

DOSY NMR EXPERIMENTS AT VARIABLE TEMPERATURE. A THOROUGH ANALYSIS OF OPTIMAL CONDITIONS FOR RELIABLE RESULTS

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ABSTRACT (up to 250 words).

Multinuclear (¹H, ³¹P, and ¹⁹F) DOSY technique has been used under variable temperature conditions, in order to establish the optimal conditions for obtaining reliable data at variable temperature, while keeping experimental settings as simple as possible (*e.g.*, using standard NMR tubes). For this goal we have used the methodology developed in 2003 by Martínez-Viviente and Pregosin, but using more modern pulse sequences

specially designed to avoid the effects of convection upon DOSY experiments. We have written new pulse sequences (modifying existing standard ones) in order to use proton/heteronuclear decoupling (in this last case, with the possibility also of adiabatic decoupling). We have performed extensive measurements, covering all possible variables: type of instrument/probe, solvent volatility, calibration of the instrument gradients, spinning/non-spinning of the sample, proton decoupling, heteronuclear decoupling (standard or adiabatic), and so on...

The ensemble of all these measurements has allowed us to design a protocol for simple measurement of DOSY at variable temperature in a standard NMR lab, without any need of special instruments/probes or glassware.

1. Introduction

Diffusion ordered spectroscopy (DOSY)¹⁻⁴ obtained through pulsed field gradient (PFG) NMR experiments⁵⁻⁶ has become a multi-purpose tool in the chemical laboratory due to its ability to gain insight of the inner behaviour of solution systems through the measurement of diffusion coefficients (D) and their correlation with properties such as molecule size, weight, shape, etc. or the relation with the interaction of molecules with other molecules (*e.g.* solvent).⁷⁻¹⁰ The application of heteronuclear magnetic resonance techniques to DOSY⁹⁻¹¹ has enormously increased the scope and reach of the technique. For instance, it allows the acquisition of diffusion data from mixtures with highly overlapped proton NMR spectra or of species that lack proton NMR signals.

The widespread use of gradient-equipped NMR spectrometers and advancements in powerful DOSY pulse sequences have significantly increased the number of publications reporting diffusion data. This trend established DOSY as a standard chemistry and pharmaceutical research tool.^{12,13} Consequently, ensuring reproducibility in DOSY experiments is crucial for comparing data obtained from different laboratories.^{3,6,14} Moreover, developing more elaborate measuring techniques or more sophisticated pulse sequences continues to be an active area of research.¹⁵⁻¹⁸

A less exploited field in DOSY NMR are the studies at variable temperature,¹⁹⁻²⁰ due mainly to the problems that arise from convection in the sample,²¹⁻²³ with potential distortion and artifacts in the spectra, which make the results unreliable. In spite of this, DOSY experiments at variable temperature have been key in studying ion-pairing systems in organometallic chemistry, especially when dealing with compounds unstable at room temperature.¹⁹

Thermal convection in the NMR sample is a key factor contributing to inaccurate DOSY data on modern high-resolution NMR spectrometers. It becomes significant when the temperature gradient along the length of the NMR tube exceeds a critical threshold. Even small horizontal temperature gradients within the sample solution tube can cause convection.²³ When temperature is not uniform throughout the sample, warmer regions may lower the solvent's viscosity, altering diffusion rates and leading to inaccurate measurements. Thermal convection is more pronounced in low-viscosity solvents, larger

sample volumes, and when the sample temperature deviates, either higher or lower, from the probe's ambient temperature.

In 2003 in what has become a classical paper in the field of variable temperature DOSY, Martínez-Viviente and Pregosin¹⁹ established how to check if convection affects your measurements and to avoid its effects.²⁵ The procedure involved the use of the standard Stejskal-Tanner pulse sequence and the Stimulated-Echo pulse sequence, derived from it through splitting of the π pulse into two $\pi/2$ pulses (*zgpgse* and *stegp1s* respectively in the Bruker pulse library).²⁶⁻²⁷ Convection effects were minimized through the use of a coaxial insert inside a 5 mm normal NMR tube. Good behaviour was checked by measuring values of diffusion coefficient (D) with different diffusion delays (Δ , *d20* parameter in Bruker nomenclature), as convection effects show up as a dependence of the diffusion coefficient value, D , on the value of the diffusion delay, Δ . Best results were obtained for a coaxial insert of 1.96 mm inner diameter, inserted in a 5 mm normal NMR tube, with just air between the tube and the insert.

This work established that the measuring of D at variable temperatures was possible, if the adequate experimental precautions were taken and the non-dependence on the Δ values was checked.

