

Difference-NMR techniques for selection of components on the basis of relaxation times

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Abstract

This work describes a numerical methodology to obtain more efficient relaxation filters to selectively retain or remove components based on relaxation times. The procedure uses linear combinations of spectra with various recycle or filter delays to obtain components that are both quantitative and pure. Modulation profiles are calculated assuming exponential relaxation behavior. The method is general and can be applied to a wide range of solution or solid-state NMR experiments including direct-polarization (DP), or filtered cross-polarization (CP) spectra. ¹³C NMR experiments on isotactic poly(1-butene) and dimethyl sulfone showed the utility of the technique for selectively suppressing peaks.

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1. Introduction

The selection or suppression of a particular component on the basis of relaxation times is a common technique in both solution and solid-state NMR spectroscopy. Typical relaxation curves are exponential, and the decay times are related to molecular dynamics and the magnetic field of the spectrometer [1,2]. The longitudinal relaxation time in the laboratory frame (T_1) is sensitive to motion in the tens to hundreds of MHz range while the longitudinal relaxation time in the rotating frame ($T_{1\rho}$) reaches a minimum when motion is in the tens of kHz. Components with a short T_1 relaxation time can be selectively excited by single-pulse experiments using a short recycle delay. Spectra containing only long T_1 relaxation time components can be obtained using a T_1 -filter [3]. Finally, components with an intermediate T_1 relaxation time can be resolved by subtracting a direct polarization (DP) spectrum with a short

recycle delay time from a spectrum obtained with a long recycle delay time [4].

The modulation profiles show the relationship between T_1 relaxation times of components and the relative signal intensity after filtering. The modulation is 1 if the component is completely retained and 0 if completely suppressed. The previously discussed filtering techniques do not result in box-function modulation profiles that can completely suppress all undesired component and quantitatively retain all desired components. For example, if a sample has 2 components with T_1 relaxation times of 1 and 10 s, a Taylor-series expansion shows that the ratio of the components in a short recycle delay single-pulse excitation experiment will be at best 10:1. Despite the large difference in T_1 values, a quantitative and resolved spectrum cannot be obtained. The selectivity using a T_1 -filter is more efficient, but quantitative-ness is limited. In the hypothetical case just mentioned, a 3.3 s T_1 -filtered experiment would provide remaining intensities of 4 and 72% for the short and long components, respectively. The 28% loss is unacceptable for quantitative experiments. Finally, the selection of components with intermediate relaxation times suffers from the limitations of selectivity and quantitative-ness that arise from

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the subtracted short and long experiments. Curve-fitting [5], or chemometric techniques [6] provide routes to separate components with different relaxation times, but these procedures require programs that are currently not standard on commercial spectrometers.

The recent successful development of the PUREX technique [7] for obtaining pure 2D exchange spectra has shown the potential for mathematical manipulation of spectra. The addition of sine wave modulated spectra with different periods resulted in a near box-function modulation profile with a sharp null at a particular frequency. The ripples in the box function were minimized by the appropriate multiplication of spectra.

The goal of this paper is the design of simple quantitative experiments that result in enhanced selectivity with minimal sacrifice of signal/noise. A mathematical approach is taken which removes first and sometimes higher order terms in Taylor-series expansions of signal modulation. Spectra obtained using different recycle delays or different lengths of T_1 -filters can then be combined using calculated multipliers to obtain short, long, or intermediate components.

2. Theoretical section

2.1. Suppression of first-order error in direct excitation spectra

Assuming exponential relaxation of magnetization, the relative signal intensity, I , for direct polarization (DP) experiments with a recycle delay, t , and a relaxation time of T_1 is

$$I = 1 - e^{-t/T_1}. \quad (1)$$

A Taylor-series expansion of this equation provides a foundation for the difference-NMR technique:

$$I = t/T_1 - \frac{1}{2}(t/T_1)^2 + \frac{1}{6}(t/T_1)^3 - \frac{1}{24}(t/T_1)^4 + \dots \quad (2)$$

In a system with two components with T_1 values of T_{1a} and T_{1b} , the selectivity can be expressed as a series calculated from long division of I_a/I_b :

$$I_a/I_b = T_{1b}/T_{1a} - \frac{(t)(T_{1b})}{2T_{1a}} \left(\frac{1}{T_{1a}} - \frac{1}{T_{1b}} \right) + \dots \quad (3)$$

Thus, the maximum selectivity when $t \ll T_{1a} \ll T_{1b}$ will be T_{1b}/T_{1a} . This linear dependence on the ratio of relaxation times prevents efficient and quantitative selection of the shorter component in most systems.

