Video Article

Spin Saturation Transfer Difference NMR (SSTD NMR): A New Tool to Obtain Kinetic Parameters of Chemical Exchange Processes

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Abstract

This detailed protocol describes the new Spin Saturation Transfer Difference Nuclear Magnetic Resonance protocol (SSTD NMR), recently developed in our group to study processes of mutual-site chemical exchange that are difficult to analyze by traditional methods. As the name suggests, this method combines the Spin Saturation Transfer method used for small molecules, with the Saturation Transfer Difference (STD) NMR method employed for the study of protein-ligand interactions, by measuring transient spin saturation transfer along increasing saturation times (build-up curves) in small organic and organometallic molecules undergoing chemical exchange.

Advantages of this method over existing ones are: there is no need to reach coalescence of the exchanging signals; the method can be applied as long as one signal of the exchanging sites is isolated; there is no need to measure T_1 or reach steady state saturation; rate constant values are measured directly, and T_1 values are obtained in the same experiment, using only one set of experiments.

To test the method, we have studied the dynamics of the hindered rotation of *N,N*-dimethylamides, for which much data is available for comparison. The thermodynamic parameters obtained using SSTD are very similar to the reported ones (spin-saturation transfer techniques and line-shape analysis). The method can be applied to more challenging substrates that cannot be studied by previous methods.

We envisage that the simple experimental set up and the wide applicability of the method to a great variety of substrates will make this a common technique amongst organic and organometallic chemists without extensive expertise in NMR.

Video Link

The video component of this article can be found at https://www.jove.com/video/54499/

Introduction

Chemical exchange commonly refers to any intermolecular or intramolecular process in which a nucleus moves from one environment to another in which its NMR parameters (chemical shift, scalar coupling, dipolar coupling, relaxation rate) differ. There are numerous examples of chemical exchange in organic and organometallic molecules (*e.g.,* rotational barriers in biaryls, ring flipping barriers and conformational equilibrium, nitrogen inversion, ligand binding, degenerate ligand exchange and tautomerization).[1-3] The chemical exchange rate is related to the thermodynamics of the barrier of the exchange process, and therefore its study is of crucial importance to understand molecular dynamics of these systems.

The classic sign of dynamic exchange in NMR is a dramatic change in the line-shape of the NMR signals as the temperature changes. At low temperatures, the process is slow and two distinct chemical shifts are observed. At high temperatures, the two signals merge into one signal, which is known as "coalescence". At intermediate temperatures, the signals become very broad. This sensitivity of the NMR spectrum to chemical exchange makes NMR a very powerful method to study the dynamics of molecules in solution. Two methods have been mainly employed in the study of dynamic processes in solution: line-shape analysis,[4-7] and spin saturation transfer experiments.[8-9] Besides, it is also worth mentioning the inversion transfer method[10] and the CIFIT program[11] for the direct extraction of rate constants, that are a relatively efficient approach for exchange measurements in simple systems. Although these methods give very good results in most cases, they, however, have a number of drawbacks. The main disadvantage of the line-shape analysis is the high temperatures needed to reach coalescence in some samples.[12] The main issues to consider when carrying out spin saturation transfer experiments are: the very long saturation times required to reach the steady state saturation transfer between the exchanging sites, and the need to determine the longitudinal relaxation time constant, T_1, which can be difficult if there is overlap of different signals in the region of study.[13]

As part of our investigations in organometallic mechanisms,[14-16] our group is studying the fluxional behavior of platinum-allene complexes in solution. This is a complex task that involves at least three different processes, one of them being the π-face exchange or rotation of the metal around one of the allene axis. We encountered that normal VT experiments and line-shape analysis techniques that have been employed before in similar systems,[17-19] were not suitable in our study, due to a very slow rotation in our platinum-allene complex that made the coalescence temperature of the signals of interest higher than the temperature of decomposition of the complex.

In order to overcome this limitation, we developed and recently reported a new NMR protocol (SSTD NMR) to study processes of mutual-site chemical exchange.[20] As the name suggests this method combines the Spin Saturation Transfer method used for small molecules, with the Saturation Transfer Difference NMR method employed for the study of protein-ligand interactions,[21-24] by measuring transient spin saturation transfer along increasing saturation times (build-up curves) in small molecules undergoing chemical exchange.

With this new method (SSTD NMR) we have shown that we can obtain the kinetic parameters of intramolecular chemical exchange in small organic and organometallic molecules with some additional advantages over traditional approaches: coalescence of the signals is not needed, so a more flexible temperature range can be used in the study; signal overlap does not interfere, although at least one of the exchanging resonances should be isolated; there is no need to measure T_1 or reach steady state saturation; rate constant values are measured directly and T_1 values are obtained in the same experiment, using only one set of experiments. Another remarkable advantage of the SSTD NMR methodology is that, in contrast to lineshape analysis, the determination of the kinetics rate constants is not limited by the increase in coalescence temperatures associated with high magnetic fields. Thus, our methodology is then very well appropriated for both low and high magnetic fields. This article is intended to help new users apply this new method to their own systems undergoing chemical exchange, and describes sample preparation, experimental set up, data acquisition, and an example of data processing and analysis in a simple organic molecule.

Protocol

Caution: Please consult all relevant material safety data sheets (MSDS) before use.

1. NMR Sample Preparation

1. Weigh 5 mg of *N,N*-dimethylacetamide, add to an NMR tube appropriate for low temperatures and dissolve in 0.6 ml of toluene-d_8.

2. NMR Experimental Setup[25]

1. NOE Spectra Acquisition
   1. Perform a one dimensional NOE (Nuclear Overhauser Effect) experiment.[26]
   NOTE: NOE effects can happen at any temperature. A one dimensional NOE spectrum irradiating the signal that will be irradiated in the SSTD NMR experiment, was recorded at -40 °C to make sure that the rotation and magnetization transfer in the sample used here was minimized, and therefore the NOE, if existent, would predominate and be measured in this experiment. Ideally, NOE effects between the two exchanging nuclei should not be present to avoid interferences with the SSTD method.

