c shows the signal decays for the signals of the \( \text{tert} \)-butyl group of 25, the CH₃CN molecules originating from the dissolved crystals of complex 25, and the signal decay of the solvent (CHCl₃). This figure clearly demonstrates that the CH₃CN molecules have a much higher diffusion coefficient than the complex, which is the

**Table 3: Diffusion coefficients \( D \) and the computed \( \lg K_a \) values of host \( \alpha \)-CD and guests 17a, 17b, 17c, 17d, 17e in D₂O at 298 K.**

<table>
<thead>
<tr>
<th>System</th>
<th>( D_{max} \times 10^{-5} \text{ cm}^2 \text{ s}^{-1} )</th>
<th>( D_{CD} \times 10^{-5} \text{ cm}^2 \text{ s}^{-1} )</th>
<th>( D_{water} \times 10^{-5} \text{ cm}^2 \text{ s}^{-1} )</th>
<th>( X^{[a]} )</th>
<th>( \lg K_a \times 10^3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )-CD [2.8 mm]</td>
<td>0.30 ± 0.01</td>
<td>1.96 ± 0.01</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>17a</td>
<td>0.76 ± 0.01</td>
<td>–</td>
<td>1.97 ± 0.01</td>
<td>0.11 ± 0.03</td>
<td>1.18 ± 0.14</td>
</tr>
<tr>
<td>17a-drCD (1:2.9)</td>
<td>0.71 ± 0.01</td>
<td>0.29 ± 0.01</td>
<td>1.97 ± 0.01</td>
<td>0.46 ± 0.04</td>
<td>1.81 ± 0.07</td>
</tr>
<tr>
<td>17b</td>
<td>0.65 ± 0.01</td>
<td>–</td>
<td>1.96 ± 0.01</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>17c</td>
<td>0.60 ± 0.01</td>
<td>–</td>
<td>1.95 ± 0.01</td>
<td>0.42 ± 0.05</td>
<td>2.83 ± 0.21</td>
</tr>
<tr>
<td>17d</td>
<td>0.55 ± 0.01</td>
<td>–</td>
<td>1.96 ± 0.01</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>17e</td>
<td>0.50 ± 0.01</td>
<td>–</td>
<td>1.94 ± 0.01</td>
<td>0.65 ± 0.06</td>
<td>4.13 ± 0.30</td>
</tr>
<tr>
<td>17e-drCD (1:0.9)</td>
<td>0.32 ± 0.01</td>
<td>0.26 ± 0.01</td>
<td>1.94 ± 0.01</td>
<td>0.75 ± 0.10</td>
<td>3.85 ± 0.27</td>
</tr>
</tbody>
</table>

[a] The bound molar fraction.
expected result if there is no interaction between the small CH$_3$CN molecule and the much larger complex. Comparison of the diffusion coefficient of the CH$_3$CN signal originating from the crystals of complex 25 with the diffusion coefficient of CH$_3$CN in CDCl$_3$ (2.01 ± 0.02 · 10$^{-5}$ cm$^2$ s$^{-1}$) led to the conclusion that, as expected, there is practically no interaction between the CH$_3$CN molecules and the complex in CDCl$_3$.

In fact, a clear visualization of the lack of interaction is obtained conclusively from Figure 12c. It should be noted that the entire diffusion experiment shown in Figure 12c, which proves unequivocally that there is no interaction between CH$_3$CN and the other part of complex 25, was acquired in 10 minutes, thus demonstrating the robustness of this approach for determining molecular interactions in solution. In contrast, the acquisition of only a single $^{133}$Cs NMR spectrum of the same sample, just to ascertain that complex 25 was indeed obtained, required several hours.

The determination of association constants by using diffusion NMR spectroscopy is based on the fact that there is a fast exchange between the free and bound states of the guest. However, there are important applications of diffusion measurements, even in the case of slow exchange—such as for the study of molecular capsules and the encapsulation phenomenon outlined in the next section.

4.1.2. Encapsulation and Molecular Capsules

Molecular capsules in general[64,65] and those obtained by self-assembly in particular, are intriguing molecular systems that have attracted considerable attention over the last decade.[64–66] Molecular capsules are molecules that can accommodate smaller molecules in their cavity, thus isolating them from the immediate bulk, and can therefore, be regarded as microreactors.[67] Indeed, in recent years, such systems have been used to stabilize reactive intermediates[67a] and enhance the rate of some reactions.[67b]

The diffusion coefficient should be a powerful tool for detecting and probing encapsulation,[68] since the encapsulated guests are generally much smaller molecular species than the capsule itself and, therefore, should have a much higher diffusion coefficient in its free state. Moreover, the encapsulated molecules, which are in slow exchange with the bulk, should have the same diffusion coefficient as the capsule itself since the capsule and the encapsulated molecules diffuse as a single molecular entity.[68]

Such a diffusion-based argument was used recently to probe benzene encapsulation in the tetraureacalix[4]arene dimer (26-26).[68] Figure 13a shows part of the $^1$H NMR spectrum of dimer 26-26 prepared in a 80:20 mixture of C$_6$H$_6$ and C$_6$D$_6$. The signal at about $\delta = 4.4$ ppm was suspected to be that of the encapsulated benzene. Figure 13b shows the decay of this signal, the signal of “free” benzene (at $\delta = 7.15$ ppm), and one representative signal of the dimer (at $\delta = 1.95$ ppm) as a function of the diffusion gradient strength $G$. This figure
demonstrates that the signal at $\delta = 4.4$ ppm has a much lower diffusion coefficient than that of the free benzene, and that the extracted diffusion coefficient for this signal ($0.34 \pm 0.01 \times 10^{-5} \text{ cm}^2 \text{s}^{-1}$) was exactly the same as the one determined for the dimer and illustrates that, as expected, the encapsulated benzene and the dimer diffuse as a single molecular entity. A titration experiment in which the diffusion coefficients of the dimer and the DMSO molecules added to the benzene solution of $26 \cdot 26$ were followed demonstrated that it was possible to determine by diffusion NMR spectroscopy that about four DMSO molecules per molecule of $26$ are required to disrupt the dimer.

Frish et al. subsequently used a similar approach to probe the encapsulation of a tropylium cation by $26 \cdot 26$ in an attempt to evaluate the relative importance of electronic effects, namely, the importance of $\pi$-cation interactions, in determining the guest affinity for the cavity of the dimer. It was found that $\pi$-cation interactions indeed play an important role in determining the relative affinity toward the dimer cavity, as it was found that the tropylium cation affinity is about four orders of magnitude larger than that of benzene, which happens to have a very similar size. The same approach was recently used to demonstrate that the affinity of the cobaltocenium cation is at least five orders of magnitude larger than that of ferrocene. The DOSY spectrum shown in Figure 14 demonstrates how easy and simple the analysis and mapping of the mutual interactions was between the different molecular species that prevail in the solution, that is, between ferrocene, the cobaltocenium cation (bound and free), and the dimer $26 \cdot 26$.

These small complexes are relatively simple systems that are assembled, in most cases, from only three molecular species. However, as will be demonstrated, the advantage of using diffusion NMR spectroscopy to map the interactions between different molecular species may be even more important in systems that contain multiple molecular species and interactions.