The main drawback of this procedure was the reduced sensitivity due to the use of the coaxial inserts, which can also lead to troubles in locking and shimming the instrument, and obviously that inserts of the adequate size are not always available in a standard NMR laboratory. This in combination with the more elaborate pulse sequences for suppression of convection artifacts, that appeared in the literature some years ago,²⁸⁻²⁹ led us to extend the original methodology to variable temperature DOSY in 5 mm normal NMR tubes.

Herein, we describe the results of our studies on the influence of several factors affecting variable temperature DOSY results, using 5 mm normal NMR tubes and the Double Stimulated Echo (DSTE) pulse sequences. The study addresses a broad range of variables, including instrument and probe type, temperature higher and lower than room temperature, sample spinning *versus* non-spinning, proton *versus* heteronuclear DOSY, heteronuclear DOSY *versus* heteronuclear with proton decoupling DOSY, proton DOSY *versus* proton with heteronuclear decoupling DOSY (writing the adequate pulse sequences and checking non-adiabatic *versus* adiabatic decoupling), the choice of Double

Stimulated Echo pulse sequence, and data representation approach (1D *versus* pseudo-2D).

The ensemble of results obtained has allowed us to elaborate a straightforward protocol, for measuring DOSY at variable temperature using standard NMR instruments and tubes, ensuring the retrieval of accurate diffusion data.

2. Experimental

2.1. NMR instrumentation

^1H , ^{19}F and ^{31}P NMR DOSY experiments spectra were carried out on a Bruker AvanceNeo spectrometer, 500 MHz, equipped with a 5-mm TBFO triple channel observe (^1H - ^{19}F -broadband) z-axis gradient probe (and a BCUII unit for low temperature measurements); a Bruker AvanceIII spectrometer, 500 MHz, equipped with a 5-mm QNP z-axis gradient cryoprobe and a Bruker AvanceI spectrometer, 400 MHz, equipped with a 5-mm BBFO observe z-axis gradient probe. Both 500 MHz instruments were equipped with vibration damping columns in order to suppress mechanical vibrations of the sample.

2.2. Samples

Three samples were prepared:

- 1) A 10% v/v sample of 4-fluorophenethyl alcohol (Aldrich 97%) in toluene-d₈ (Deutero 99.5%)
- 2) A 10% v/v sample of diethyl 2-phenylethylphosphonate (Aldrich 98%) in toluene-d₈ (Deutero 99.5%).
- 3) A sample of pure deuterium oxide (Aldrich 99.9%) with nothing added.

Toluene-d₈ was chosen as solvent for the samples studied due to its great range of temperatures (melting point -85 °C, boiling point 109 °C), which allows us to use a single sample for all studies at different temperatures. In this way, when comparing studies made

at temperature higher and lower than room temperature, we may discard any effect due to different properties of the solvents used (viscosity, density, polarity, etc.). Anyway, as we are aware that a lot of samples may need other solvents, we have started other studies on real-life host-guest systems, involving solvents such as acetone-d₆ and dms_o-d₆.³⁰

All three samples were prepared in 5 mm normal NMR tubes. An exact sample height of 7 cm was kept in all tubes,³¹ to ensure that the liquid/air interface was far away from the probe coils and minimize convection effects. All tubes were sealed to avoid evaporation. For sample 1), in addition to the sample in a normal 5 mm NMR tube, another sample was prepared filling an insert of 1.96 mm inner diameter and put into a 5 mm NMR tube with air between the two tubes. Again a 7 cm sample height was kept and both tubes were sealed. This sample was prepared in order to replicate the conditions used by Martínez-Viviente and Pregosin.¹⁹ The inset is adjusted concentrically into a standard NMR tube through a plastic o-ring which fits the insert exactly into the center of the standard tube. This fitting, in combination with the vibration damping columns installed in the 500 MHz instruments, eliminates any possibility of mechanical vibration of the insert into the NMR tube. No other sample setting like the use of capillary tubes were tried, as they resulted in much lower sensitivity and its use was strongly discouraged by Martínez-Viviente and Pregosin.¹⁹

Compounds 1) and 2) were chosen for their high stability and the presence of high sensitivity NMR active heteronuclei, ³¹P and ¹⁹F. Stability of samples was checked through their ¹H, ¹⁹F{¹H} and ³¹P{¹H} spectra at 298 K, the spectra remained unchanged alongside all the study.