An improved selectivity can be obtained from the combination of multiple spectra. Spectra are multiplied by coefficients that are chosen to cancel terms in the Taylor-series expansion shown in Eq. (2). A basic example is subtracting a spectrum with a recycle delay of $d = 2t$ from twice a $d = t$ spectrum yielding:

$$2 * I_{(d=t)} - I_{(d=2t)} = (t/T_1)^2 - (t/T_1)^3 + \frac{7}{12}(t/T_1)^4 - \dots \quad (4)$$

Eq. (4) lacks the first-order term thus yielding enhanced selectivity: the maximum selectivity when $t \ll T_{1a} \ll T_{1b}$ will be $(T_{1b}/T_{1a})^2$. The quadratic dependence on the ratio allows greater resolution of the components compared with the linear dependence typically obtained in experiments.

An example of a theoretical modulation profile for the difference technique is shown in Fig. 1. For comparison, the curves of DP experiments with 0.5, 1, and 2 s recycle delays are also plotted. Cancellation of the second-order terms in Eq. (4) results in enhanced selectivity. The combination of $3*1s - 3*2s + 3s$ spectra succeeds in removal of the first and second-order terms from the Taylor expansion. All curves are quantitative for fast-relaxing components, but only the difference technique achieves effective suppression of long T_1 signals. Traditional DP experiments are capable of exciting 97% of a fast relaxing component but just 3% of undesired longer relaxing carbons *only* if the ratio of the two T_1 values is greater than 115:1. Using the difference methodology, on the other hand, pure spectra can be obtained if the ratio is 23:1 for the two spectra technique and 13:1 for the three spectra technique. This improved efficiency can be important for many practical systems. An applied example is shown in Section 3.

The method can be generalized for any 2 spectra with recycle delays of t and yt :

$$I = (yI_{(d=t)} - I_{(d=yt)})/(y - 1). \quad (5)$$

The selection of y is dependent on the desired goals. A large value of y will optimize the signal/noise ratio because little intensity is subtracted from the original

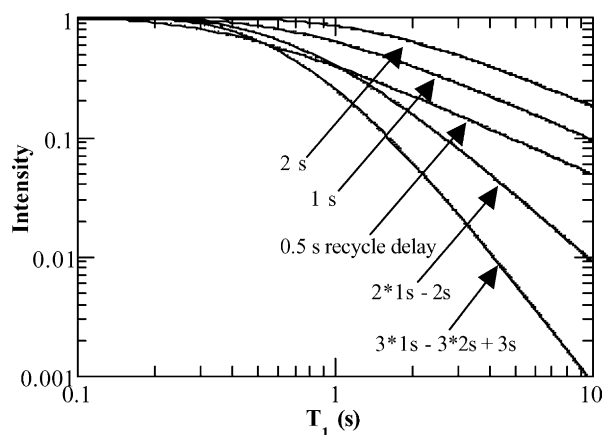


Fig. 1. Theoretical modulation curves calculated for DP experiments with 0.5, 1, and 2 s recycle delays. The difference-NMR method obtains more efficient suppression of long T_1 components from the subtraction of 2 s curve from twice the 1 s curve. A more complex method is also shown with a curve calculated by the combination of $3*1s - 3*2s + 3s$ spectra.

spectrum, but a small value of y will maximize efficiency of the filter. A y value of 2 provides a reasonable combination of both desired features for typical experiments.

The procedure will have only moderate selectivity if the relaxation behavior is non-exponential but will not generate artifacts. For example relaxation dominated by physical or spin diffusion can result in a \sqrt{t} dependence. Isolating slowly relaxing components in this specific example could potentially be addressed by the difference of $(y * I_{(d=t)} - I_{(d=y^2t)})/y - 1$. This procedure fails if exponential relaxation and diffusion are both experimentally significant.