2. SSTD NMR Experiments Setup
   1. Insert the sample in the magnet by first typing *ej* in the command line of the software to turn on the air flow. Then, put the sample on top of the magnet and then type *ij*. Wait until the sample is inside the magnet.
   2. Once the sample is in the magnet, type *edte* in the command line. Change the temperature to the first selected temperature to carry out the experiment (295.5 K in this case). Let the sample stabilize at the chosen temperature for at least 20 min.
   3. Perform a 1D ¹H-NMR experiment on the sample.
      1. Create a new dataset of a ¹H-NMR experiment. For this click on FILE/NEW and name the new experiment.
      2. Type sequentially and waiting for the previous command to finish: *lock*, *atma*, *topshim*, *getprosol* and *rga*.
      3. Type *zg* to acquire the proton experiment. Once it is finished type *efp* and *apk* to Fourier transform it and adjust the phase.
   4. Create a new dataset of, for example, a ¹H NMR experiment. For this click on FILE/NEW and name the new experiment.
   5. In this new dataset, type *rpar* in the command line. Select one of the "STDDIFF" parameter sets from the list, for example STDDIFFESGP, and click "read" and then "read all" (**Figure 1**). Alternatively, do this by typing *rpar STDDIFFESGP all*.
      NOTE: The experiment can be performed with this pulse sequence. However, the pulse program used in our experiment was STDDIFF.
   6. To select the STDDIFF pulse sequence, click the button with three dots in the PULPROG line (**Figures 2** and **3**).
   7. Before carrying out the SSTD NMR experiment, calibrate the ¹H 90° hard pulse (*p1*). For this purpose, ensure that the sample is in the magnet at the desired temperature (Step 2.2.2). Type *pulsecal* in the command line and copy the value of the 90° pulse at the higher power (pl1 = -1 dB in this case), *i.e.,* the one that gives the shortest pulse (**Figure 4**).
   8. Introduce the values for the calibrated hard pulse in the experiment. Type *getprosol 1H* (value for p1 obtained in step 2.2.7) (value for pl1) (**Figure 5**).
   9. Set the length of the shaped pulse. Type *p13* and introduce a value of 50,000 μsec (**Figure 6**).
   10. Set the selective pulse shape. To do this, go to Power and click the "Edit…" button next to SHAPE (**Figure 7**). Go to the shaped pulse 13 and choose: *Gaus 1.1000* (**Figure 8**).
   11. Set the selective pulse power (*SP13*). Set it to something appropriate, *i.e.*, on this system between 40-60 dB (corresponding to a field strength of approximately 120 Hz) (**Figure 8**). Excessive field strengths can lead to unacceptable saturation effects.[27-29]
      NOTE: 50 dB was optimum in our case. Take into account that this is an attenuation scale, so the smaller the value the higher the power of the radiofrequency. As it corresponds to the saturating Gaussian cascade, which is applied for long time (several seconds), *sp13* should not go below 40 dB (if needed, consult the instrument specifications, as long pulses at high power could damage the

probehead). In our experience 41-61 dB above the attenuation of the hard [1]H 90° pulse (-1 dB in this work) works fine. Try to always select the highest attenuation possible leading to similar saturation level.

12. Type *ns* and set it to 8 and type *ds* and set it to 4.

3. NMR Data Acquisition and Processing[25]

1. SSTD NMR Experiment Acquisition
    1. Open the ^{1}H NMR experiment performed in step 2.2.3 to check where the signal that will be irradiated is. For this, search the experiment in the software browser, right click in the dataset and click "Display in a new window".
    2. Move the cursor line to the center of the signal to irradiate and write down the chemical shift in ppm. Select the spectral width that will be used in the experiment.
    NOTE: In this case, the signal which will be irradiated is at 2.17 ppm, and the spectral width used was 1.46 ppm. Ensure that no chemical shift correction is used or the irradiation frequency can be set incorrectly.
    3. Go to the previously created SSTD NMR experiment with the setup mentioned in section 2.2.
    4. Create a list with the frequencies of irradiation. For this, type *fq2list* in the command line and select an existing list.
    5. Edit the list of irradiation frequencies including the following data in the 3 first rows (**Figure 9**): *Row 1. P (*indicates that the following data is in ppm); *Row 2. Frequency of the signal to be irradiated in ppm, as measured in 3.1.1; Row 3.40 ppm* (a frequency that is far from the ^{1}H signals of the compound so the irradiation in that frequency does not affect the spectra).
    6. Save the list with a new name and then type *fq2list* in the command line and select the list just created.
    7. To center the experiment on the signals under study, type *o1p* and select as the center of the experiment the chemical shift of the signal that will be irradiated.
    8. Type *sw* to select the spectral width (1.46 ppm in this case, but any other spectral width can be chosen).
    NOTE: If the acquisition time obtained after changing the spectral width is too long (which will introduce more noise in the spectra) it can be adjusted by typing *AQ* to provide the desired Free Induction Decay (FID) resolution (FIDRES, 0.25 Hz in this case).
    9. Choose the value for the interscan relaxation delay *D1*. Ensure that it is at least 1 to 5 times the value of the T_1 of the slowest relaxing proton.
    NOTE: We set it up to 40 sec, which is the longest saturation time (*D20*) in the experiment. In this way all of the experiments will keep the same total "per scan" time (delay + saturation time + pulses + acquisition time).
    10. Type *D1* and set it to 40 sec.
    11. Set the first value for the saturation time by typing *D20* and setting it to 40 sec. Determine the receiver gain (*rg*) automatically by typing *rga*.
    12. Create the next experiment by typing *iexpno*. Type *D20* and select a saturation time of 20 sec in this experiment. Type rga to automatically determine *rg*.
    13. Repeat the last step for *D20* = 10, 5, 2.5, 1.25, 0.625, 0.3 sec.
    14. Once all the experiments are created, open the first one and in the command line type *multizg* and specify the number of experiments, 8 in this case (*i.e.*, *multizg 8*).