Figure 1 here

2.3. DOSY experiments.

All DOSY ^1H , ^{31}P and ^{19}F experiments were obtained using a DSTE (double stimulated echo) and LED (longitudinal Eddy current delay) and 3 spoil gradients pulse sequence,²⁸⁻²⁹ in both versions, with and without bipolar gradient pulses (*dstegp3s* and *dstebpgp3s* in the standard Bruker pulse sequence library). Without and with decoupling of proton/heteronucleus (see supplementary material). Without and with (20 Hz) spinning of the sample. For gradient calibration experiments on the deuterium oxide sample, the standard LED bipolar gradient and 2 spoil gradients pulse sequence was used (*ledbpgp2s* in the standard Bruker pulse sequence library). The gradient shape was smoothed square (*SMSQ10.100* in the standard Bruker gradient library), acquiring 32 gradient levels. A gradient recovery delay of 0.5 ms and an eddy current delay of 5 ms were used.

Decoupling for proton, when necessary, has been done with a *waltz16* sequence. Decoupling for heteronucleus, when necessary, has been done either with a standard *garp* sequence or with an adiabatic *bi_p5m4sp_4sp2* sequence involving two Smoothed Chirp shapes (*Crp120,0.75,30.4_500p* and *Crp120,1.5,30.4_500p*)

The diffusion time Δ was set at different values, going from 100 to 1000 ms. After setting the Δ value, the gradient length (δ , *p30* parameter in Bruker nomenclature) was adjusted in such a way that the measured signal decreased around 90-95% in intensity when going from low gradient strength (2%) to high gradient strength (95%). Between 8 and 32 transients were acquired depending on the instrument used, 8 when using a cryoprobe-equipped instrument, 32 when using a standard probe-equipped instrument. The gradient recovery delay (*d16* parameter in Bruker nomenclature) used was 200 μs for all different instruments and probes. This is the default value set in *edprosol* (Bruker nomenclature for the probe pulses and parameters) after the probe installation. In order to ensure that the value was adequate, some preliminary tests were run with a bigger value, 400 μs . As

no effect was observed, obtaining the same well-behaved DOSY with the same measured D value in both cases, the default value of 200 μs was used in all experiments. Previous studies have used even lower values (100 μs) also without any problem.³²

Experiments were processed with the standard Bruker DOSY (2D fitting) and T_1/T_2 (1D fitting, peak area integration) processing modes (both of them included in the Bruker TopSpin software package, version 3 and 4), and D values were calculated. For a ^1H DOSY experiment, all relevant signals were integrated for D calculation, and an average value was taken, those signals showing peak enlargement (evidence of chemical exchange) were ignored. The decay was fitted to the equation:

$$I = I_0 e^{-D Q}$$

Where I and I_0 are the signal intensity, D is the diffusion coefficient and:

$$Q = \gamma^2 G^2 \delta^2 (\Delta - \delta/3)$$

Where γ is the gyromagnetic ratio of the nucleus involved, G is the gradient strength, δ is the gradient length and Δ is the diffusion time.

In a typical well-behaved experiment, where convection is kept under control, the standard deviation obtained with 1D fitting was always lower than $1 \cdot 10^{-2} \text{ m}^2 \text{ s}^{-1}$. In experiments where convection is significant the standard deviation may reach between $5 \cdot 10^{-2} \text{ m}^2 \text{ s}^{-1}$ and $10 \cdot 10^{-2} \text{ m}^2 \text{ s}^{-1}$ (see Table S2 in the supplementary material for more detailed information about errors).

The temperatures used in different experiments were 253 K, 298 K and 333 K. In all cases the temperature was calibrated, either with a 4% methanol in methanol- d_4 sample (for 253 K and 298 K) or with 80% ethylene glycol in $\text{dms}\text{-}d_6$ sample (for 333 K). The nitrogen gas flow for each temperature was adjusted increasing its value until complete temperature stability along time was reached (no drift at all of the adjusted temperature

value, not even 0.1 °C for 24 hours or longer). Typical values used were 1000 l/h for 253 K, 535 l/h for 298 K and 600/670 (depending on the instrument used) l/h for 333 K. In all cases a ceramic spinner was used, to prevent the sample being partially lifted by the high gas flow, and to ensure an stable rotation of the sample.

The use of a ceramic spinner, prevents the use of an autosampler to introduce/extract samples in/of the magnet. As these spinners are much heavier than the standard ones (henceforth their utility for this type of experiments), a whole recalibration of the autosampler would be needed. Therefore, no automation software modules like ICON-NMR have been used. The samples were introduced in the magnet manually and then the whole series of experiments for that sample were acquired with an automatic acquisition routine (*multizg* in Bruker language).