2.2. Signal/noise and reproducibility

An important limitation of the described method is the ratio of signal/noise and restricts the choice of y in Eq. (5). In a typical difference-NMR experiment where a spectrum with a recycle delay of $d_1 = 2t$ is subtracted from twice the spectrum obtained with $d_1 = t$, the signal will remain the same, but the noise will be $\sqrt{5}$ times larger than in the original spectra. In addition, the number of scans used to obtain each spectrum must be reduced to preserve the total experiment time. The reduced number of scans decreases the signal/noise by a factor of approximately $\sqrt{3}$. Thus, the expected ratio will be just 26% of the original signal/noise. Only slight improvements can be made if the restriction of equal number of scans for each spectrum is lifted. More scans are optimal for the shorter recycle delay experiment, but the ratio is dependent on the choice of y .

Irreproducibility also becomes an important issue in difference NMR. Experiments become long and drifts in both power levels and tuning can result in systematic variations. To minimize these factors, experiments can be run in loops where additional scans are added to each spectrum with each pass. Another possible method to reduce error is writing an extended pulse program which combines both recycle delays and multiple acquisition periods with appropriate receiver phases.

2.3. Application to T_1 -filter experiments

Experiments attempting to quantitatively filter short T_1 components suffer from partial loss of the long relaxation components. The relative magnetization of a component with a relaxation time of T_1 after a filter time of t is

$$I = e^{-t/T_1}. \quad (6)$$

Similar to the approach discussed for the direct polarization experiments, the difference-NMR technique relies on the Taylor-series expansion:

$$I = 1 - t/T_1 + \frac{1}{2}(t/T_1)^2 - \frac{1}{6}(t/T_1)^3 + \dots \quad (7)$$

This equation, of course, is similar to Eq. (2) and the first-order term can be removed by appropriate combination of two spectra with filter times of t and yt . Again, Eq. (5) can be used to determine the prefactors for the subtraction. Using $y = 2$, the relaxation time and relaxation delay dependence of the intensity will be

$$2 * I_{(d=t)} - I_{(d=2t)} = 1 - (t/T_1)^2 + (t/T_1)^3 - \frac{7}{12}(t/T_1)^4 + \dots \quad (8)$$

The lack of the linear term in Eq. (8) is the important modification to the series. Fig. 2 shows the modulation curves for a T_1 -filtered spectrum with the traditional methodology and with the difference-NMR technique. The improved quantitiveness of the modified procedure is readily apparent. A component with a 100 s T_1 relaxation time loses 10% of its intensity when subjected to a 10 s filter. The combination of the 10 and 20 s filters allows the component with the 100 s T_1 relaxation time to be 99% retained while still completely suppressing quickly relaxing components.

2.4. Shaped modulation profiles

Modulation profiles can be designed with specific shapes to suppress unwanted components with either shorter or longer relaxation times. An applied example goes through the calculations necessary to design an experiment which removes fast-relaxing components ($T_1 \leq 0.5$ s) and slow-relaxing components ($T_1 \geq 50$ s) in $T_{1,C}$ -filtered CP spectra, leaving only the intermediate components ($T_1 \approx 2$ s). Five filter times are selected on the basis of the expected relaxation times: 0.3, 0.6, 5, 10, and 20 s. The five prefactors used to combine these spectra are calculated from simultaneous equations that define points on the modulation curve. Specifically, components with $T_{1,C}$ values of 0.37 or 50 s are