Recently, the potential of simple diffusion NMR experiments in mapping the different molecular interactions in the spectacular resorcinarene hexamer capsule, first characterized by Atwood and co-workers, was demonstrated. Atwood’s group showed that $27a$ forms a hexamer of the type $[(27a)_6(H_2O)_8]$ in the solid state. It was shown subsequently by Shivanyuk and Rebek that $27b$ forms such a hexamer in the presence of suitable guests in water-saturated CDCl$_3$. It was found that the cation of tetrahexylammonium bromide (THABr, $29$) is encapsulated in the large cavity of the hexamer. The addition of THABr to $27b$ resulted in a change in the chemical shifts of $27b$ and the appearance of very high field signals at $\delta = -1.00$ ppm. On the basis of chemical shifts arguments, it was logically assumed that guest $29$ induces the formation of the hexameric capsule. However, simple diffusion measurements demonstrated that this is not the case in these systems. Diffusion measurements of $27b$, before and after the addition of $29$, showed that the
same diffusion coefficients (that is, \(0.28 \pm 0.01 \times 10^{-5} \text{ cm}^2 \text{s}^{-1}\) for a 3 mM solution at 298 K) are obtained, within experimental error, for the two systems. This result clearly contradicted the notion that \(27b\) is a monomer that self-assembles into a hexamer in the presence of \(29\) in CDCl\(_3\). Therefore, it was suggested that \(27b\) self-assembles spontaneously into a hexameric capsule in water-saturated CDCl\(_3\).\(^{[71]}\) Indeed, when \(27b\) was subsequently dissolved in water-saturated CHCl\(_3\), new high-field singlets appeared at about 4.8–5.1 ppm. The peaks were tentatively assigned to encapsulated CHCl\(_3\) molecules. These singlets were found to have the same diffusion coefficient as that of the resorcinarene moiety, thus further supporting the assignment of these singlets to encapsulated chloroform molecules. Moreover, addition of \(29\) to this chloroform solution resulted in the immediate disappearance of these signals, thus corroborating the conclusion that \(27b\) spontaneously self-assembles into a hexameric capsule in water-saturated CDCl\(_3\). Very recently Shivanyuk and Rebek came to the same conclusion by using a different methodology.\(^{[74]}\)

The results of titration experiments in which the diffusion coefficient of the resorcinarene moiety was measured as a function of the amount of DMSO added to the CDCl\(_3\) solution are shown in Figure 15.\(^{[71]}\) This study demonstrated that there is an increase in the diffusion coefficient of \(27b\) with the addition of DMSO. Very similar titration curves were found for the hexamer in the presence and absence of \(29\).\(^{[73]}\) The fact that the minor changes in the chemical shifts observed upon addition of DMSO is accompanied by an increase in the diffusion coefficient of \(27b\) (an increase in the viscosity of the solution is expected because of this addition) strongly supports the interpretation that the process followed is the disruption of the hexamer into its monomer.

The role of water in the formation of the hexameric capsule of \(27b\) and \(28\) in water-saturated chloroform was also studied by measuring the diffusion coefficient of the water signal and the hexamer signals in solutions with different \(27b, 28/\text{H}_2\text{O}\) ratios. Only a single water signal was observed in these samples, thus indicating that the different observable water pools are in fast exchange on the NMR time scale.\(^{[75a]}\) However, since the chemical shift and width of the water signal were found to be very sensitive to different parameters and to conditions other than the \(27b/\text{H}_2\text{O}\) ratio (Figure 16a), the measurement of diffusion coefficients was selected as an alternative method to evaluate the role of water molecules in these hexameric capsules. Figure 16b shows the effect of the \(27b/\text{H}_2\text{O}\) ratios on the diffusion coefficients of water and of \(27b\) in the presence and absence of THABr.\(^{[75a]}\) It was found that there was a decrease in the diffusion coefficient of the water signal as the relative amount of H\(_2\)O decreased. Interestingly, the diffusion coefficient for the water signal was nearly twice that of the hexamer even when the \(27b/\text{H}_2\text{O}\) ratio was 6:8.4. However, the diffusion coefficient of the water signal was found to be equal to that of the hexamer when the ratio was less than 6:8 (for example, 6:7.2). It was found for the \(29@27b\) system that the diffusion coefficient of the water signal is still several times that of the hexamer, even when the \(27b/\text{H}_2\text{O}\) ratio was less than 6:8 (Figure 16b).\(^{[75b]}\) The diffusion NMR measurements seem to indicate that the water molecules play a different role in the two capsules. In CDCl\(_3\) solution and in the absence of THABr the water molecules seem to be part of the supramolecular structure of the capsule. Indeed, it was found that about eight water molecules per six molecules of \(27b\) have the same diffusion coefficient as \(27b\) in the hexamer. It should be noted, however, that diffusion measurements cannot distinguish between encapsulated water molecules and water molecules which are part of the hexamer since the water diffusion coefficient in both situations should be equal to the diffusion coefficient of the hexamer when slow exchange occurs. However, because of the fast exchange of H\(_2\)O with bulk water and the fact that [\(27b\),\(\text{H}_2\text{O}\)]\(_6\)-type capsules were observed in the solid state,\(^{[75]}\) the more plausible explanation seems to be that the eight water molecules which have the same diffusion coefficient as the hexamer are part of the supramolecular structure in the solution. However, it seems that in the presence of THABr there is no need for water molecules for the construction of the supramolecular capsule.\(^{[75b]}\) By using the same methodology (Figure 16c) it was found that water has no role in the construction of the hexameric capsule of \(28\) in CDCl\(_3\) solutions and to conditions other than the \(27b/\text{H}_2\text{O}\) ratio (Figure 16a),
this system.\textsuperscript{[75d,e]} In addition, it was found by combining the results from chemical shift experiments and diffusion NMR spectroscopy that 
27b encapsulates trialkylamines and tetraalkylammonium salts. However, 
28 was shown to encapsulate only trialkylamines which are ejected from the cavity of 
the hexamer of 28 following addition of DCl and the in situ formation of ammonium salts.\textsuperscript{[75f]} These examples demonstrate the ability of diffusion NMR spectroscopy to provide a simple means to map the molecular interactions in such multicomponent systems. The maps of some of the molecular interactions that prevail in these systems are summarized graphically in Figure 17.\textsuperscript{[71,75a–c]}

4.1.3. Hydrogen Bonds and Water Hydration in Organic Solutions

Many NMR methods have been used to study the interaction of water molecules with biomolecules, since such interactions are of prime importance.\textsuperscript{[76]} Hydration of biomolecules has been approached by NMR methods based on the nuclear Overhauser effect (NOE) and magnetic relaxation dispersion (MRD).\textsuperscript{[77,78]} In principle, the interaction of water molecules with a biomolecule should be easy to probe by diffusion NMR spectroscopy because of the large difference in the sizes and molecular weights of the small water Figure 16a. a) Sections of the $^1$H NMR spectra from different studies of 
27b as a function of the 27b/H$_2$O ratios for CDCl$_3$ solutions. Both the 
line shape and chemical shift may be very different even when the 
27b/H$_2$O ratio is very similar. b) Diffusion coefficients of 27b and the 
water signal in the presence of 29 (○ and ⃝, respectively) and absence of 
29 (● and □, respectively) as a function of the number of water 
equivalents ($N_{\text{H}_2\text{O}}$) per six equivalents of 27b.\textsuperscript{[79c]} c) Diffusion coefficients of 28 and the water signal (● and □, respectively) as a function of the number of water equivalents ($N_{\text{H}_2\text{O}}$) per six equivalents of 28. 
Figure 16b and c are adapted with permission from Ref. \textsuperscript{[75b,c]}. 

Figure 17. Schematic representation of some of the different molecular interactions and aggregation modes identified in these capsules by diffusion NMR spectroscopy.\textsuperscript{[71,75a–c]}
molecules and the large biomolecule. Interactions between water molecules and biomolecules in an aqueous solution is beyond the scope of the present review, and we shall restrict ourselves to the few studies that measured the interaction of water with organic species in non-aqueous media.