Even if 253 K may seem a not low enough temperature, keeping in mind that a lot of compounds require lower temperatures.¹⁹ We chose this limit, because it allowed us to work in an environment where the low temperature was provided by a BCUII cooling unit. These settings allowed us to work with a very stable low temperature over an indefinite period of time, which allowed us to perform very long measurements on one sample (like 20 DOSY experiments in a row, which takes between one-two days). Going for lower temperatures requires cooling with a dewar of liquid nitrogen and an exchanger unit. At the high gas flows required for these experiments, these settings would only allow us to run experiments for around 5-7 hours, having to stop the measurements in order to refill the liquid nitrogen dewar several times. This technical constraint decided us to settle for the compromise temperature of 253 K, which even if is not low enough for a lot of molecules, it has allowed us to stablish the optimal conditions for acquiring DOSY

experiments at low temperature. With this knowledge, now we are proceeding to study real-life systems³⁰ in other solvents and at lower temperatures. All the knowledge baggage acquired will allow us to study the systems without the need of acquiring so many experiments.

Some series of experiments were acquired by duplicate, a first series increasing the value of Δ and a second series decreasing the value of Δ , in order to discard some possible effect on the instrument electronics of increasing/decreasing Δ values. In all cases, calculated values of D were the same within the experimental error.

3. Results and discussion

Diffusion coefficient values, D , were calculated for all DOSY experiments. A typical DOSY experiment, processed both with the DOSY and the T₁/T₂ software packages is shown in Fig. 2

Figure 2 here

The values of D for all experiments were analyzed, looking for dependency or non-dependency on the diffusion time Δ . This allowed us to establish the effect of all different aspects involved and its possible effect over the reliability of calculated D values.

3.1. Gradient calibration

This should be always the first step if you want to obtain accurate D values from a DOSY experiment. We performed this calibration measuring ¹H DOSY at 298 K for sample 3) (pure deuterium oxide 99.9% with nothing added) in three different instruments. The theoretical value of D at 298 K for such a sample is established as $1.9 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$.³³ Deviations from this value are caused by small deviations of the electronics of the instrument from the theoretical values of gradient power and duration. This calibration

should be performed for each instrument/probe ensemble if we want to measure absolute D values.

Table 1 here

As all the analyses reported in this article are based on the variation of D values and not on its absolute values, the values are given as obtained from calculation, without any additional correction.

3.2. Sample spinning versus non-spinning

It has been established in the literature^{32,35} that sample spinning helps to minimize convection artifacts on DOSY experiments. As constant rotation of the sample may be tricky to obtain in cryoprobes (requiring fine adjustment of the sample height regulator of the cryoprobe by a skilled operator), we have performed DOSY experiments with and without rotation of the sample, to observe any possible effects. This is shown in Fig. 3

Figure 3 here

As we can see in the figure, all experiments done with spinning of the sample present non-dependence of D values on Δ values. When we stop spinning the sample, problems start to appear. In case b) there is a small dependence for the sequence with bipolar gradients and a huge dependence on the sequence without bipolar gradients. While in case c) both sequences show a huge dependence. It is worth pointing that case a) manages to avoid dependence even for non-spinning experiments. So, even if spinning the sample is highly recommended when performing variable temperature DOSY,³⁶ if by any instrumental mishap we are unable to, we can still try to run the experiments but more check and control will be needed.

3.3. 2D versus 1D processing

Typically, a DOSY experiment may be processed in two ways.

2D processing (as performed by the Topspin DOSY module) analyzes all acquired spectra together and gives a pseudo-2D display, where D appears calculated as a horizontal line in the F1 dimension on a logarithmic scale. The only indicator of the quality of the data/fit is the projection extracted from the data in the F1 dimension. And this is not a reliable quality indicator as shown further, data with high scattering may give a fairly good-looking F1 projection.

1D processing (as performed by the Topspin T₁/T₂ module) analyzes each acquired spectrum individually, taking the intensity/integration of a given signal and plotting it against the gradient intensity for each spectrum. In this case the plot/fit gives a very clear idea of the quality of your data.

Figure 4 here

In Fig. 4 we can see for a typical DOSY experiment, the comparison of both 2D and 1D processing. While 2D processing (upper) shows a sharp projection in the F1 dimension, apparently suggesting good data quality, we must be aware that this sharp projection in the F1 dimension is no indicator at all of the data quality and the suggestion is wrong. This projection is the result of a mathematical algorithm and even data with a lot of scattering (as we will see further in the 1D processing) may give a sharp F1 projection if the scattering is distributed evenly along the mathematical fit.³⁷ 1D processing (lower) shows clearly a huge amount of scattering in the fit, indicating low data quality (probably through convection effects due mainly to the non-spinning of the sample). Even if a D value is obtained for this experiment, it will have to be taken with a pinch of salt. Looking at the scattering, we may see that is symmetrically distributed along the fit, with the same approximate number of points lower than the fit and points higher than the fit. That is the

reason that the algorithm for 2D processing gives a sharp F1 projection, resulting in a false impression of good data quality.

