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Communication

A new detection scheme for ultrafast 2D *J*-resolved spectroscopy

Patrick Giraudeau *, Serge Akoka

Université de Nantes, Nantes Atlantique Universités, Laboratoire d'Analyse Isotopique et Electrochimique de Métabolismes, UMR CNRS 6006, Faculté des Sciences, B.P. 92208, 2 rue de la Houssinière, F-44322 Nantes Cedex 03, France

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Abstract

Recent ultrafast techniques enable 2D NMR spectra to be obtained in a single scan. A modification of the detection scheme involved in this technique is proposed, permitting the achievement of 2D ^{1}H *J*-resolved spectra in 500 ms. The detection gradient echoes are substituted by spin echoes to obtain spectra where the coupling constants are encoded along the direct v_2 domain. The use of this new *J*-resolved detection block after continuous phase-encoding excitation schemes is discussed in terms of resolution and sensitivity. *J*-resolved spectra obtained on cinnamic acid and 3-ethyl bromopropionate are presented, revealing the expected 2D *J*-patterns with coupling constants as small as 2 Hz.

Keywords: Ultrafast 2D NMR; ^{1}H NMR; *J*-resolved spectroscopy; Resolution enhancement; Spatial encoding

1. Introduction

The introduction of two-dimensional (2D) spectroscopy [1,2] has played a major role in the evolution of nuclear magnetic resonance (NMR) techniques. One of the first 2D NMR experiments proposed was homonuclear *J*-resolved spectroscopy [3], which has become a powerful tool for the analysis of complex proton NMR spectra. The pulse sequence, 90°—$t_1/2$—180°—$t_1/2$—acquisition (t_2), gives rise to a phase-modulated signal which allows, after 2D Fourier transform (FT), to separate chemical shift and coupling constant information along two distinct axes. Unfortunately, numerous t_1 increments and the collection of several independent transients are required to obtain a spectrum with a good resolution, which leads to very long acquisition times.

Recently, a novel method based on ultrafast imaging techniques was proposed by Frydman and co-workers [4,5], enabling the acquisition of 2D NMR spectra within a single scan. In this so-called "ultrafast 2D NMR" technique, the usual t_1 encoding is replaced by a spatial encoding, which is decoded during a detection period by an echo planar imaging (EPI) scheme [6]. However, the discrete encoding mode proposed suffers from practical drawbacks as it requires fast gradient switching carefully synchronized with RF irradiation. Moreover, it leads to the appearance of undesirable "ghost peaks" in the indirect domain [7]. To deal with these limitations, a continuous encoding scheme was proposed by Shrot et al. [8], using a pair of adiabatic pulses applied during a bipolar gradient. Unfortunately, this method involves an amplitude modulation which is incompatible with the phase-modulated *J*-resolved spectroscopy. Alternatives were then proposed [9–11] to obtain a phase-modulated encoding which provides the opportunity to achieve ultrafast *J*-resolved spectroscopy. Moreover, a modification of Tal's excitation scheme was proposed [9] to obtain a continuous phase encoding of homonuclear couplings along the indirect k/v_1 domain, leading to a 2D *J*-resolved spectrum in less than 250 ms. However, this technique suffers from limitations which are discussed below.

A new acquisition scheme is proposed, substituting detection gradient echoes by spin echoes, to obtain ultrafast *J*-resolved spectra where the coupling constants are encoded along the direct v_2 domain. This scheme is coupled to

* Corresponding author. Fax: +33 (0)2 51 12 57 12.
E-mail address: patrick.giraudeau@univ-nantes.fr (P. Giraudeau).

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Pelupessy's and Tal's excitation patterns and the resolution of both methods is compared to the conventional *J*-resolved technique.

2. Results and discussion

The excitation scheme proposed by Pelupessy [11] is described in Fig. 1a. It starts with a 90° non-selective pulse, followed by a 180° adiabatic pulse of duration τ_{ad} with a linear frequency ramp, applied during a magnetic field gradient $+G_e$. It is followed by an identical adiabatic pulse applied during an inverted gradient $-G_e$. At the end of the encoding process, the phase evolution of the system possesses a *z*-dependent term given by

$$\phi(z) = -\frac{4\tau_{ad}\Omega_1\gamma_e G_e}{\Delta\Omega_{ad}}\cdot z \approx -\frac{4\tau_{ad}}{L}\Omega_1\cdot z, \quad (1)$$

if we assume that the frequency range $\Delta\Omega_{ad}$of the adiabatic pulse is approximately adjusted to the one induced by the gradient, $\gamma_e G_e L$.