In addition to the studies described in Section 4.1.2 regarding the role of water molecules in the hydrogen-bonded capsule,[76a–c] an early report dealt with the hydration, or more accurately the interaction of water molecules with crown-6 (4) and its KI complex (4 Ki) in water-saturated CDCl3 solutions.[79] In this study, the process was followed by comparing the changes observed in the chemical shift and the diffusion coefficient of the water signal upon changing the H2O:4 ratio. It was found that the average fraction of bound water molecules was independent of the KI ratio but increased considerably when the H2O:4 ratio was increased. In this study, similar conclusions were obtained from the chemical shift titrations and the diffusion NMR measurements.[79] The results of this study (Figure 18) show that the bound fraction of water molecules was higher in the case of the noncomplexed crown ether. An average of about 0.3 water molecules per complex molecule were obtained for the KI system, which was consistent with the finding of Iwachido et al. who found that the potassium complex of 4 can extract on average 0.3 water molecules per complex molecule into nitromethane while 4 can extract up to 1.6 water molecule per molecule.[80] These results imply that 4 acts as an efficient solvent shell for the potassium ion. In the case of 4, the fraction of bound H2O was found to depend on the H2O:4 ratio. In this case, it was suggested that complexation of the water molecules into the empty cavity of 4 cannot be ruled out.

The hydration of macromolecules in aqueous media is very complex and different types of hydration spheres have been evoked on the basis of their average lifetime in the bound state.[76–78] One of the main problems in aqueous media is to avoid the large signal of the bulk water, which is in most cases in fast exchange with the water molecules that do interact with the (bio)molecules. This problem is much less severe in organic solvents, however. Nevertheless, it should be noted that diffusion measurements give only an average picture. A bound fraction of 0.3 may well be one molecule that spends 30% of its lifetime in the bound state or two molecules that each spend only 15% of their lifetimes in the bound state and so on.[79]

Hydrogen bonds have well-established effects in NMR spectroscopy,[81] such as downfield shifts,[82a] an intermolecular NOE effect,[82b] and spin–spin coupling which may be observed through such hydrogen bonds in some cases.[82c] Recently, Berger and co-workers demonstrated how DOSY could be used to qualitatively study the strength of hydrogen bonds between different species in solutions.[83] The principle behind the measurement is clear: The formation of a hydrogen bond will decrease the diffusion coefficient of a certain molecule from that expected from its molecular weight and shape in a given medium at a given temperature. DOSY was used for this purpose because it has the advantage of visualizing the effect very clearly even in complex mixtures.[83a]

It was shown that the addition of DMSO (a known H-bond acceptor) results in a more pronounced decrease in the diffusion coefficient of phenol than that of cyclohexanol. These different responses were attributed to the higher tendency of phenol, which is the more acidic compound, to form hydrogen bonds with the added DMSO molecules.[83a] A similar phenomenon was observed for a mixture of phosphorus-containing compounds: The four compounds studied by using the 31P DOSY technique were trimethyl phosphate (30a), triphenylphosphate oxide (30b), triethylphosphine oxide (32), and ditymphosphate (31). The results of the DOSY spectra of these systems obtained before and after the addition of triethanolamine are shown in Figure 19a and b, respectively. It can be seen that the largest change in the diffusion coefficients were observed for 32, which is expected to form the strongest hydrogen bonds. However, a closer look at the data shows that the addition of triethanolamine caused a decrease in the diffusion coefficients of all the solutes in both samples thus suggesting that the addition of the H-bond acceptor affected the solutions viscosity. More recently, the same research group suggested the use of tetramethylsilane (TMS) as a reference for studying this effect in a more quantitative manner by using the ratios of the diffusion coefficients relative to the diffusion coefficients of TMS before and after addition of an H-bond acceptor. This was done since TMS is considered to be unaffected by hydrogen bonds and can therefore be used to report on the effect of the change in viscosity on the measured diffusion coefficients.[83b]
4.2. Molecular Size and Shape

Diffusion coefficients, being intuitively related to the effective radius of the molecular species through the Stokes–Einstein equation [see Eq. (5)], have also been used to determine the size and shape of different supramolecular entities called rosettes.\(^{[84,85]}\) The pulsed gradient spin echo (PGSE) sequence was used to characterize several rosettes prepared by the research group of Reinhoudt.\(^{[86]}\) In this study, single, double, and tetrarosettes of the type shown in Scheme 1 were classified on the basis of their diffusion coefficients.\(^{[86]}\) Relatively good agreement was found between the hydrodynamic diameter extracted from diffusion NMR measurements and those obtained from gas-phase-minimized structures.\(^{[86]}\) More importantly, by measuring the diffusion coefficient of the single rosette \((33a,34)\) to which \(33a\) was added, it was possible to show that these rosettes are kinetically labile and in fast exchange with their components on the NMR time scale under the given conditions (2 mM sample in CDCl\(_3\), 298 K, 500 MHz). In addition, it was also possible to determine by using this approach which of the double rosettes were in fast exchange with their components and which were kinetically less labile. This information was difficult to obtain from conventional NMR spectroscopic analysis of these systems.

In another recent application, Stang and co-workers\(^{[87a]}\) used the PGSE sequence to corroborate the formation of their spectacular dodecahedra constructed from 50 predesigned components (Scheme 2). The diffusion coefficients of the two dodecahedra \(40\) and \(41\), the molecular weights of which could be characterized by their diffusion coefficients.

**Figure 19.** a) The \(^{31}\)P DOSY spectrum of a mixture of trimethyl phosphate \((30a)\), triphenylphosphine oxide \((30b)\), triethylphosphine oxide \((32)\), and dibutyl phosphite \((31)\); b) the \(^{31}\)P DOSY spectrum of the same mixture containing triethanolamine as an additional component. The spectrum was recorded using the BPLED sequence with gradient strengths of 2 ms in 32 steps. Reproduced with permission from Ref. [83a].

**Scheme 1.** Single \((33,34)\), double \((35,34)\), and tetrarosettes \((36,34)\), which could be characterized by their diffusion coefficients.

**Scheme 2.** Synthesis of the dodecahedra \(40\) \((R = \text{Et}, n = 1, M_r = 41656 \text{ Da})\) and \(41\) \((R = \text{Ph}, n = 2, M_r = 61955 \text{ Da})\) from 20 monomers of \(37\) with 30 monomers of \(38\) \((R = \text{Et}, n = 1)\) and \(39\) \((R = \text{Ph}, n = 2)\), respectively.\(^{[87a]}\)
which were calculated to be 41,656 and 61,955 Da, were found to be $0.18 \pm 0.005 \times 10^{-5}$ and $0.13 \pm 0.006 \times 10^{-5}$ cm$^2$s$^{-1}$, respectively, in a mixture of acetone and dichloromethane.\[87a\] Simulation of the motion with such diffusion coefficients through that medium (taking into account its viscosity) enabled them to extract diameters of 5.2 and 7.5 nm, which are in good agreement with the estimated calculated diameters of 5.5 and 7.5 nm, respectively. Recently, Viel et al. demonstrated by using DOSY that π-π stacking interactions occurred in the hydrophobic compound metolachlor (42) in concentrated aqueous solutions.\[87b\] These authors reached the conclusion that the polymer formed was held together by π-π interactions from the much lower diffusion coefficient of the extra signals observed at high concentration.\[87b\] Comparison of the surprising DOSY data with other NMR methods (such as 2D NOESY) led to the conclusion that, indeed, the formed aggregate is not a dimer or a trimer but a polymer.\[87b\]

Diffusion NMR spectroscopy was used to corroborate the formation of double-stranded helicates in solutions in several cases.\[88a,b\] Larive and co-workers recently used diffusion NMR measurements to characterize a series of ligands (43–47) and rhenium complexes that were used as building units for the construction of molecular squares, which could not be characterized by mass spectrometry.\[89\] In this case, a good correlation was found between the diffusion coefficient and the reciprocal of the estimated stokes radii ($1/r_s$), as shown in Figure 20. The authors also concluded on the basis of the diffusion NMR measurements that the complexity of some of the spectra are intrinsic characteristics of the supramolecular systems prepared rather than contamination from species of low-molecular weight.\[88c\] The data extracted from these diffusion NMR experiments are presented in Table 4.