For this reason, all D values presented in this article, have been obtained from 1D processing which allows us a much better view of data quality.³⁷

From the two possibilities offered in 1D processing, peak intensity or peak integration, we have always used the second one. In our experience integration of the peak gives always a much better plot/fit with less scattering than intensity of the peak.

3.4. Proton versus heteronuclear DOSY

When a sample contains NMR active nuclei of high sensitivity (like ^{19}F or ^{31}P), it may be beneficial to acquire heteronuclear DOSY instead of proton DOSY. The reduced number of signals/multiplets when acquiring heteronuclear DOSY results in higher quality data with less noise.

Figure 5 here

As seen in Fig. 5 the data fit for ^{19}F DOSY has less scattering than the one obtained from ^1H DOSY. Even if both experiments give almost the same value of D ($2.50 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ for ^1H versus $2.58 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ for ^{19}F), ^{19}F DOSY is preferable as there is only one signal of interest, while in ^1H DOSY one has to calculate D for all relevant signals (provided there is no signal overlapping) and calculate an average value. The greater scattering in the ^1H experiment is caused by the signals in the spectrum being less intense (even if they still had a very good signal/noise ratio) than in the ^{19}F experiment, due to great number of ^1H - ^1H couplings present in all multiplets. This increases the scattering present in the values obtained from integration (specially the zone concerning the satellites).

3.5. Proton decoupling

If the acquisition of an heteronuclear signal gives better plot/fit results than acquisition on proton signals, decoupling of proton in order to turn the heteronuclear signals into singlets should give even better data.

Figure 6 here

As shown in Fig. 6 the introduction of proton decoupling improves the plot quality, reducing scattering, without an increase of convection effects, as D values remain independent of Δ values in all cases.

3.6. Heteronuclear decoupling

Following the previous point, the introduction of heteronuclear decoupling (if high sensitivity, $I = \frac{1}{2}$, NMR active nuclei are present) should simplify the proton signals, and again allow for better plot/fit results when proton DOSY is performed.

But for heteronuclear decoupling there is a shortcoming present which does not appear in proton decoupling. Proton decoupling is achieved with power low enough such as to have no detectable influence in the sample temperature, and as consequence no detectable convection effects. In contrast, heteronuclear decoupling often demands higher power levels, which can lead to fluctuations of the sample temperature. These temperature variations may induce convection currents within the sample, distorting diffusion measurements and compromising the accuracy of ^1H DOSY data. Therefore, careful temperature control and optimized decoupling conditions are essential to mitigate these effects and ensure reliable diffusion measurements.

Figure 7 here

As seen in Fig. 7 a), when using proton with heteronuclear decoupling the dispersion of the D values obtained is bigger than when proton without heteronuclear decoupling is used. This indicates a bigger effect of convection, even if in this case it does not result in

D values increasing with Δ values. The effect appears to be particularly pronounced when sample-spinning is not used, as the lack of rotation can further exacerbate the convection-related artifacts (Fig. 3 b). In addition to this general trend, in a great deal of the experiments it is observed that the signal decay profile deviates from what would be expected in a well-behaved DOSY experiment. Presenting both an irregular decay profile and an increased data scattering.

Increasing of the thermostating air flux and spinning of the sample are not capable of solving this problem, as seen in Fig. 7 b) where it is clearly seen that each pulse goes together with an increase of the sample temperature.

3.7. Adiabatic heteronuclear decoupling versus non-adiabatic heteronuclear decoupling

Given the developments in the last years on adiabatic decoupling,³⁸⁻³⁹ we tried to re-analyze the use of heteronuclear decoupling for variable temperature DOSY using adiabatic sequences/shapes (either from the standard Bruker library or obtained from Bruker support). There was no relevant change in what we had previously observed. D values showed a bigger dispersion than the equivalent ones without heteronuclear decoupling and each pulse was accompanied by an oscillation of the temperature.

3.8. Temperature higher than room temperature versus temperature lower than room temperature

We have already presented some well-behaved examples of variable temperature DOSY at temperature higher than room temperature (333 K, see Figs. 3, 5 and 6), where good quality data are obtained, provided there is a good adjusting of the experimental conditions.

The same can be applied to DOSY at temperature lower than room temperature. With a good adjustment of the experimental conditions, good quality data can be obtained.