2. SSTD NMR Experiment Processing
    1. Open the PROCNO 1 (Process Number) from EXPNO 1 (Experiment Number) of the set (the one with the higher saturation time).
    2. In the command line type *lb* and set the value to 1.5.
    NOTE: For spectra with very high signal-to-noise ratios this value could be decreased; inversely, it could be increased for noisy experiments, if the spectral resolution is not severely affected.
    3. In the command line type *efp* and process FID # = 1 ("on-resonance" spectrum) in PROCNO = 2 (**Figure 10**).
    4. Correct the phase of the experiment by clicking the *Interactive phase correction* button and save it as a 2D experiment. Save and exit (**Figure 11**).
    5. Type *rep 1* in the command line to go to the PROCNO 1.
    6. In the command line type *efp* and process FID # = 2 ("off-resonance" spectrum) in PROCNO = 3 (**Figure 12**).
    7. In the command line type *.md* and then *rep 2* to show a multiple display window with both processed spectra: 2 (the one with the signal in the middle saturated) and 3 (the one in which the saturating pulse train was applied at 40 ppm) (**Figure 13**).
    8. Click the button with a delta sign (**Figure 13**) to calculate the difference spectra and save it in PROCNO 4. Exit the multiple display window.
    9. Select an integration range for the signal on the left (the signal in which the transfer of saturation due to the chemical exchange process will be observed). Always integrate the same region in PROCNO 3 and PROCNO 4.
    NOTE: The integration range used in this experiment was 2.55 - 2.67 ppm.
    10. Once integrated, go to the "Integrals" tab in each of the experiments and copy the value of "Integral [abs]" (**Figure 14**).
    11. Divide the integral in PROCNO 4 by the integral in PROCNO 3. That is the value of η_{SSTD} for a saturation time of 40 sec (η_{SSTD} =Spin Saturation Transfer Difference parameter)[21]
    12. Repeat the procedure for the rest of the experiments with different saturation times.

4. Data Analysis[30]

1. Analysis of the Data to Get the Kinetic Parameters
    1. Plot the obtained η_{SSTD} values versus the saturation time.[21]
    2. Perform an exponential fit to adjust the obtained curves to the equation
    $$\eta_{SSTD} = \eta_{SSTD}{}^{MAX}(1 - \exp(-\delta \cdot t))$$
    δ = dynamic constant

$\eta_{SSTD}{}^{MAX} = \eta_{SSTD}$ at very long saturation time
t = time

3. Calculate the values of $\eta_{SSTD}{}^{MAX}$ and δ and use them to calculate the values of the rate constants (*k*) and relaxation times (T_{1A}) according to the following equations:

$$\eta_{SSTD}{}^{MAX} = \frac{k}{(1/T_{1A} + k)} \quad and \quad \delta = 1/T_{1A} + k$$

T_{1A} = longitudinal relaxation time constant of spin A
k = mutual-site exchange kinetic rate constant

    1. Obtain the kinetic rate constant by: $k = \eta_{SSTD}{}^{MAX} \cdot \delta$

2. Eyring Plot to get the Thermodynamic Parameters
   1. Plot ln(*k*/T) versus 1/T (T = absolute temperature), using the values of the kinetic rates at different temperatures.
   2. Perform a linear fit to adjust the data obtained to the Eyring equation:

$$\ln\left(\frac{k}{T}\right) = \ln\left(\frac{k_B}{h}\right) - \frac{\Delta H^{\ddagger}}{RT} + \frac{\Delta S^{\ddagger}}{R}$$

$\Delta H^{\ddagger}$ = entalphy of activation
$\Delta S^{\ddagger}$ = entropy of activation
h = Plank's constant
R = gas constant
k_B = Boltzmann constant
T = absolute temperature

   3. Calculate the thermodynamic parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$.
   4. Calculate the values for $E_{A(298)}$ and $\Delta G^{\ddagger}{}_{(298)}$ using the following equations:

$$E_{A(298)} = \Delta H^{\ddagger} + RT$$
$$\Delta G^{\ddagger}_{(298)} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$$

$E_{A(298)}$ = Activation Energy at 298 K
$\Delta G^{\ddagger}_{(298)}$ = Gibbs Energy at 298 K

Representative Results

The SSTD NMR technique was applied for the calculation of the kinetic parameters in the rotation of the amide bond of *N,N*-dimethylacetamide.[21] This is a simple example for which extensive data for comparison can be found in the literature.[31]

The hindered rotation around the amide bond, due to the partial double bond character in the resonance form, differentiates both methyl groups into two signals in the ^{1}H-NMR spectra (2.61 and 2.17 ppm at 22.5 °C). Spin saturation of the signal of the methyl group at 2.17 ppm (Me[B]) leads to the disappearance of its signal in the ^{1}H NMR. Upon saturation of Me[B], transfer of saturation to the other methyl group (Me[A]) due to the internal rotation process can be observed by a decrease in ^{1}H intensity in the signal at 2.61 ppm. The magnitude of this decrease will depend on the saturation time. **Figure 15** shows the ^{1}H NMR spectra of the *N,N*-dimethylacetamide at 22.5 °C, and the expansions show the spectra without (a) and with (b) saturation of the methyl group at 2.17 ppm, as well as the difference spectrum (c), used to calculate the values of η_{SSTD}. The η_{SSTD} factor is calculated dividing the value of the integral of Me[A] in the SSTD NMR spectrum (c) by the value of the integral of the Me[A] in spectra (a), as explained in the protocol. The obtained values of η_{SSTD} for each saturation time at different temperatures are gathered in **Table 1**. The plot of the obtained values of η_{SSTD} versus the saturation time gave exponential curves in which a plateau was reached at higher saturation times. For a certain temperature, the exponential fit of the curve permits the calculation of the rate constant (*k*) and the relaxation time of the ^{1}H of the measured signal (T_{1A}) (**Figure 16**). **Figure 17** shows all the obtained curves along with the *k* and T_{1A} values obtained in the fits.

Finally, the plot of ln(*k*/T) versus 1/T and the fit to the Eyring equation (**Figure 18**) were used to calculate the enthalpy and entropy of activation. The determined activation parameters are shown in **Table 2**, together with the previously reported parameters calculated using different methodologies.