A specific field where size determination by diffusion NMR spectroscopy can assist the characterization of the obtained system in solution is dendrimer chemistry, in which generations can be probed easily by using diffusion NMR spectroscopy, as demonstrated briefly in the next section.

**Figure 20.** a) Approximated molecular radii of ligands 43–47 as well as their respective monomers (corners) and square complexes; b) the correlation between their diffusion coefficients and the reciprocal of the estimated Stokes radii ($1/r_s$) for ligands (●), corners (▲), and squares (○). Reproduced with permission from Ref. [88c].

### 4.3. Dendrimers and Dendrons: Size, Shape, and Function

Diffusion NMR measurements may assist in characterizing dendrimers\[89\] and it is surprising that, until recently, relatively few studies have used this technique to characterize...
Molecular diffusion coefficients of the ligands 43–47 and their rhenium complexes.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>$D_g \times 10^4$ cm$^2$s$^{-1}$</th>
<th>$D_d \times 10^4$ cm$^2$s$^{-1}$</th>
<th>$D_i \times 10^4$ cm$^2$s$^{-1}$</th>
<th>$D_q \times 10^4$ cm$^2$s$^{-1}$</th>
<th>$D_5 \times 10^4$ cm$^2$s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ligand</td>
<td>9.68 ± 0.09</td>
<td>6.03 ± 0.04</td>
<td>3.53 ± 0.04</td>
<td>2.24 ± 0.02</td>
<td>1.94 ± 0.06</td>
</tr>
<tr>
<td>corner</td>
<td>3.62 ± 0.08</td>
<td>2.69 ± 0.02</td>
<td>1.61 ± 0.03</td>
<td>1.28 ± 0.04</td>
<td>1.47 ± 0.04</td>
</tr>
<tr>
<td>square</td>
<td>2.37 ± 0.11</td>
<td>1.42 ± 0.05</td>
<td>0.87 ± 0.02</td>
<td>0.80 ± 0.04</td>
<td>0.94 ± 0.03</td>
</tr>
</tbody>
</table>

The last two examples demonstrate how diffusion NMR spectroscopy gives a better insight into the solution structure of dendritic materials, which, in turn, may affect their properties, and demonstrate the potential of such measurements to establish the structure/functional activity of such complex systems.

4.4. Reactive Intermediates, Ion Pairing, and Organometallic Systems

The interactions of different ions with different systems and polyelectrolytes, their binding to micelles, and their involvement in the formation of microemulsions have been studied extensively by diffusion NMR spectroscopy and are
Scheme 3. A strong influence of the solvent on the hydrodynamic radii was found for the flexible electroactive dendrimers 48–52 (compact structure in DMF, slightly swollen structure in THF).

Scheme 4. The rigid electroactive dendrimers 53–57 probably have a nonspherical and noncompact structure.
Table 5: Diffusion coefficients $D$, radii $r$, volumes $V$, and densities $\rho$ from pulsed-field-gradient NMR experiments

<table>
<thead>
<tr>
<th>Compound</th>
<th>$M_r$</th>
<th>$D_{ff}$ [cm$^2$ s]$^{-1}$</th>
<th>$r$ [Å]$^{[c]}$</th>
<th>$V_{\text{stokes}}$ [Å]$^{[d]}$</th>
<th>$\rho_{\text{stokes}}$ [Da Å$^{-3}$]$^{[e]}$</th>
<th>$R_{\text{stokes}}$ [Å]$^{[f]}$</th>
<th>$V_{\text{theory}}$ [Å]$^{[j]}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>pyrene</td>
<td>82</td>
<td>1.7 \times 10^{-5}</td>
<td>2.8</td>
<td>92</td>
<td>2.19</td>
<td>(3.5)</td>
<td>180</td>
</tr>
<tr>
<td>58</td>
<td>308</td>
<td>1.3 \times 10^{-5}</td>
<td>3.7</td>
<td>210</td>
<td>1.47</td>
<td>7.2</td>
<td>1560</td>
</tr>
<tr>
<td>59</td>
<td>521</td>
<td>0.97 \times 10^{-5}</td>
<td>4.9</td>
<td>490</td>
<td>1.06</td>
<td>10.1</td>
<td>4320</td>
</tr>
<tr>
<td>60</td>
<td>932</td>
<td>0.78 \times 10^{-5}</td>
<td>6.1</td>
<td>950</td>
<td>0.99</td>
<td>13.0</td>
<td>9200</td>
</tr>
<tr>
<td>61</td>
<td>1822</td>
<td>0.48 \times 10^{-5}</td>
<td>10</td>
<td>4200</td>
<td>0.43</td>
<td>15.9</td>
<td>16800</td>
</tr>
<tr>
<td>62</td>
<td>3495</td>
<td>0.34 \times 10^{-5}</td>
<td>14</td>
<td>11500</td>
<td>0.30</td>
<td>18.8</td>
<td>27800</td>
</tr>
<tr>
<td>acetonitrile</td>
<td>82</td>
<td>2.3 \times 10^{-5}</td>
<td>2.6</td>
<td>74</td>
<td>2.72</td>
<td>(3.5)</td>
<td>180</td>
</tr>
<tr>
<td>58</td>
<td>308</td>
<td>1.8 \times 10^{-5}</td>
<td>3.3</td>
<td>150</td>
<td>2.06</td>
<td>7.2</td>
<td>1560</td>
</tr>
<tr>
<td>59</td>
<td>521</td>
<td>1.4 \times 10^{-5}</td>
<td>4.1</td>
<td>289</td>
<td>1.80</td>
<td>10.1</td>
<td>4320</td>
</tr>
<tr>
<td>60</td>
<td>932</td>
<td>1.2 \times 10^{-5}</td>
<td>4.9</td>
<td>490</td>
<td>1.92</td>
<td>13.0</td>
<td>9200</td>
</tr>
<tr>
<td>61</td>
<td>1822</td>
<td>1.1 \times 10^{-5}</td>
<td>5.4</td>
<td>660</td>
<td>2.72</td>
<td>15.9</td>
<td>16800</td>
</tr>
<tr>
<td>cyclohexane</td>
<td>82</td>
<td>9.4 \times 10^{-6}</td>
<td>2.6</td>
<td>74</td>
<td>2.73</td>
<td>(3.5)</td>
<td>180</td>
</tr>
<tr>
<td>58</td>
<td>308</td>
<td>8.7 \times 10^{-6}</td>
<td>2.8</td>
<td>92</td>
<td>2.52</td>
<td>7.2</td>
<td>1560</td>
</tr>
</tbody>
</table>

[a] Calculated from NMR diffusion data. [b] Calculated from Equation (4) using, $q = 4.56 \times 10^{-4}$ Pas for THF. [c] Calculated from $R_{\text{stokes}}$ given above. $V_{\text{stokes}} = \frac{4}{3} \pi (R_{\text{stokes}})^3$. [d] Calculated from $V_{\text{stokes}}$ given above and $M_r \rho_{\text{stokes}} = M_r/V_{\text{stokes}}$. [e] Radii of fully extended structures calculated from models. [f] Volume of fully extended structures, from radii given above, $V_{\text{theory}} = \frac{4}{3} \pi (R_{\text{theory}})^3$. Measurements were performed at 298 K.