Figure 8 here

As seen in Fig. 8 a) very good quality plot/fit can be obtained at low temperature and with no dependence at all on Δ values, even without sample spinning (Fig. 8 b))

3.9. Normal 5 mm NMR tube versus insert

We have compared our working method (normal 5 mm NMR tubes) with the one described by Martínez-Viviente and Pregosin¹⁹ (an insert of 1.96 mm inner diameter and put into a normal 5 mm NMR tube with air between the two tubes). Both methods give the same results within the experimental error, with the same good quality D values.

Figure 9 here

As seen in Fig. 9, both plots are of very good quality and give the same value of D within the experimental error (as indicated in section 2.3, DOSY experiments in a typical well-behaved case, after 1D processing have a standard deviation lower than 1%).

Let us keep in mind that for this study we have used fairly concentrated samples. When dealing with real life samples, concentration may be lower and then sensitivity (directly related to sample diameter) may begin to be an important issue.

3.10. DSTE sequence versus DSTE sequence with bipolar gradients

Double Stimulated Echo (DSTE) pulse sequences, can be used without or with bipolar gradients (*dstegp3s* and *dstebpgp3s* in the Bruker pulse library). According to the literature,²⁸⁻²⁹ the sequence with bipolar gradients has a better tolerance towards convection caused artifacts, at the cost of a decreased signal-noise sensitivity. In this study we have not detected any difference between using one type of sequence or the other one. In both cases we have obtained very well-behaved data, with no dependence at all of D

values on Δ values (when using the right conditions, like sample spinning, avoiding heteronuclear decoupling, etc.).

The only difference we have observed between both types of pulse sequences is a tendency when using bipolar gradients to obtain slightly (around 3-5%) higher absolute values of D . This is a consistent trend, especially when working with low values of Δ (100 ms). We attributed this tendency to the presence of 180° pulses in the sequences with bipolar gradients

Figure 10 here

Figure 10 illustrates the pulse sequence diagrams used in this study. The sequence without bipolar gradients uses only 90° pulses, while the one with bipolar gradients uses a combination of 90° and 180° pulses. It is well known that 180° pulses are much more affected by imperfections than 90° pulses,^{28-29,40} so a calibration of the pulse with the sample we are going to work with would be needed.

Obviously having to calibrate pulses before any measurement would make the method cumbersome and the opposite of what we are looking for in this study, good reliable data with simple instrumental settings.

If we are not interested in absolute D values, but just in its variation, this pulse calibration will not be necessary and the method is still valid and simple.

If we are interested in absolute D values, again we can avoid having to calibrate pulses just selecting the adequate pulse sequence for gradient calibration. If we are going to use DSTE without bipolar gradients (*dstegp3s*) for variable T DOSY, we should use for gradient calibration at 298 K a Longitudinal Eddy Current Delay (LED) sequence without bipolar gradients (*ledgp2s* in the Bruker pulse sequence library). If we are going to use DSTE with bipolar gradients (*dstebpgp3s*) for variable T DOSY, we should use for

gradient calibration at 298 K a Longitudinal Eddy Current Delay (LED) sequence also with bipolar gradients (*ledbpgp2s* in the Bruker pulse sequence library). With this procedure, possible pulse imperfections will be included in the gradient calibration procedure, and no additional pulse calibration is needed.

3.11. Standard probes versus cryoprobes

It is well known that cryoprobes are more sensitive than standard probes to problems with sample spinning/thermostating temperature. So, one would expect to have more well-behaved data when using a standard probe, and to obtain less reliable data (specially without spinning the sample) when using a cryoprobe.

This is not the case in this study, we observe approximately the same behaviour in the data obtained from a standard probe and a cryoprobe. In both cases data is well-behaved, provided we take the precautions explained in reference 36.

Both probes show also more or less the same type of badly behaving data when sample spinning is not used.⁴¹

The only conclusion we can point to is that any instrument/probe system must be tested carefully as we cannot predict in advance which type of instrument/probe will give optimal data. All instruments/probes are capable of measuring good variable temperature DOSY, provided we do the adequate checks and take the adequate precautions (mainly measuring some previous DOSY experiments on some available standard sample, a good initial test would be measuring 2-3 quick DOSY experiments at different values of Δ , and check for dependence of D values on Δ values).

3.12. Type of solvent

Another factor to take into account will be the type of solvent. Different solvents with different viscosity/volatility, will behave differently towards variable temperature DOSY.

Low viscosity/high volatility solvents will be more prone to convection, specially at temperatures higher than room temperature. Each new solvent will have to be tested carefully, following the guidelines given in this article.

All this study has been done in deuterated toluene-d8 by the reasons stated in **2.2 Samples**. We have started a study of real-life systems,³⁰ in deuterated acetone-d6, and deuterated dmsd-d6. So far, using the presented protocols, we have been able to obtain well-behaved variable temperature DOSY data.