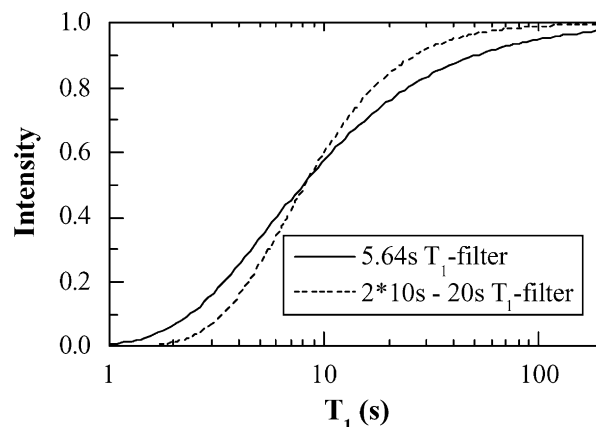


Fig. 2. Modulation curves calculated for a 5.64 s T_1 -filtered experiment and a 2*10 s – 20 s T_1 -filtered experiments. The selectivity is improved using the difference technique.

completely removed. Two additional equations are defined by the requirements that components between 1 and 5 s should be near unitary. The fifth and final simultaneous equation is that the sum of the 5 prefactors should be zero to suppress extremely long relaxing components. Thus the requirements are

$$Ae^{-0.3/0.37} + Be^{-0.6/0.37} + Ce^{-5/0.37} + De^{-10/0.37} + Ee^{-20/0.37} = 0,$$

$$Ae^{-0.3/1} + Be^{-0.6/1} + Ce^{-5/1} + De^{-10/1} + Ee^{-20/1} = 1,$$

$$Ae^{-0.3/5} + Be^{-0.6/5} + Ce^{-5/5} + De^{-10/5} + Ee^{-20/5} = 1,$$

$$Ae^{-0.3/50} + Be^{-0.6/50} + Ce^{-5/50} + De^{-10/50} + Ee^{-20/50} = 0,$$

$$A + B + C + D + E = 0.$$

The prefactors are calculated with linear algebra. The resulting values are then normalized so that the maximum intensity value is 1: $A = -1.46$; $B = 3.29$; $C = -2.11$; $D = -0.512$; $E = 0.800$. The modulation curve using these prefactors, and a second curve optimized to retain signals between 1 and 3 s are shown in Fig. 3. Both curves reveal that the desired modulation goals have been obtained; a negative region exists below 0.37 s, but the significance is reduced if excitation is through CP because the excitation efficiency for carbons with fast dynamics is reduced. Interestingly, the negative values of the same prefactors can be used to obtain a

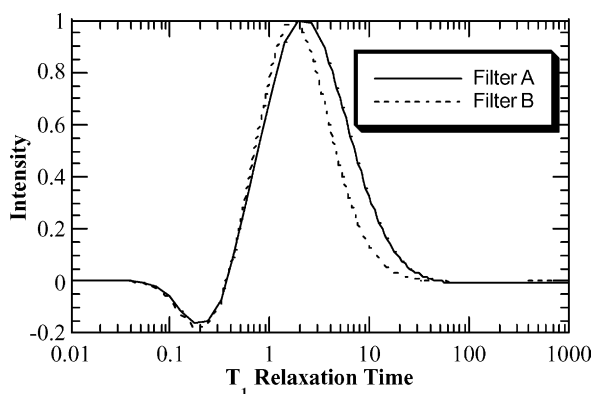


Fig. 3. Shaped modulation curves to suppress short and long components. Filter A uses coefficients of -1.46 , 3.29 , -2.11 , -0.512 , and 0.800 for 0.3, 0.6, 5, 10, and 20 s T_1 -filtered experiments, respectively. Filter B uses similar filter lengths, but coefficients of -1.66 , 3.74 , -4.62 , 2.91 , and -0.372 to achieve a narrower shape.

similar modulation profile of DP spectra using multiple recycle delays instead of filter delays.

3. Experimental section

3.1. Samples and experimental parameters

The demonstration of the difference-NMR technique was performed using two model samples. Isotactic poly(1-butene) (iPB-1), $[-CH_2CHR-]_n$, with $R = CH_2CH_3$, a semicrystalline polymer with two backbone and two side-group carbon sites, of $M_w = 570,000$ was obtained from Aldrich. The as-received polymer pellets were prepared for NMR studies by melting at approximately 423 K, followed by slow cooling and storage at room temperature for several weeks to ensure complete conversion to crystal form I (iPB-1). Dimethyl sulfone (DMS; $O_2S(CH_3)_2$) was used in powder form as received from Aldrich.