Tal et al. [9] proposed another method leading to a continuous phase encoding, described on Fig. 1b. It consists of a 90° continuous excitation performed by a chirp pulse of duration $\tau_{ad}^{\pi/2}$, with a linear frequency ramp, applied during a gradient $G_e^{\pi/2}$. This excitation is immediately followed by a 180° adiabatic pulse of duration τ_{ad}^{π}, applied during a magnetic field gradient G_e^{π}. This encoding process leads to an overall evolution phase containing constant, linear and quadratic terms. The latter can be removed if the condition

$$\tau_{ad}^{\pi/2}G_e^{\pi/2} = 2\tau_{ad}^{\pi}G_e^{\pi} \quad (2)$$

is respected [9]. Under this condition, the *z*-dependence of the phase evolution can be written

$$\phi(z) = \left(\frac{\gamma_e\tau_{ad}^{\pi/2}G_e^{\pi/2}}{2} + \frac{\tau_{ad}^{\pi/2}}{L}\left(1 - \frac{G_e^{\pi/2}}{G_e^{\pi}}\right)\Omega_1\right)\cdot z, \quad (3)$$

assuming that the frequency range of each chirp pulse matches the one of the corresponding gradient ($\Delta\Omega_{ad}^P \approx \gamma_e G_e^P L$, with $P = \pi/2$ or π). In Eq. (3), the Ω_1-independent contribution is an offset that can be easily compensated by an appropriate gradient G_c prior to acquisition. For both encoding schemes, the evolution phase also contains a *z*-independent term which leads to a first-order phase distortion. Spectra are represented in magnitude mode to avoid dealing with this distortion.

Both excitation schemes lead to phase-modulated encoding, which is compatible with *J*-resolved spectroscopy. It is interesting to note that Tal's scheme presents a $[1 - G_e^{\pi/2}/G_e^{\pi}]$ scaling factor that allows to adjust the level of spatial encoding. Moreover, Tal et al. have shown [9] that when the conditions $G_e^{\pi/2} = G_e^{\pi}$ and $\tau_{ad}^{\pi/2} = 2\tau_{ad}^{\pi}$ are fulfilled, the internal chemical shifts are refocused, but the homonuclear *J* evolution is not affected by the 180° pulse and is still encoded in the indirect k/v_1domain. After the usual EPI detection block and FT in v_2 dimension, a *J*-resolved spectrum is then obtained, with chemical shifts and *J*-couplings expressed along the v_2 axis, and *J*-couplings only in the k/v_1domain.