As can be observed in **Table 2**, the values of the activation parameters obtained with the Spin Saturation Transfer Difference technique (SSTD NMR) are in excellent agreement with the data previously reported using other techniques, such as SST NMR or line shape analysis. The wide range of values reported for $\Delta S^{\ddagger}$ is due to the difficulty in the measurement of this parameter with NMR techniques.[31] As for the rest of the activation parameters, the values obtained with our method are not only really similar to the ones already reported but also more accurate, since our errors (SD) are smaller in all the cases.
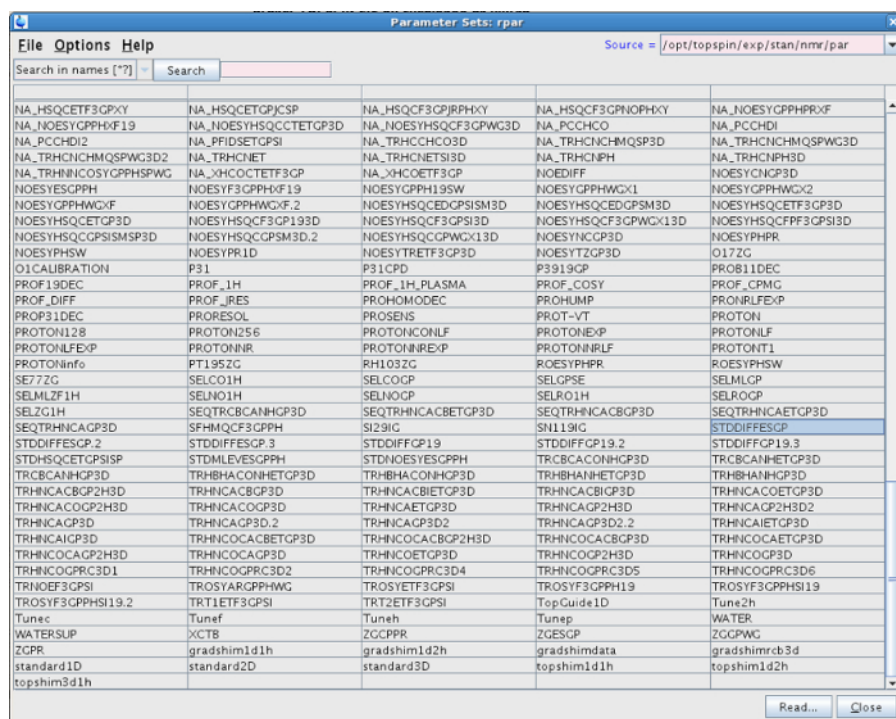
Figure 1: List of experiments after typing _rpar_. The figure shows the different parameter sets among which STDDIFFESGP should be selected. Please click here to view a larger version of this figure.
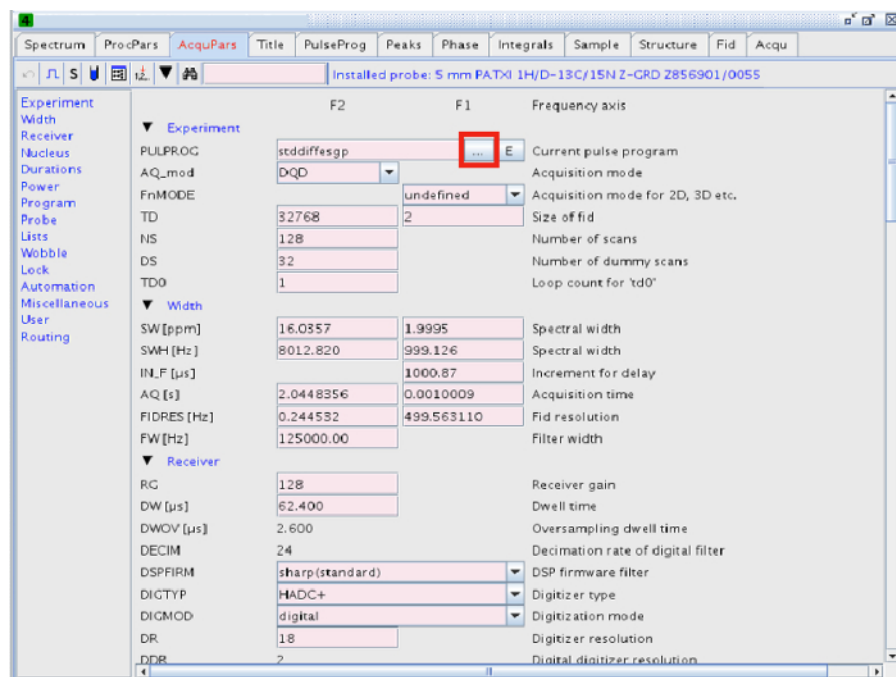