The experiments were performed using a VARIAN INOVA spectrometer at ^{13}C and 1H frequencies of 100.5 and 400.0 MHz, respectively. A VARIAN 7-mm MAS double resonance variable temperature probe head was used. $\pi/2$ pulse lengths of 3.8 and 4.0 μs were applied for ^{13}C and 1H , respectively. Proton decoupling field strength of approximately 65 kHz, cross-polarization time of 1.0 ms were used. Standard inversion-recovery experiments were used to measure the T_1 values of all samples.

3.2. T_1 relaxation times

In order to show the details of the difference-NMR technique it is desirable to estimate the values of the relaxation times of the sample. For this reason, the T_1 -relaxation times of the different carbon sites in iPB-1 and DMS were measured using a standard inversion-recovery pulse sequence performed under magic-angle-spinning (MAS) and high power decoupling. The obtained values are shown in Table 1. The iPB-1 sample is particularly useful for demonstrating the efficiency of the difference NMR technique because of the reasonable difference between the T_1 relaxation times of the backbone (~ 10 s) and side groups (~ 2 s) carbons in the crystalline regions. Additionally, the T_1 value of the amorphous region is also quite short as compared to the backbone crystalline carbons, making possible the

Table 1
Longitudinal relaxation times of the different groups in the samples as measured by standard inversion recovery experiments

Sample	IPB-1					DMS
Site	b-CH (crystalline)	b-CH ₂ (crystalline)	sc-CH ₂ (crystalline)	sc-CH ₃ (crystalline)	sc-CH ₃ (amorphous)	CH ₃
T_1 (s)	11.0 ± 0.5	10.0 ± 0.5	1.2 ± 0.1	1.7 ± 0.1	1.0 ± 0.1	0.180 ± 0.001

b, backbone; sc, side-chain.

use of the proposed techniques to selectively excite or suppress the signal arising from fast relaxing groups. In the case of demonstrating the suppression using modulated profiles, it is necessary to have another component with an even shorter T_1 . This was achieved using a physical mixture of iPB1 with DMS, which has a very short T_1 of 0.18 s.

3.3. Suppression of long T_1 -components in direct excitation spectra

Fig. 4 shows a set of direct-polarization magic-angle-spinning (DPMAS) Spectra obtained with different recycle delays, t . The spectra obtained with $t = 100$ s shows sharp CH, CH₂, and CH₃ peaks and also a broad signal which is attributed to backbone carbons in the amorphous region of the polymer. The T_1 values of the five observed peaks range from 1.0 (amorphous side-chain CH₃) to 11 s (backbone crystalline CH). Quantitatively selecting the CH₃ peaks while fully suppressing the backbone CH₂ carbons is not theoretical possible with a simple direct excitation experiment. Fig. 4b shows a spectrum acquired with a recycle delay sufficient to fully relax the CH₃ components ($t = 6$ s). It is evident that the signal from the CH and CH₂ are not fully

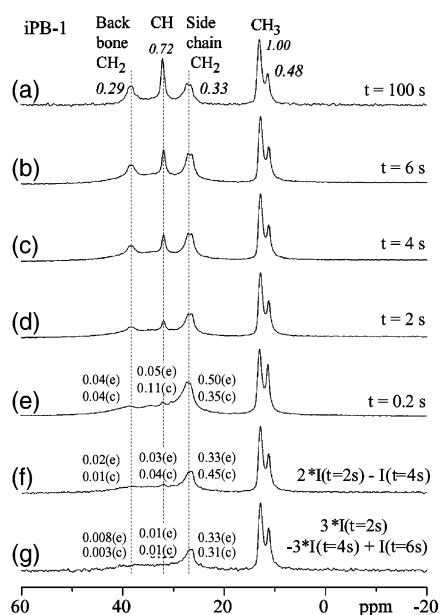


Fig. 4. Direct-polarization magic-angle-spinning spectra of iPB-1. (a) Reference spectrum with recycle delay of 100 s. (b) Spectrum with recycle delay of 6 s. (c), (d), and (e) Spectra with recycle delays of 4, 2, and 0.2 s, respectively. (f) Result of the combination of the spectra shown in (c) and (d) according to Eq. (4). (g) Combination of (b), (c), and (d) using weighing factors of 1, -3, and 3. The spectra show in (e), (f), and (g) where acquiring within the same total experimental time. The relative line intensities, normalized to the maximum height of each spectrum, are also shown and compared with the calculated values. The peaks at 32 and 39 ppm are affected by a broad, fast-relaxing and required baseline subtraction to quantify peak intensity.