Beyond the scope of the present Review. Some selected examples of the applications of diffusion NMR spectroscopy in the field of organic reactive intermediates, ion pairs, and organometallic systems which bear some relevance to supramolecular chemistry are outlined below. In fact, diffusion NMR spectroscopy should make an important contribution in the field of reactive intermediates since most of these species are unstable and therefore classical methods for measuring diffusion, such as Rayleigh interferometry or radioactive tracer techniques, are impractical. Diffusion NMR spectroscopy, which provides a means for studying diffusion without perturbing the systems or the need for any chemical modification, is much more suitable for measuring the diffusion characteristics of such unstable species. In addition, many ions and organometallic systems tend to dimerize and self-aggregate, thus making diffusion an intuitively important parameter for such systems. Therefore, it seems surprising that diffusion NMR spectroscopy of such systems only became popular over the last few years.

One of the first applications of high-resolution diffusion NMR spectroscopy in the study of reactive intermediates was the determination of the diffusion coefficients of a series of polycyclic systems, such as compounds 63-69, and their respective doubly and quadruply charged derivatives. In this study, both external and internal references were used since it was anticipated that the reduction of the polycyclic systems into their respective charged derivatives would affect the solution viscosity and hence the diffusion coefficient, and would limit the utility of the diffusion coefficient as a characterizing parameter of the systems. Indeed, a pronounced decrease in the diffusion coefficients of the polycycles was observed upon reduction (Table 6), with only a small change in the viscosity seen. Since the two-electron reduction has no effect on the molecular weight of the systems, it is clear that the decrease in the diffusion coefficients should be attributed to a superposition of the higher solvation and the probable partial self-aggregation of the charged systems. The dilithium, disodium, and dipotassium salts of the tetracene dianion ($65^{-2}$), for example, were found to have very similar diffusion coefficients, thus implying that the ion-pair phenomenon had only a marginal effect on these systems under the experimental conditions used.

An interesting point that emerged from the comparison of the diffusion coefficients of the charged systems with their respective noncharged parent systems was that a larger decrease in the diffusion coefficients was observed for systems that are more suited to self-aggregation. A decrease in the diffusion coefficients to nearly 50% of their initial values was found and attributed to partial self-aggregation in these systems. Indeed, the corannulene tetraniun $G3^{+}$, which showed a pronounced decrease in the diffusion coefficient, was previously shown to form dimers. The conclusion of this study was that self-aggregation and dimerization in these systems might be more important than previously thought.

Rabinovitz and co-workers subsequently showed through the use of diffusion NMR spectroscopy that there is a fair to good correlation between $1/D$ and the van der Waals radii of neutral polycyclic compounds. A somewhat weaker correlation was found between the Stokes–Einstein radii...
This may be a consequence of the different solvation of the extracted Stokes–Einstein radii for the charged systems. \[100a\] 

The Stokes–Einstein equation does not hold very well when the dimer of \(63^+\) is 0.76 \(\times\) \(10^{-3}\) \(\text{cm}^2\text{s}^{-1}\) in \([\text{D}_8]\)THF solutions, and concluded that \(69^–\) forms an intermolecular sandwich.\[100b\]

Pochapsky et al., who studied the phenomenon of ion pairing by NMR spectroscopy quite extensively,\[100b\] recently used diffusion NMR spectroscopy to study the aggregation of tetrabutylammonium chloride (70).\[100c\] In this study the diffusion coefficient ratios of 70 were compared with that of tetrabutylsilane (71), a non-aggregating standard with a similar molecular weight and shape, as a function of the concentration. Pochapsky et al. found that this ratio decreased when the concentration was decreased, and achieved a value of nearly 1.0 in very dilute solutions (\(<10^{-4}\)M). The aggregation number for 70 was estimated to be 3 at a concentration of about 30 mM in CDCl\(_3\).\[102b\] Subsequently, Pochapsky et al. used the same rationale to study the ion pairs of tetrabutylammonium tetrahydroborate (72) by simultaneously monitoring the diffusion coefficients of the cation and the anion.\[102b\] They showed that the diffusion coefficients of the cation and anion in CDCl\(_3\) are not very different and are much lower than that of tetrabutylsilane (71). The interpretation was that, indeed, a tight ion pair is formed.

The fact that the diffusion coefficient ratio was found to be concentration-dependent clearly indicated that this ion pair forms aggregates in CDCl\(_3\), which is to be expected because of the low solvating power of the solvent.\[102b\] Recently, Keresztes and Williard demonstrated by diffusion NMR experiments that the tetramer and dimer of n-butyllithium can be identified in \([\text{D}_8]\)THF.\[103\] This was accomplished by comparing the diffusion coefficients obtained experimentally from diffusion NMR measurements with the those expected from known X-ray parameters of these species. The data was also presented in a DOSY form.\[103\]

Organometallic chemistry, where ligand insertion and/or dissociation and aggregation may play an important role, is an additional field in which diffusion NMR spectroscopy may complement other NMR methods for the characterization of species in solution. Since this field was recently reviewed by Pregosin and co-workers,\[104\] we shall only outline a few examples here. Beck et al. have claimed on the basis of diffusion NMR experiments that the formation of ion quadruples (B) from simple ion pairs (A), as shown in Scheme 6, is probably more important than previously thought in zirconocene-based catalytic systems.\[105\] This was concluded by measuring the diffusion coefficient of the zirconocenes 73–77 (Scheme 6), and by comparing the findings with the diffusion coefficient obtained for a series of ion pairs such as 73ab and 77ab with the diffusion coefficient of 73c, for which the binuclear structure was previously established by X-ray crystallogra-

**Table 6:** Diffusion coefficients \(D\) of polycyclic systems and their anions in \([\text{D}_8]\)THF at 298 K.\[98\]

<table>
<thead>
<tr>
<th>System</th>
<th>(D) (CH(_2)Cl(_2); ext. stand.) ([10^{-5} \text{cm}^2\text{s}^{-1}])</th>
<th>(D) (benzene; int. stand.) ([10^{-5} \text{cm}^2\text{s}^{-1}])</th>
<th>(D) (polycyclic system) ([10^{-5} \text{cm}^2\text{s}^{-1}])</th>
</tr>
</thead>
<tbody>
<tr>
<td>63</td>
<td>3.73 ± 0.002</td>
<td>2.87 ± 0.017</td>
<td>1.57 ± 0.017</td>
</tr>
<tr>
<td>63(^+)/4Li(^+)</td>
<td>3.73 ± 0.002</td>
<td>2.90 ± 0.041</td>
<td>0.84 ± 0.017</td>
</tr>
<tr>
<td>65</td>
<td>3.83 ± 0.031</td>
<td>2.96 ± 0.027</td>
<td>1.83 ± 0.032</td>
</tr>
<tr>
<td>65(^+)/2Li(^+)</td>
<td>3.79 ± 0.006</td>
<td>2.86 ± 0.039</td>
<td>0.96 ± 0.038</td>
</tr>
<tr>
<td>66</td>
<td>3.78 ± 0.000</td>
<td>2.39 ± 0.043</td>
<td>0.71 ± 0.020</td>
</tr>
<tr>
<td>66(^+)/2Na(^+)</td>
<td>(20 mg)</td>
<td>3.78 ± 0.000</td>
<td>2.86 ± 0.039</td>
</tr>
<tr>
<td>66(^+)/2K(^+)</td>
<td>(2 mg)</td>
<td>3.78 ± 0.001</td>
<td>2.86 ± 0.025</td>
</tr>
</tbody>
</table>

<sup>[a]</sup> After correcting for viscosity changes. <sup>[b]</sup> Very broad signal for benzene, undetectable in the spin echo.

obtained from the diffusion coefficients and the van der Waals radii for the neutral polycyclic systems. The authors suggested that this is partially a result of the fact that the Stokes–Einstein equation does not hold very well when the diffusing particles are less than about five times the radius of the solvent molecules in which the diffusion takes place. There was practically no correlation between \(1/D\) and the extracted Stokes–Einstein radii for the charged systems.\[100b\] This may be a consequence of the different solution of the anions studied, which also differed in their number of charges.