Figure 2: Acquisition parameters. The button highlighted in a red square leads to a list of the different pulse programs. Please click here to view a larger version of this figure.
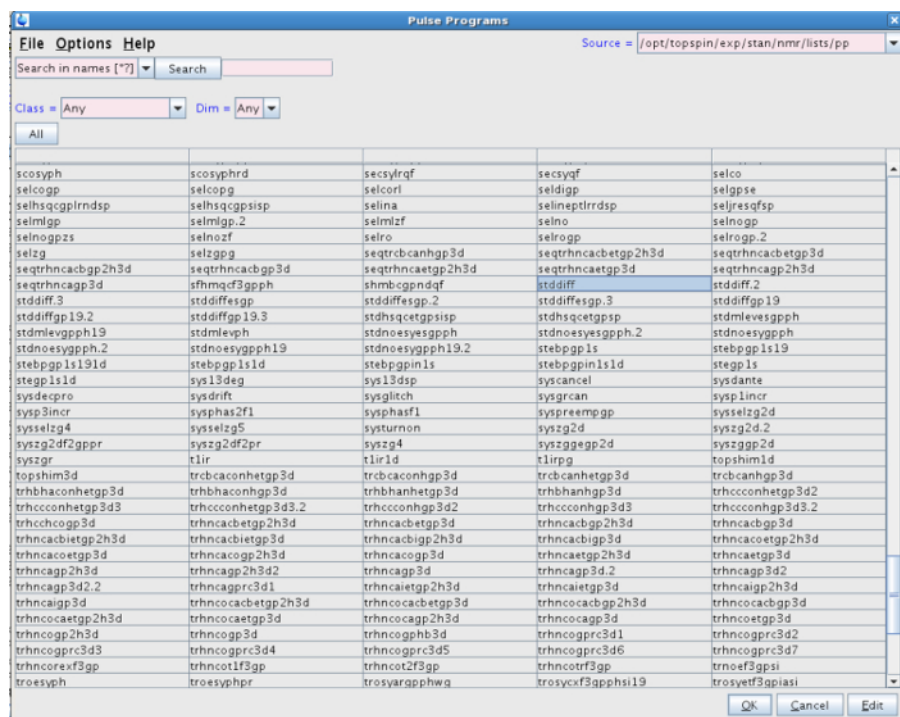
Figure 3: List of pulse programs. The figure shows the selected pulse program in the experiment (STDDIFF). Please click here to view a larger version of this figure.
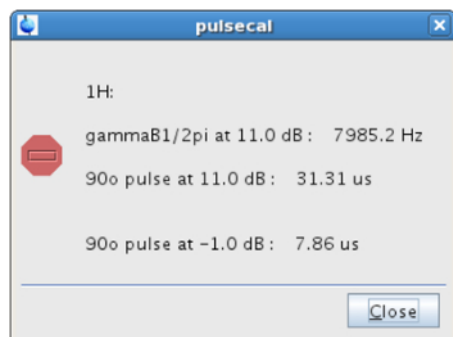
Figure 4: Pop-up window appeared after the 90° pulse calibration. The figure shows the values of the calibrated 90° pulse at different power levels. Please click here to view a larger version of this figure.
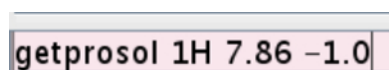
Figure 5: Screenshot of the command line. The figure shows how to introduce the value for the calibrated hard pulse. Please click here to view a larger version of this figure.
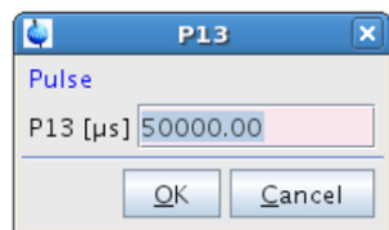
Figure 6: Value for the length of the shaped pulse. The figure shows how to introduce the value for the length of the shaped pulse. Please click here to view a larger version of this figure.
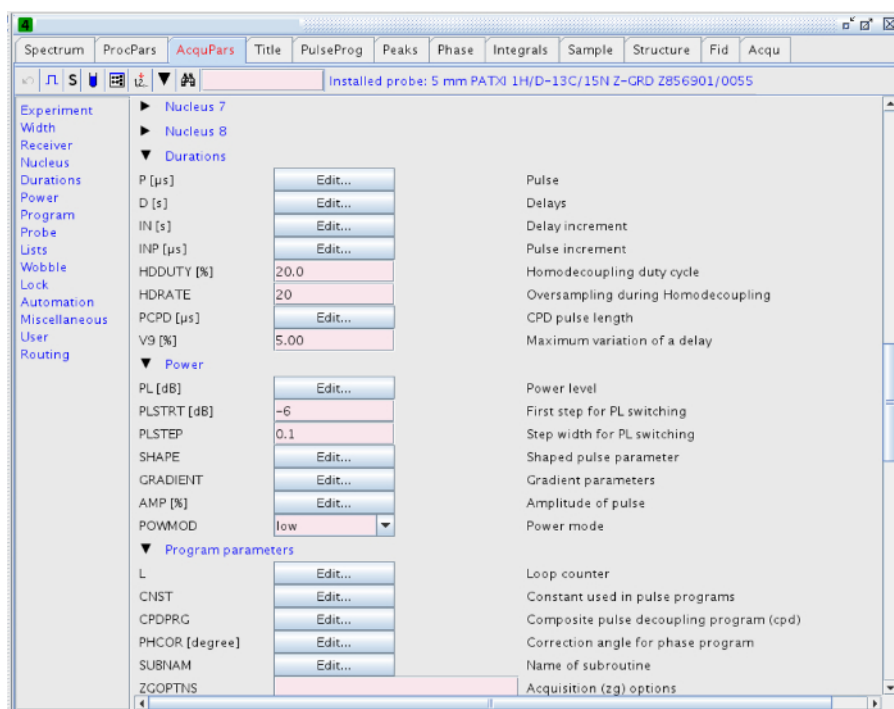
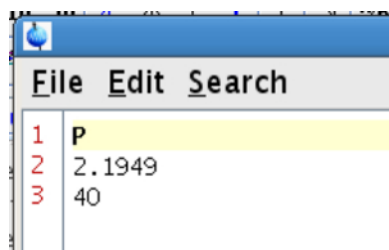**Figure 7: Acquisition parameters.** The figure shows the power parameters. Please click here to view a larger version of this figure.

Figure 8: Parameters for the shaped pulse. The values for the shaped pulse will be introduced in line 13. Please click here to view a larger version of this figure.

Figure 9: List of irradiation frequencies. The figure includes the following data in the 3 first rows: *Row 1. P (indicates that the following data is in ppm); Row 2. Frequency of the signal to be irradiated in ppm, as measured in 3.1.1; Row 3.40 ppm* (a frequency that is far from the ^{1}H signals of the compound so the irradiation in that frequency does not affect the spectra). Please click here to view a larger version of this figure.
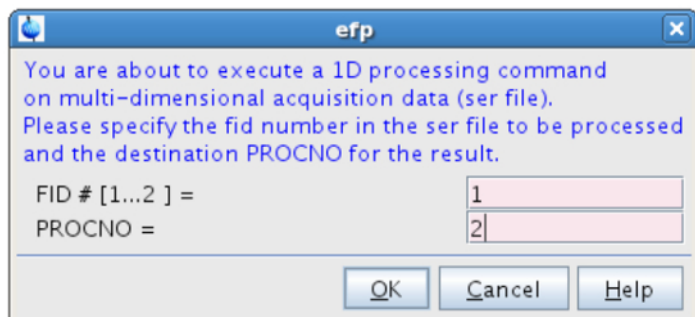