suppressed. A reasonable suppression of the crystalline peaks is only achieved if the recycle delay is decreased to 0.2 s, Fig. 4e. However, compared to the $t = 100$ s spectrum, the ratio between the two CH₃ peaks and also between the CH₂-side chain and CH₃ signals is noticeably changed. Much better result is obtained using the difference technique, as shown in Figs. 4f and g. In Fig. 4f, two spectra acquired with $t = 2$ s and $t = 4$ s were combined according to Eq. (4). The result is a better suppression of the long- T_1 components with less modification in the intensity ratios of the remaining peaks. Even better suppression is achieved by using a combination of $3 \cdot 2$ s - $3 \cdot 4$ s + 6 s, Fig. 4g. The intrinsic decrease of signal-to-noise ratio in the difference-NMR technique is also demonstrated in Fig. 4. The spectra shown in 4e, 4f, and 4g were acquired with the same total experimental time, showing the progressive decrease of signal-to-noise ratio.

In the present situation the T_1 of the samples were measured in order to better understand the effects of the difference-NMR technique in the spectrum. However, in practical situations these T_1 measurements may become unfeasible. An experimental procedure to choose the recycle delays used in the difference spectrum is performing a standard variation of the recycle delay and taking the recycle delay where the fast relaxing component with minimum T_1 has fully relaxed. Then, this recycle delay is chosen as the $2t$ -signal in Eq. (4), and the recycle delays for the other spectra are chosen according to the number of spectra in the combination. The recycle of delay of 2, 4, and 6 s used in the experiments shown in Fig. 4 were chosen according to this procedure and not based on the measured T_1 values.

3.4. Suppression of fast relaxing groups in $T_{1,C}$ filtered CPMAS experiments

Fig. 5 shows the spectra obtained after the application $T_{1,C}$ filtered CPMAS experiments. Fig. 5a shows the standard CPMAS experiments for comparison, while Figs. 5b and c show the results of the application of a standard T_1 -filter (filter time of 5.64 s) and the difference-NMR technique (filter times of 10 and 20 s) respectively. These filter times were chosen in order to compare with the theoretical prediction shown in Fig. 3. The intensity lost of the CH signal is 42 and 37% in the standard T_1 -filter and in the difference-NMR technique, respectively. Moreover, for the fast relaxing components 7 and 4% of the signal are retained in each experiment. These relative intensity values of the CH peak are in good agreement with the theoretical curve shown in Fig. 2, but the CH₃ peak is unexpectedly intense. This is due to non-exponential relaxation. A more optimal combination of spectra could have been selected to quantitatively retain the long T_1 components while still suppressing most of the short T_1 components, but the purpose of this

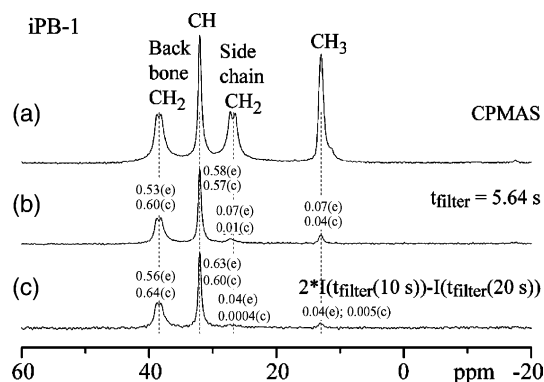


Fig. 5. $T_{1,C}$ filtered CPMAS experiments on iPb-1. (a) Reference CPMAS spectrum. (b) Spectrum with filtering time of 5.64 s. (c) Combination of spectra with filtering times of 10 and 20 s according to Eq. (4). The plots are in the same vertical scale and the numbers indicate the experimental and calculated intensity of the peak relative to the reference CPMAS spectrum.

experiment was comparison of the calculated curve in Fig. 2 to experiment.