In a different study,\[100b\] the same research group used diffusion NMR spectroscopy to try to verify whether or not the octaanion of 69 forms dimers. They found that the diffusion coefficient of \(69^–\) is 0.55 \(\times\) \(10^{-5}\) \(\text{cm}^2\text{s}^{-1}\), while that of the dimer of \(63^+\) is 0.76 \(\times\) \(10^{-3}\) \(\text{cm}^2\text{s}^{-1}\) in \([\text{D}_8]\)THF solutions, and concluded that \(69^–\) forms an intermolecular sandwich.\[100b\]

Kaupp et al. used diffusion NMR spectroscopy to study the aggregation of tetrabutylammonium chloride (70)\[100c\] and the octaanion of 69 in [D\(_8\)]THF at 2005, 520–554 www.angewandte.org © 2005 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

453
Since the diffusion coefficient of 73b deviated from that of 73 and 74, and was similar to that of the dinuclear complex of 73c, it was concluded that 73b is an ion quadruple (structure 8 in Scheme 6). These measurements were performed in benzene in the 1.6–4.7 mM concentration range. However, a very recent study on very similar complexes (78–82) and with (p-tolyl)4Si (83) as a reference (Scheme 6) demonstrated that there is a good correlation between the hydrodynamic volumes of these complexes, as obtained from the PGSE diffusion data, and the van der Waals volumes computed for 1:1 ion pairs from the crystal structures of these complexes (Figure 21)[107]. The concentrations of the studied metalocenes were well above the $10^{-4}$–$10^{-3}$ M range generally used in polymerizations and it was therefore concluded that aggregation of the metalloccenium catalysts is unlikely to be of major importance for chain growth in olefin polymerization.[106b]

Zuccaccia et al. measured the diffusion coefficients of the cationic complex 84 and its neutral analogue 85 in different solvents at various concentrations.[107] In this study, the simple STE pulsed gradient sequence was used for the diffusion measurements. The results clearly indicated that compound 84 exists as a tight ion pair in CDCl3. It seems that the equilibrium is shifted to a looser ion pair in CD3NO2, as witnessed by the higher diffusion coefficient of BPh4 relative to the cation. In this solvent, there was only about a 10% difference between the diffusion coefficient of 84 and 85,[107] in the CDCl3 and CD2Cl2 solutions, however, the difference between these diffusion coefficients was much more pronounced and found to be concentration-dependent.[107] The authors suggested on the basis of these results that higher aggregates were involved in these solutions. It should be noted that reference 85 has a lower molecular weight than 84, for which the molecular weight of the ion pair (cation and anion) should be taken into account. In addition, compound 85 is probably less solvated than 84 since 85 is an uncharged molecule and therefore should have a somewhat higher diffusion coefficient. However, the fact that the changes in diffusion coefficients are smaller in solvents with higher solvating power and the fact that the difference in diffusion coefficients are concentration-dependent favors the explanation of higher aggregates in the case of 84 in the chlorinated solvents under the experimental conditions used.[107]
In a recent application Pregosin and co-workers reported the diffusion coefficients of a series of Pd(II)–arsine complexes of the type [PdCl₂L₂] (86–89; L = AsMe₂Ph₃, x = 3–0) and some other organometallic complexes of different sizes (90–93; Tp’ = hydrotris(3,5-dimethylpyrazolyl)borate, Ar = p-tolyl, dba = trans,trans-dibenzylideneacetone), from which they calculated their hydrodynamic radii $r_s$ by using the Stokes–Einstein equation [see Eq. (5)]. They found a good correlation between the $r_s$ values and the estimated radii compiled from the X-ray structures of these systems (Figure 22). This approach was further extended to ruthenium(II) complexes through the use of ¹⁹F and ¹H PGSE measurements.

A recent demonstration of how diffusion NMR spectroscopy could be used to characterize organometallic reactive intermediates in solution was recently given by Berger and co-workers, who provided a snapshot of the reaction of ¹³CO₂ with [Cp₂Zr(Cl)H] (94) by using ¹³C diffusion NMR spectroscopy (Scheme 7). The authors used ¹³CO₂ to increase the sensitivity, and combined the DOSY sequence with the INEPT sequence to enhance the signal of the protonated carbon atoms formed during the course of the reaction. The dimeric nature of intermediate 96 was elucidated by comparing the diffusion coefficients, and hence the hydrodynamic radii, of the formed intermediates with those of a series of known zirconocenes, such as 97–99 (see Table 7).

### Table 7: Experimental and calculated diffusion coefficients $D$ and hydrodynamic radii $r_s$ of the studied zirconium complexes

<table>
<thead>
<tr>
<th>Experimental</th>
<th>Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_s$ [Å]</td>
<td>$D$ [cm² s⁻¹]</td>
</tr>
<tr>
<td>99</td>
<td>3.0</td>
</tr>
<tr>
<td>97</td>
<td>3.7</td>
</tr>
<tr>
<td>98</td>
<td>4.2</td>
</tr>
<tr>
<td>96</td>
<td>6.3</td>
</tr>
</tbody>
</table>

[a] Calculated from the Stokes–Einstein equation. [¹] Calculated from X-ray structure data under the assumption of a spherical shape. [c] Calculated from the minimized gas-phase structure (PM3).

![Scheme 7](image-url) \n
**Scheme 7.** The combination of DOSY and INEPT sequences enabled the structure of the dimeric intermediate 96 in the reaction of CO₂ with [Cp₂Zr(Cl)H] to be elucidated.
This study demonstrates the tremendous utility of diffusion NMR spectroscopy in probing the structure of organometallic complexes where aggregation, dimerization, and ligand insertion may occur.

5. Applications of Diffusion NMR Measurements in Combinatorial Chemistry

Combinatorial chemistry has become an important tool in organic and pharmaceutical research in recent years.[3,110,111] One of the main consequences of combinatorial chemistry is the generation of mixtures of a large number of products in minute concentrations that require high-throughput NMR methods. Therefore, methods that can be used to assign mixtures of compounds, preferably without their isolation, are needed. In addition, as one of the main concerns of combinatorial chemistry is finding new lead compounds and specific ligands for different receptors, efficient screening methods for the identification of the interaction of tentative ligands with their specific receptors are required. In addition, the kinetics of solid-phase reactions may depend on the ability of the solid support to swell and the ability of reagents to reach the interface of the solid support. Therefore, it seems only logical to use diffusion NMR spectroscopy in the context of combinatorial chemistry where mixture characterization and screening for potential new ligands are central issues. Herein, we outline some of these applications, emphasizing DOSY applications that were originally developed by the group of Johnson[33–36] and applied by Shapiro and co-workers[37,112] and several other research groups.[113] The next section emphasizes the potential and limitations of diffusion NMR techniques in the context of combinatorial chemistry by focusing on only two issues, mixture characterization and ligand screening.