Figure 10: Processing of the first FID. The figure shows the pop up window that appears after typing *efp.* Please click here to view a larger version of this figure.
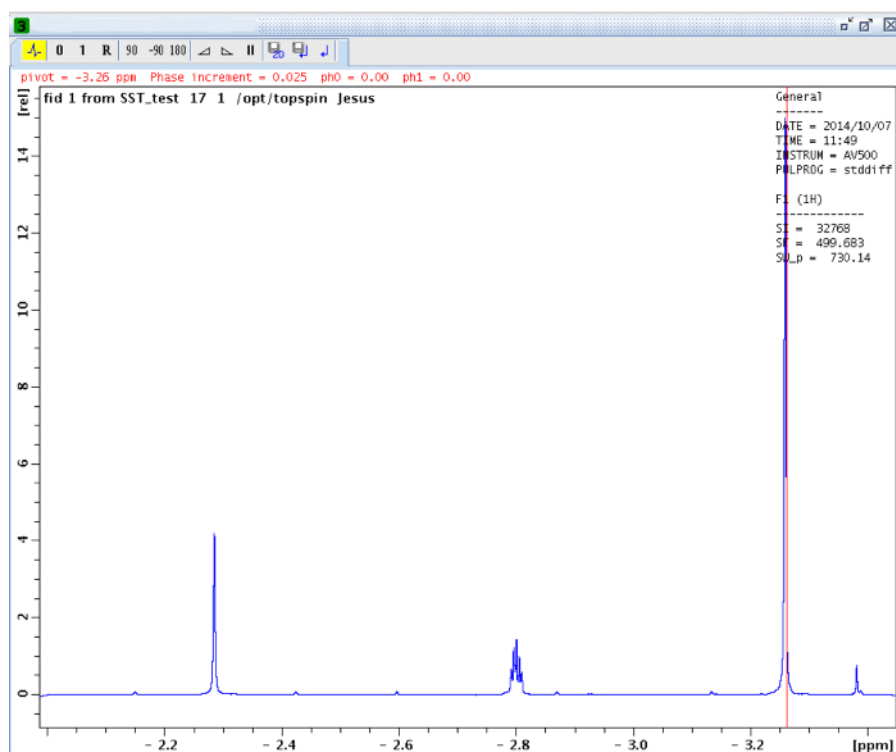

Figure 11: Phase correction. Screenshot showing the window for the manual phase correction. Please click here to view a larger version of this figure.
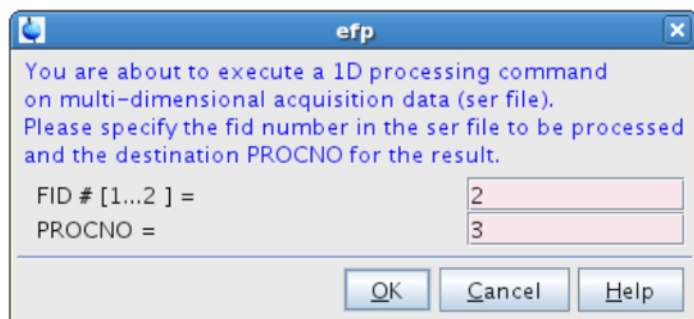
Figure 12: Processing of the second FID. The figure shows the pop up window that appears after typing *efp*. Please click here to view a larger version of this figure.
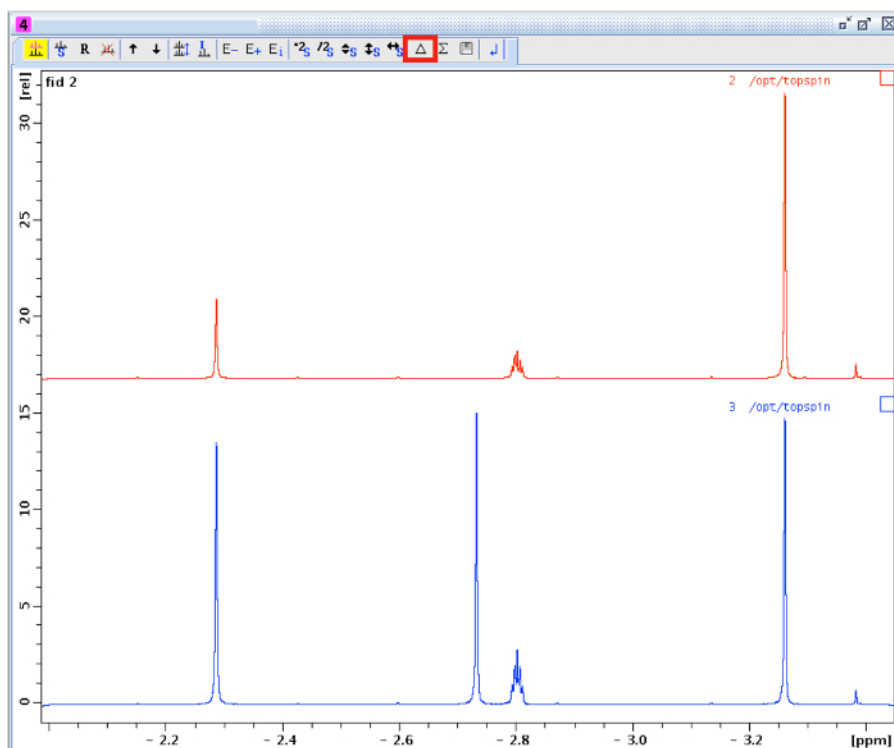

Figure 13: Multiple display of spectra 2 and 3. The button highlighted in a red square is the one to calculate the difference spectra. Please click here to view a larger version of this figure.