3.5. Suppression of very fast and slow relaxing groups using shaped modulation profiles

The feasibility of the simultaneous suppression of very fast and slow relaxing segments was demonstrated in a physical mixture of iPb-1 and DMS. This sample

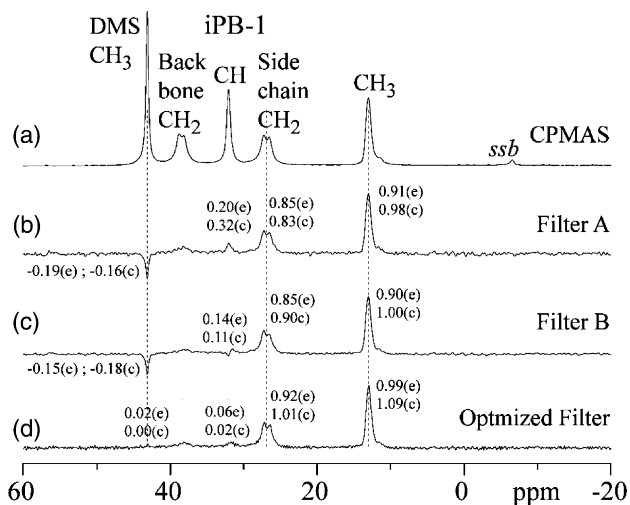


Fig. 6. $T_{1,C}$ -filtered CPMAS experiments on a physical mixture of DMS and iPb-1. (a) Reference CPMAS spectrum. (b) Combination of spectra with filtering times of 0.3, 0.6, 5.0, 10.0, and 20.0 s using the weighing factor for Filter A. (c) Same as (b) using the weighing factor for Filter B. The weighing factor for Filter A and B are explicitly shown in the caption of Fig. 3. (d) Optimized filter to suppress DMS and long T_1 components. The weighing factors used are -0.44 , $+2.30$, -3.04 , and $+0.02$, -1.16 . The plots are in the same vertical scale and the numbers indicate the experimental and calculated intensities of the peaks relative to the reference CPMAS spectrum.

is constituted of groups with basically three distinct relaxation times: CH₃-DMS ($T_1 = 0.18$ s), amorphous and crystalline CH₃ and CH₂ iPb-1 side chain iPb-1 groups ($T_1 \sim 2.0$ s) and crystalline backbone carbons of iPb-1 ($T_1 \sim 10.0$ s). Figs. 6b and c show the resulting $T_{1,C}$ filtered CPMAS spectra obtained after the application of the filters A and B described in Fig. 3. Five individual spectra acquired with filter times of 0.3, 0.6, 5.0, 10.0, and 20.0 s were combined using the weighing factors shown in the caption of Fig. 3. Compared with the standard CPMAS spectrum of Fig. 6a, the remaining intensities are $\sim 15\%$ for the short, $\sim 15\%$ for long, and $85\text{--}90\%$ for the intermediate T_1 components. Additionally, a negative (CH₃-DMS) peak with an intensity of 0.19 for Filter A and 0.15 for Filter B is observed. The observed peak intensities are in agreement with the theoretical prediction of Fig. 3. Optimized weighting factors were calculated for the specific system to suppress the DMS and long T_1 components. The modified spectrum, Fig. 6d, shows almost complete suppression of undesired peaks while quantitatively retaining the desired components. This shows that under favorable conditions (distinct relaxation times) the combination of spectra according to the described procedure may be efficient in simultaneously removing very fast and slow relaxing components. If relaxation times are known prior to the experiment, a modulation can be designed to more effectively suppress specific peaks while retaining desirable components.

4. Conclusions

Difference-NMR can be a convenient and effective technique to achieve superior selection of components on the basis of relaxation times. Although not as effective as more sophisticated mathematical algorithms such as chemometrics, the method can be readily conducted. The main drawback is the reduction of the signal/noise ratio. Suppression can be limited if relaxation is non-exponential, but will not completely fail.

Acknowledgments

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