5.1. DOSY for Mixture Evaluation

DOSY provides an efficient means for the “virtual separation” of compounds.[34,35,37,113] As already shown in Section 3.3, DOSY provides 2D maps in which one axis corresponds to the chemical shifts and the other the diffusion coefficients. In fact, the validation of the DOSY approach was first demonstrated on different mixtures.[34,37,112-113] Figure 23 shows one of the first applications of DOSY, where it was demonstrated that one could indeed “virtually separate” compounds on the basis of their molecular weights. In this study, compounds of significantly different sizes, such as water, glucose, ATP, and sodium dodecylsulfate SDS micelles, were identified.[36a] Subsequently, with the advance in gradient hardware as well as DOSY acquisition and processing schemes, more demanding mixtures were characterized[34,113-117] (Figure 24) by using high-resolution DOSY (HR-DOSY).[113]

In this study, a handful of different metabolites, some of which are not very different in their molecular weights, were resolved.[115]

Diffusion coefficients that differ by only few percent can be resolved with the current conventional technology for non-overlapping peaks (in the same sample). However, it should be noted that, for overlapping peaks, one needs at least a factor of 2–3 in the diffusion coefficients to render them resolvable in a conventional DOSY spectrum.[34,112] The DOSY approach requires the acquisition of a number of 1D spectra with relatively good signal-to-noise ratios (SNR) and are therefore more easily performed by using proton NMR spectroscopy. Overlapping signals may present a real problem when mixtures of large numbers of compounds or complex compounds having many types of protons are present in the mixture. One way to alleviate this problem is to couple the DOSY approach with a 2D NMR sequence where overlapping signals are less of a problem; this affords a 3D sequence, referred to as 3D DOSY. Since diffusion can easily
be coupled to a 2D sequence, many 3D DOSY sequences were developed with relative ease.⁴³,⁷⁸,⁸⁰,¹¹²

However, there are only limited applications of these techniques in real chemical or combinatorial systems besides those of the systems used to demonstrate that these sequences are indeed operable. One of the main reasons for this might be the fact that these 3D DOSY sequences require long acquisition times and larger data storages, with the former being the much more important. Figure 25 shows a 3D DOSY-HMQC spectrum obtained on a simple mixture consisting of quinine (100), camphene (101), and geraniol (102) in CD₃OD as an example.⁴³ This 3D DOSY spectrum shows no overlap, and it is therefore much easier to extract the structural information from these maps and assign the different signals to the actual compounds present in the mixture. Although there is considerable potential for this type of spectrum, the acquisition schemes are only practical for relatively concentrated samples. A better solution is to use hybrid sequences, which introduce diffusion weighting into the 2D NMR spectrum without elongation of the acquisition time.⁴³,⁸⁰ Another way to overcome this problem is to use different nuclei whose chemical shifts extend over a larger range. An example was recently provided by the research group of Morris, who demonstrated that a mixture of silicon compounds can indeed be identified and assigned by performing a ²⁹Si DOSY experiment.¹¹⁷ There was clear resolution of the four principle species in the system, namely, the monomeric silicate 103, the cyclic trimer 105, the prismatic hexamer 107, and the cubic octamer 108 (Figure 26). It also seems that the dimer 104 and the cyclic tetramer 106 are also resolved. A good resolution of compounds was achieved in this study, but it should be noted that it was performed on a concentrated sample using enriched silicates (99.35% ²⁹SiO₂) and required several hours.

These recent examples, which were obtained by using conventional instruments, demonstrate that the current technology is already suitable for identifying relatively subtle differences in molecular weights as a means to virtually separate different compounds spectroscopically.

5.2. Ligand Screening by Diffusion and Affinity NMR Spectroscopy

A number of NMR parameters that change upon complex formation, such as chemical shift, transferred nuclear Overhauser effect (NOE), relaxation, and diffusion, were suggested as a screening method.¹¹⁸ The most widely used NMR screening method is the structure/activity relationships (SARs) introduced by Fesik and co-workers.¹¹⁹ Diffusion coefficients can be used to screen for the interaction between small molecules and specific receptors, thus separating the spectra of different compounds without really physically separating the compounds in the mixture. As already demonstrated in Section 4.1.1, one can translate the fast change in the diffusion coefficient into an association constant. The calculation of the association constant requires...
quantitative determination of the diffusion coefficients, and hence requires the full characterization of the signal decay as a function of the diffusion weighting. However, for screening purposes, where the indication of binding or relative binding is the required information, one can use only a few gradient points to screen for the possible binding of a certain ligand to a given receptor.\cite{120a,b}

An approach where only one gradient point is measured was provided by Shapiro and co-workers and is depicted in Figure 27.\cite{120b} These researchers first measured the 1D $^1$H spectrum of the mixture of the eight potential ligands (109–116) and hydroquinine 9-phenanthryl ether (117) as the receptor (Figure 27a). They then found the experimental PFG conditions (gradient strength and duration) needed to eliminate the spectrum of the mixture of the eight tentative ligands in the absence of 117 (Figure 27b). They then repeated the same PFG 1D $^1$H NMR spectrum after addition of 117 to the mixture and indeed observed, in addition to the signals of 117, signals originating from compounds 109 and 110 that interact with the receptor molecule 117 (Figure 27c). Only the compounds which interact with the receptor experience a decrease in their diffusion coefficients and, hence, their signals reappear under the previously selected PFG conditions.

This simple method for screening potential ligands and receptors has the following advantages: The measured parameter, that is, the diffusion coefficient, is intuitively related to the followed phenomenon; the expected decrease in the diffusion coefficient for the compounds that interact with the receptor is indeed observed. This is in contrast to other (non-NMR) parameters and NMR chemical shifts and relaxation times, where the sign of the changes can be both positive or negative. Additionally, this quantitative result can be obtained in a matter of a few minutes from a mixture of several ligands. An important advantage of screening by diffusion NMR spectroscopy is the fact that the method is applicable to all receptors, even to systems of unknown ligands and receptors. This is in clear contrast to the main competing NMR approach for ligand screening, namely, SAR-NMR spectroscopy,\cite{118–119} which is based on monitoring the changes in the $^{15}$N/$^1$H hetreonuclear single quantum coherence (HSQC) of a uniformly $^{15}$N-labeled receptor after the addition of a mixture of potential ligands. This method is, therefore, only applicable to known receptors for which a large quantity (about 200 mg) of labeled compounds is available. The receptor should have a relatively high solubility, with a molecular weight not more than about 20000–30000 Da.\cite{121} Diffusion NMR spectroscopy, on the other hand, is a nonspecific method and, as such, can be used for the screening of possible ligands for possible receptors with practically no limitation in the size of the receptor. This technique can, in principle, also be applied in combination with other NMR sequences and using all NMR-active nuclei. As in all NMR methods, screening by diffusion NMR spectroscopy is less prone to complications arising from impurities as compared with non-NMR methods. In addition, it should be noted that the larger the difference in the size of the screened ligands and receptors, the higher the sensitivity of the method and, in fact, it may even be easier to monitor ligand association to larger receptors which are NMR silent.

The major drawback of the simple approach for ligand screening by diffusion NMR spectroscopy, as outlined in Figure 27, is the fact that both the signals of the ligands interacting with the receptor and the receptor signals are all observed in the final spectrum.\cite{120a,b} This implies that there is a good chance for signal overlap that will preclude the identification of the interacting ligand(s). However, the
identification of interacting ligands may be even simpler in cases where relatively large receptors, which have a short \( T_2 \) value that renders them NMR silent under the conditions used, are screened by this diffusion NMR screening method.\(^{[121]}\) In such systems only the signals of the interacting ligand(s) will appear in the final spectrum.