Figure 14: Integrals tab. The figure shows the values of the absolute and relative integrals. Please click here to view a larger version of this figure.
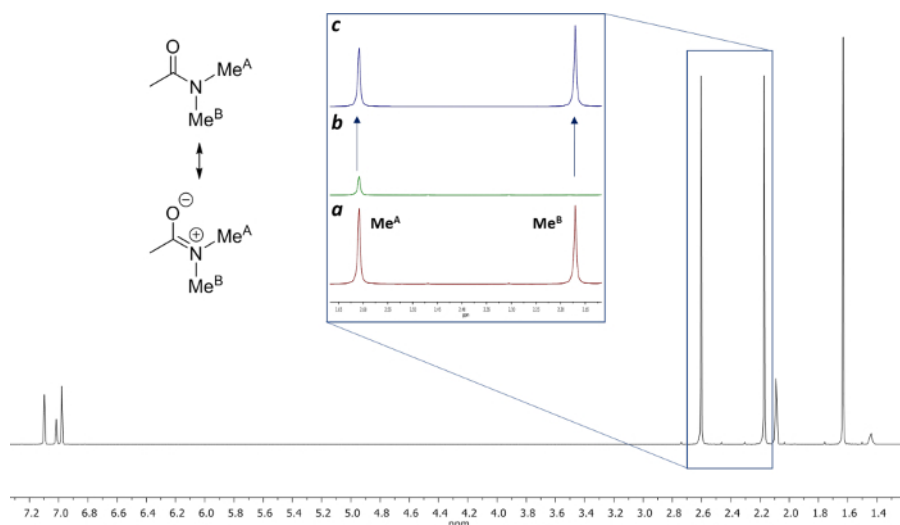
Figure 15: Structure and ¹H NMR spectra of *N,N*-dimethylacetamide at 22.5 °C in toluene-d₈. (**a**) ¹H NMR expansion of the region from 2.13 to 2.66 ppm before irradiation. (**b**) Expansion of the same region after irradiation of the methyl group at 2.17 ppm. (**c**) Difference spectrum [(a)-(b)]. Please click here to view a larger version of this figure.
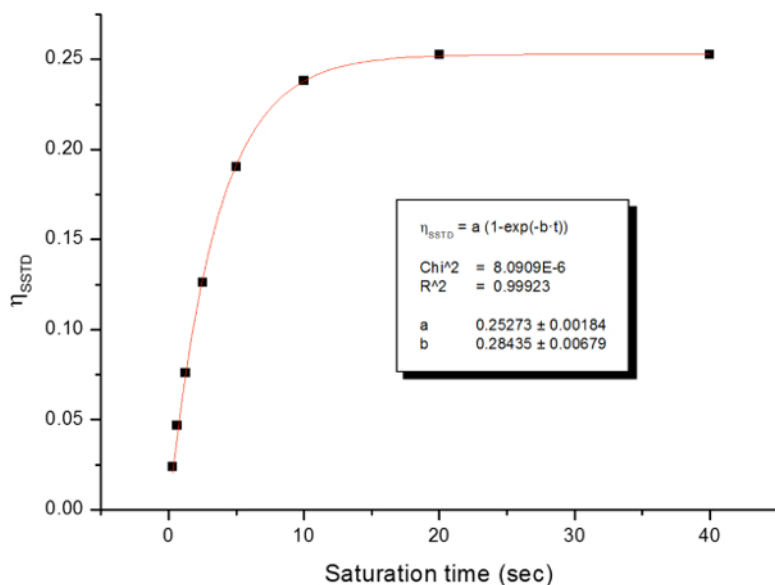
Figure 16: Example of the plot of η$_{SSTD}$ and its exponential fit at 278 K. Reproduced from the supporting information of reference[21] with permission from the Royal Society of Chemistry. Please click here to view a larger version of this figure.
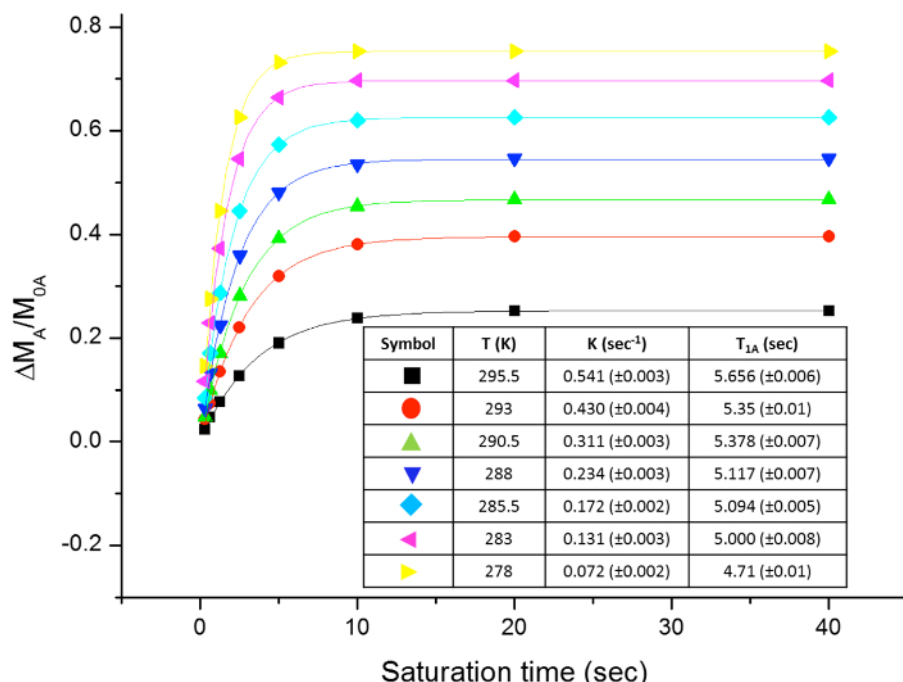
Figure 17: Plots of η_{SSTD} vs. saturation time at different temperatures. The figure shows the plot for *N,N*-dimethylacetamide and the table with the obtained rates constants and relaxation times. Please click here to view a larger version of this figure.

Figure 18: Eyring plot. The figure shows the plot for *N,N*-dimethylacetamide. Please click here to view a larger version of this figure.