4. Conclusions

We have studied the different aspects related to convection and we have established that it is possible to measure variable temperature DOSY, in normal 5 mm NMR tubes and obtaining good quality results. Provided the next protocol is followed:

1. Prepare a sealed sample in a 5 mm NMR tube, 5-7 cm sample height to minimize convection.
2. If absolute D values are needed, it is necessary to calibrate the gradients of the instrument/probe used. This is done with a sample of deuterium oxide 99.9% at 298 K. Make sure to use the same type of pulse sequence (with or without bipolar gradients) that it will be used later. If absolute D values are not required, this step may be skipped.
3. Work always with sample spinning (if possible). Ensure good temperature/rotation stability with a ceramic spinner.
4. Start always working with the *dstegp3s* pulse sequence that will ensure optimal signal/noise. Use the *dstebpgp3s* pulse sequence only if the previous one does not provide good enough data/results.

5. If a high sensitivity, $I = \frac{1}{2}$, heteronucleus is present, acquire $X\{^1\text{H}\}$ DOSY. If not, acquire ^1H DOSY. Avoid always heteronuclear decoupling.
6. Acquire a minimum of 3 experiments with different and separated Δ values (*e.g.*, 200, 400 and 800 ms.).
7. Process the previously acquired experiments with 1D processing software (or similar) and check for non-dependence of D value on Δ value. Calculate an average D value.

Supplementary material

Acknowledgments

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25. A lot of times in the literature we find mentioned “the suppression of convection”. As Prof. Gareth Morris clearly states in ref. 24, the only thing we can do is minimize its effects so it does affect minimally our experiments, in order to obtain reliable data.
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37. We are taking a leaf out of Prof. Gareth Morris book. As he states in ref. 23, you should always mistrust “black box processing software” which does not let you see clearly the process involved in the calculation, and try to use software that allows you to see how the values are obtained.

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41. One possible reason because our normal probe and cryoprobe behave towards rotation/temperature in such a similar way, may be that our “normal” probe is a three channel TBFO probe (^1H - ^{19}F -BB). Having three channels/coils in the space usually occupied by two channels, may cause a different behaviour with respect to the thermostating/spinning gas.

Let us keep in mind that NMR instruments have a more “artisanal” component that the manufacturers would like to acknowledge sometimes. Even if is not related to the present work, it is worth mentioning the case of our 300 MHz instrument with two identical

probes, one just for spare purposes. Both identical probes (same part number) behave in a way completely different towards routine shimming. One probe gives very good routine results, in a short time, with standard shimming involving Z1, Z2, Z3, Z4 and Z5 shims. The other probe, in order to give the same good results, in the same time, needs to use a shimming routine with convection compensation and involving only Z1, Z2 and Z3 shims.

CAPTIONS FOR TABLES

Table 1. D values for a sample of pure deuterium oxide 99.9% with nothing added at 298 K. Experiments have been measured twice for different values of Δ , using the *ledbpgp*³⁴ pulse sequence and with sample spinning. Values were calculated with the T_1/T_2 module (1D processing). Observing no dependence of D values from Δ , an average was calculated and its deviation from the theoretical value of $1.9 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ calculated. Average standard deviations are given between parenthesis, (*e.g.*, 2.14 (0.23) means a D value of $2.34 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ with a SD of $0.23 \times 10^{-2} \text{ m}^2 \text{ s}^{-1}$). The standard deviation values are obtained from 1D processing with the T_1/T_2 module and an average for the different values is calculated.

CAPTIONS FOR FIGURES

Figure 1. Structures of the two molecules used in this study: 1) 4-fluorophenethyl alcohol, 2) diethyl 2-phenylethylphosphonate.

Figure 2. Typical ^{31}P NMR DOSY experiment for sample 2) (10% v/v diethyl 2-phenylethylphosphonate in toluene- d_8). The experiment was acquired at 333 K, with sample spinning, in an AvanceIII instrument equipped with a QNP cryoprobe, using the *dstegp3s* pulse sequence and a Δ value of 200 ms. A normal 5 mm NMR tube was used.

Figure 3. Graph of D values versus increasing Δ values. All experiments have been acquired at 333 K in an AvanceNeo 500 MHz instrument with a TBFO probe. a) ^1H DOSY on sample 1) (10% v/v 4-fluorophenethyl alcohol in toluene- d_8), b) $^1\text{H}\{^{19}\text{F}\}$ DOSY on sample 1) (10% v/v 4-fluorophenethyl alcohol in toluene- d_8), c) ^1H DOSY on sample 2) (10% v/v diethyl 2-phenylethylphosphonate in toluene- d_8). ■) *dstegp3s* sequence with sample spinning, ●) *dstegp3s* sequence without sample spinning, ▲) *dstebpgp3s* sequence with sample spinning, ◆) *dstebpgp3s* sequence without sample spinning. Normal 5 mm NMR tubes were used.