Several approaches to increase the assignment capability of the diffusion NMR screening method were proposed to identify the interacting ligand(s). Fesik and co-workers suggested using diffusion editing to simplify the spectrum of the interacting ligand by eliminating the signals of the receptor, thus leaving only the signals of the interacting ligands after few spectra subtractions.\(^{[122]}\) They demonstrated this approach for ligands of the FK506 binding protein and the catalytic domain of stromelysin. For the stromelysin system, the ligand 118 and an additional eight small molecules 119–126 that were not expected to interact with FK506 were examined (Figure 28). First, they measured the \(^1\)H NMR spectra at low gradient strengths of compounds 118–126 in the absence (Figure 28a) and presence of stromelysin. The signal of the protein was then eliminated by subtracting the spectrum of the mixture of the ligands in the presence of stromelysin, obtained at high gradient strengths (data not shown), from the spectrum of the same mixture obtained at low gradient strengths (Figure 28b). Then by subtracting the spectra shown in Figure 28a and Figure 28b, they could identify the peaks of the interacting ligand, namely, compound 118 as shown in Figure 28c. This approach is likely to be operational only in cases in which there are no dramatic changes in the chemical shifts of the compounds involved in the complexation, and it is likely to be more efficient for systems in which the ligands and the receptors differ greatly in size. In fact, Shapiro and co-workers reported that the Fesik approach did not work well in the screening of a mixture of ten different tetrapeptides (two ligands and eight nonbonding tetrapeptides) for their affinity with respect to vancomycin.\(^{[123a]}\)

They suggested another approach to identify the interacting ligands, namely, to couple the diffusion weighting with a 2D TOCSY sequence (TOCSY = total correlation spectroscopy) which they named DECODES (diffusion encoded spectroscopy).\(^{[123b]}\) They showed by comparing the DECODES and TOCSY spectra of the series of ten tetrapeptides in the presence of vancomycin that only signals from amino acids D, F, S, and A remain in the spectrum, thus indicating that only the two peptides DDFA and DDFS bind to vancomycin (see Figure 29). This system, in which there is relatively weak binding and in which the different ligands are of very similar size and not always very different from that of the small receptor of the vancomycin, clearly demonstrates the potential of this simple approach.\(^{[123b]}\)

Recently, such an approach was used to decode the binding affinity to a DNA dodecamer.\(^{[123c]}\)

In fact, few DOSY spectra can, in principle, be used to screen for potential ligands for known or unknown receptors.\(^{[124]}\) All that has to be done is obtain the DOSY spectra of a mixture of the potential ligands in the absence and presence of the alleged receptor—it must, however, be ensured that the addition of the receptor has no effect on the viscosity of the
sample. It should then be possible to identify the interacting ligands by comparing the change in the DOSY spectra. In this approach, the signal separation should be significantly increased since the information is now spread in two dimensions and one has to compare the two 2D spectra of the DOSY experiment. If signal overlap does occur, one can resort to any of the 3D DOSY sequences to try to alleviate the signal overlap at the cost of a longer acquisition time.

6. Summary and Outlook

High-resolution diffusion NMR spectroscopy is a very simple, flexible, and accurate method to obtain diffusion coefficients in solution. With this method it is possible to analyze ensembles of signals simultaneously by using standard NMR spectroscopic technology, thus allowing the addition of the diffusion coefficient into the set of parameters used to characterize systems in solution. If signal overlap does occur, one can resort to any of the 3D DOSY sequences to try to alleviate the signal overlap at the cost of a longer acquisition time.

Figure 29. a) TOCSY NMR spectrum for the tetrapeptide mixture containing DDFA, YPFV, GLGG, GPRP, RGDF, GRGD, RCFF, KDEL, DASV, and DDFS in the presence of vancomycin. b) The corresponding DECODES spectrum highlighting the amino acids that remain: D, F, S, and A. Adapted with permission from Ref. [123b].

The main problem of diffusion NMR spectroscopy, like all NMR spectroscopic techniques in general, is its relative low sensitivity, which requires relatively concentrated samples and a long acquisition time. In addition, diffusion weighting means that a filtering out of the signal occurs. Moreover, in conventional DOSY sequences 1D and 2D NMR sequences are transformed into 2D and 3D NMR experiments, respec-
Diffusion NMR Spectroscopy

tively. This implies that relatively long acquisition times are required, but this will probably be partially alleviated in the future by using gradient systems on high-field magnets (more than 600 MHz) in combination with cryoprobes. This limitation is not very severe, however, and with current technology the PGSE or the STE diffusion experiments can be performed on host–guest or supramolecular systems having molecular weights of a few kilodaltons and a reasonable lineshape (that is, \( \Delta \nu_0 \approx 10 \text{ Hz} \)) within one hour on a conventional 400 or 500 MHz spectrometer when the concentration is in the range of about 5 mM.

Two additional areas of application of diffusion NMR spectroscopy, not dealt within the present Review because of space limitations, that deserve mentioning are: biochemistry and protein research,\cite{124,126} as well as the newly emerging field of semisolid samples, where diffusion NMR spectroscopy is used in combination with magic angle spinning (MAS).\cite{125} Diffusion measurements have been used in the context of biochemistry and protein research to study self-aggregation, the association of different ligands and DNA, as well as protein folding.\cite{124,126} However, a very exciting recent development is the use of diffusion NMR spectroscopy to study protein folding in real time.\cite{127} Buevich and Baum used the lineshape (that is, \( D \approx \Delta )\) of the diffusion time depends on the shape of the pulse gradients used, as expected from Equation (9). For example, for the PGSE or the STE diffusion sequence with sine-shape gradient pulses, the effective diffusion time is \( D \approx \Delta )\). When \( D \approx \Delta \) the diffusion time is \( D \approx \Delta \).

The other field which is currently emerging is the measurement of diffusion by MAS probes on semisolid beads.\cite{125} These probes, which only recently became available, have been used to distinguish between trapped or coerently bonded small molecules in swollen Wang resin. The combination of the bipolar LED and CPMG sequences (CPMG = Carr–Purcell–Meiboom–Gill) is useful for studying the interaction of different molecules with resins—an area vital to combinatorial chemistry.\cite{125a,b}

With the above applications already published in the literature and in view of the rapid advancement of instruments and software, which will make diffusion NMR spectroscopy more accurate and efficient, it is possible to speculate that diffusion NMR spectroscopy will, in the near future, become a popular tool in the hands of chemists interested in molecular interactions in solution. The triumph of diffusion NMR spectroscopy will be when it becomes as commonplace as other NMR methods. Our aim in this Review was to convince organic, inorganic, organometallic and supramolecular chemists to add diffusion NMR spectroscopy to their arsenal of analytical tools used to study molecular interactions in solution.

We thank the Israel Science Foundation administered by the Israel Academy of Science and Humanities, Jerusalem, Israel, for supporting the early stage of this research. We also wish to thank Professors S. E. Biali, V. Böhmer, R. Ungaro, and D. N. Reinholdt for collaborative efforts and Professor S. E. Biali for reading and commenting on the manuscript.

Received: October 23, 2003


\[5\] a) O. E. Stejskal, J. E. Tanner, J. Chem. Phys. 1965, 42, 288–292; b) it should be noted that the diffusion time depends on the shape of the pulse gradients used, as expected from Equation (9). For example, for the PGSE or the STE diffusion sequence with sine-shape gradient pulses, the effective diffusion time is \( D \approx \Delta \). When \( D \approx \Delta \) the diffusion time is \( D \approx \Delta \).


For example, fluorescence was used to calculate the association constant of 5,10,15,20 tetrakis(4-carboxyphenyl) porphyrin with β-CD. The discrepancy was not only in the values obtained but also in the sign of the changes in the fluorescence observed upon complex formation: in one report, S. S. Zhao, J. H. T. Luong, _J. Chem. Soc. Chem. Commun._ 1994, 2307–2308, an increase in the fluorescence is reported, while in another, F. Venema, A. E. Rowan, R. Nolte, _J. Am. Chem. Soc._ 1996, 118, 257–258, a decrease in fluorescence was reported.


Reviews

Y. Cohen et al.