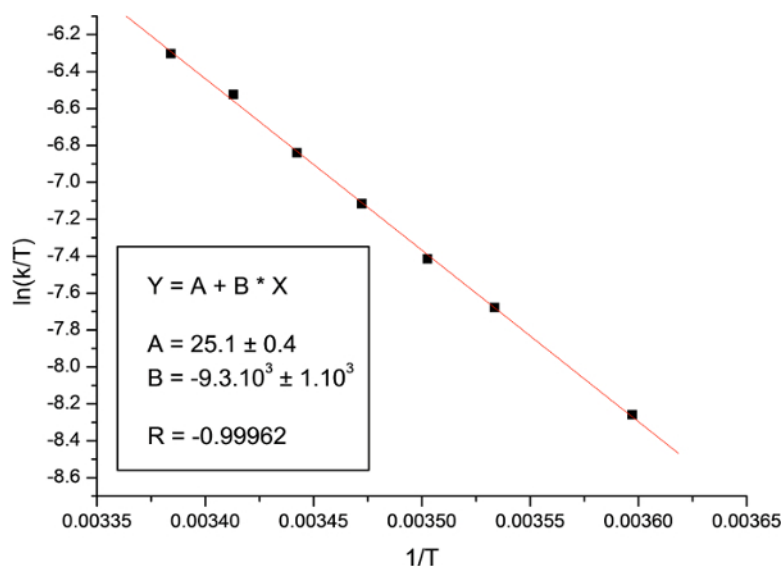| t_{sat} (sec)	η_{SSTD} (T = 278 K)	η_{SSTD} (T = 283 K)	η_{SSTD} (T = 285.5 K)	η_{SSTD} (T = 288 K)	η_{SSTD} (T = 290.5 K)	η_{SSTD} (T = 293 K)	η_{SSTD} (T = 295.5 K)
40	0.2526	0.3957	0.4671	0.5461	0.626	0.6969	0.7535
20	0.2526	0.3957	0.4671	0.5461	0.626	0.6969	0.7535
10	0.2383	0.3806	0.4537	0.5355	0.6199	0.6969	0.7535
5	0.1904	0.3193	0.3919	0.481	0.5734	0.6638	0.7318
2.5	0.1263	0.2204	0.2812	0.3589	0.4449	0.5461	0.626
1.25	0.0761	0.1353	0.171	0.2247	0.2868	0.3732	0.4449
0.625	0.0467	0.0739	0.099	0.1327	0.171	0.2291	0.2758
0.3	0.0238	0.044	0.0472	0.0644	0.0847	0.1169	0.1463

Table 1: Values of η_{SSTD}. The table shows the values obtained at different saturation times for *N,N*-dimethylacetamide in the range of temperatures 278-295.5 K.

Method	SSTD NMR	SST NMR[31]	Line-shape analysis	Line-shape analysis	Line-shape analysis
Parameter	(This work)		(^{1}H NMR)[4]	(^{1}H NMR)[5]	(^{13}C NMR)[6]
$E_{a\ 298}$ (KJ mol^{-1})	79.7 ± 0.1	73.1 ± 1.4	70.5 ± 1.7	82.0 ± 1.3	79.5 ± 0.4
$\Delta H^{\ddagger}$ (KJ mol^{-1})	77.2 ± 0.1	70.6 ± 1.4	68	79.5 ± 0.4	76.6 ± 0.4
$\Delta S^{\ddagger}$ (J mol^{-1} K^{-1})	11.5 ± 0.4	-10.5 ± 5.0	-15.0 ± 5.1	13 ± 8	3 ± 4
$\Delta G^{\ddagger}_{298}$ (KJ mol^{-1})	73.8 ± 0.1	73.7 ± 2.0	72.5	75.3 ± 0.4	75.7 ± 0.4
Solvent	Tol-d_8	Tol-d_8	CCl$_4$	Acetone-d_6	neat

Table 2: Activation parameters. The table shows the activation parameters for the internal rotation of *N,N*-dimethylacetamide obtained by the SSTD NMR method compared with the same parameters obtained using different NMR methods for analysis.[4,5,6] Errors in this table refer to standard deviation errors (SD). (Reproduced from reference[21] with permission from the Royal Society of Chemistry).

Discussion

One of the more obvious advantages of this methodology is that the rate constants and the relaxation time for a given temperature can be obtained with a single set of experiments, with a robust pulse sequence (the same used for STD experiments to study protein-ligand interactions, which is typically found within the available set of experiments from the spectrometer manufacturer). This simplifies the experimental setup since there is no need to measure T_1 or reach steady state saturation. Besides, it is remarkable that this method does not depend on the magnet strength, as coalescence methods. On the other hand, the main limitation is that this technique cannot be applied to chemical exchange processes too fast or too slow, which would depend on the temperature range of the NMR machine or the solvents used.

This new technique for the calculation of kinetic parameters can be applied to a great variety of substrates and its applicability has already been demonstrated with some interesting molecules.[21] The kinetic parameters of the 4-*N,N*-dimethylamido[2.2]paracyclophane, a challenging substrate in which the signal of one of the methyl groups of interest is overlapped with other signals from the molecule, were successfully calculated using SSTD NMR. Interestingly, this methodology can be applied as long as one of the signals of study is isolated. SSTD NMR is also a useful protocol for the calculation of kinetic parameters in molecules in which the coalescence temperature is so high that the molecule decomposes before reaching it. This is the case with PtCl$_2$(dimethylallene)(pyridine), in which the methodology was successfully applied without the need of reaching coalescence. The choice of solvents and temperatures is critical to obtain good results, since the chemical exchange rates can vary significantly with these parameters. Moreover, in addition to the criteria in a normal NMR experiment, key steps in a SSTD NMR experiment are the selectivity of the irradiation as well as the temperature control. Both factors have to be precise to guarantee the success of the experiment.

The representative results presented here are for the kinetics of intramolecular chemical exchange, but the technique can also be applied to study the kinetics of intermolecular chemical exchange and also ligand exchange, common processes in the dynamic behavior of transition metal complexes.

Finally, providing a proper modification of the equations is made,[32] this method could be extended to deal with multi-site exchange and unequal populations, as it has been done in former double resonance experiments,[8-9] increasing the usefulness of this technique for the study of chemical exchange processes in challenging compounds.

Disclosures

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