Figure 4. $^{31}\text{P}\{^1\text{H}\}$ NMR DOSY experiment for sample 2) (10% v/v diethyl 2-phenylethylphosphonate in toluene- d_8). The experiment was acquired at 333 K, without sample spinning, in an AvanceIII instrument equipped with a QNP cryoprobe, using the *ICIQdstebpgp3sdec* pulse sequence and a Δ value of 400 ms. A normal 5 mm NMR tube was used. 2D processing is shown in the upper part and 1D processing in the lower part, showing that even data with high scattering can give a sharp F1 projection in 2D processing, making it less reliable than 1D processing.

Figure 5. ^1H and ^{19}F NMR DOSY experiments for sample 1) (10% v/v sample of 4-fluorophenethyl alcohol in toluene- d_8). The experiments were acquired at 333 K, with

sample spinning, in an AvanceIII instrument equipped with a QNP cryoprobe, using the *dstepp3s* pulse sequence and a Δ value of 300 ms. Normal 5 mm NMR tubes were used.

Figure 6. a) and b) ^{19}F DOSY and $^{19}\text{F}\{^1\text{H}\}$ DOSY respectively on sample 1) (10% v/v 4-fluorophenethyl alcohol in toluene- d_8). The experiments were acquired at 333 K, with sample spinning, in an AvanceIII instrument equipped with a QNP cryoprobe, using the *dstepp3s* (^{19}F DOSY)/*ICIQdstepp3sQNP* ($^{19}\text{F}\{^1\text{H}\}$ DOSY) pulse sequence and a Δ value of 600 ms. c) Graph of D values versus increasing Δ values, ■) ^{19}F DOSY and *dstepp3s* sequence, ●) $^{19}\text{F}\{^1\text{H}\}$ DOSY and *ICIQdstepp3sdecQNP* sequence, ▲) ^{19}F DOSY and *dstepp3s* sequence, ◆) $^{19}\text{F}\{^1\text{H}\}$ DOSY and *ICIQdstepp3sdecQNP* sequence. Normal 5 mm NMR tubes were used.

Figure 7. a) Graph of D values versus increasing Δ values for ^1H DOSY and $^1\text{H}\{^{19}\text{F}\}$ DOSY on sample 1) (10% v/v 4-fluorophenethyl alcohol in toluene- d_8). The experiments were acquired at 253 K, with sample spinning, in an AvanceNeo instrument equipped with a TBFO probe, using the *dstepp3s* and *ICIQdstepp3sdec* pulse sequences. ■) ^1H DOSY, ●) $^1\text{H}\{^{19}\text{F}\}$ DOSY. Normal 5 mm NMR tubes were used. b) Oscillation on the sample temperature when heteronuclear decoupling is applied. The green line is the requested temperature and the white line is the measured temperature, which oscillates with each decoupling pulse (relaxation delay has been set to 120 s. for clarity of view).

Figure 8. a) $^{31}\text{P}\{^1\text{H}\}$ DOSY on sample 2) (10% v/v diethyl 2-phenylethylphosphonate in toluene- d_8). The experiments were acquired at 253 K, with sample spinning, in an AvanceNeo instrument equipped with a TBFO probe, using the *ICIQdstepp3sdec* pulse sequence and a Δ value of 100 ms. b) Graph of D values versus increasing Δ values, for $^{31}\text{P}\{^1\text{H}\}$ DOSY at 253 K, ■) *ICIQdstepp3sdec* sequence with sample spinning, ●) *ICIQdstepp3sdec* sequence without sample spinning, ▲) *ICIQdstepp3sdec* sequence

with sample spinning, ♦) *ICIQdstebpgp3sdec* sequence without sample spinning. Normal 5 mm NMR tubes were used.

Figure 9. $^{19}\text{F}\{^1\text{H}\}$ DOSY on sample 1) (10% v/v 4-fluorophenethyl alcohol in toluene-d8). The experiments were acquired at 333 K, with sample spinning, in an AvanceIII instrument equipped with a QNP cryoprobe, using the *ICIQdstegp3sdecQNP* pulse sequence and a Δ value of 100 ms. The upper plot shows the result for a normal 5 mm NMR tube and the lower plot shows the results for an insert of 1.96 mm inner diameter and put into a 5 mm NMR tube with air between the two tubes.

Figure 10. Pulse sequences for DSTE DOSY without (upper, *dstegp3s*) and with (lower, *dstebpgp3s*) bipolar gradients.