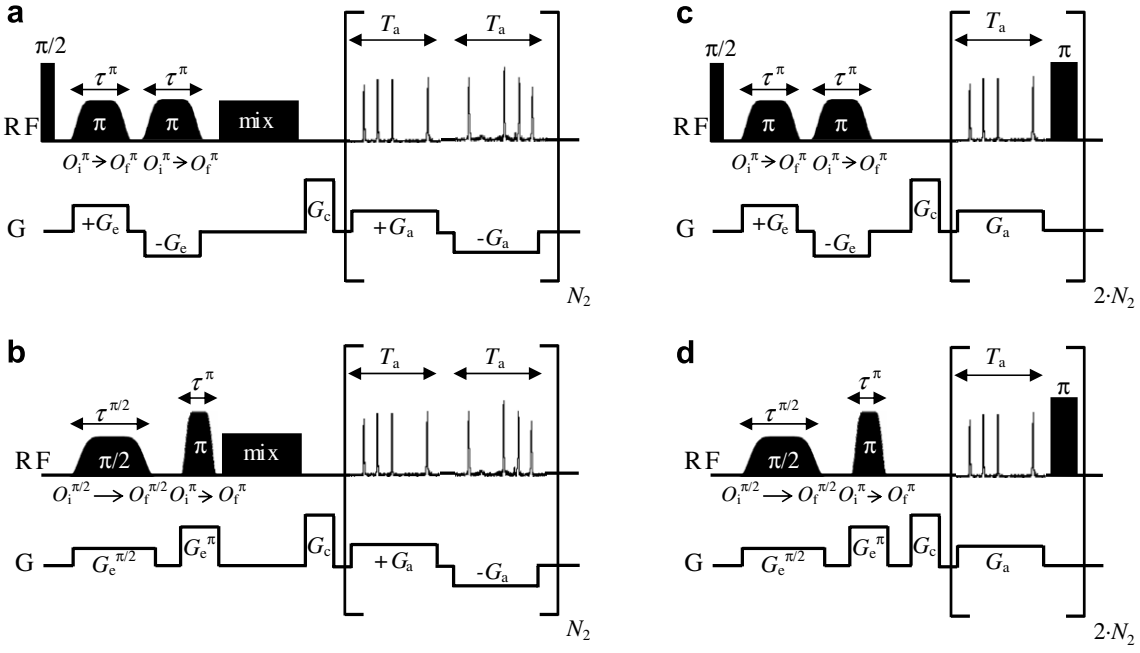

Fig. 1. (a and b) Pulse sequences for the acquisition of ultrafast 2D spectra, with phase-modulated encoding schemes proposed by Pelupessy (a) and Tal et al. (b), followed by the usual EPI detection block. (c and d) New ultrafast *J*-resolved detection scheme preceded by phase-modulated encoding schemes proposed by Pelupessy (c) and Tal et al. (d). The G_c gradient prior to acquisition is adjusted to set the middle of the chemical shift range in the middle of the detection period T_a. The 180° pulse phase is alternated as described in the text. Note that the detection loop is repeated $2{\cdot}N_2$ times in the new scheme to obtain the same number of FIDs than with the original EPI scheme.

It must be noted that this method may be more sensitive to molecular diffusion than the other excitation schemes, as it relies on a constant acquisition gradient applied during the encoding phase [9]. Consequently, stronger motion-related attenuation effects can be expected [12]. For this reason, it seems useful to elaborate a method where coupling constants are obtained in the direct v_2 dimension. To achieve this encoding, we propose a modification of the EPI detection block that can be applied to Pelupessy's (Fig. 1c) or Tal's (Fig. 1d) excitation schemes. The excitation blocks are unchanged compared to Fig. 1a and b. They lead to a continuous phase-encoding of chemical shifts and J-couplings in the k/v_1domain. The acquisition block must be modified to refocus the effect of chemical shifts. To achieve this goal, the acquisition gradients are separated by non-selective 180° pulses that refocus the effect of internal chemical shifts while the homonuclear J-evolution remains unaffected. As the 180° pulses also refocus the effect of acquisition gradients, there is no need for gradient inversion, contrary to the initial EPI pattern. This scheme, repeated $2{\cdot}N_2$ times, results in a $2{\cdot}T_a{\cdot}N_2$ total digitization time. Two interleaved mirror-image datasets are obtained and can be separated in the same manner as the ones obtained by the usual EPI block, to lead to the 2D spectrum after FT in the v_2 dimension.

This new J-resolved detection scheme presents several advantages. The main one is the small spectral width SW_2 required in the v_2 dimension. It leads to long acquisition times T_a (typically a few ms) which permit the observation of a large chemical shift range in the k/v_1 dimension with a good resolution. Moreover, there is no need for strong acquisition gradients, contrary to the schemes that have been presented before. Finally, there is no alternation between the successive decoding gradients, contrary to the initial EPI block. That is an important feature, as $+G_a$ and $-G_a$ are rarely of identical magnitudes because of the possible gradient amplifier offset. These nonidealities had to be compensated by data processing, which is not the case here.

However, the cumulated effects of the 180° hard pulse imperfections induce the formation of spurious stimulated echoes [13] for a series of three successive pulses, giving rise to undesirable signals at $v_2 = 0$ Hz. To compensate for it, we introduced a variation of the 180° pulse phase which is: y, y, $-y$, $-y$. It actually corresponds to a simple phase alternation $(y, -y)$ for each of the two interleaved datasets. Its effect is to move the artefacts towards the edge of the v_2 J-coupling range.

The new J-resolved detection scheme was first tested together with Pelupessy's phase encoding scheme [11] (Fig. 1c). We applied it to a 100 mmol L^{-1} solution of cinnamic acid in DMSO-d_6 to compare our results with those obtained by Tal et al. on this molecule [9]. The resultant spectrum is presented on Fig. 2. The components of the olefinic doublets, characterized by a 16 Hz coupling constant, are clearly better separated than in the aforementioned work, thanks to the good resolution obtained in

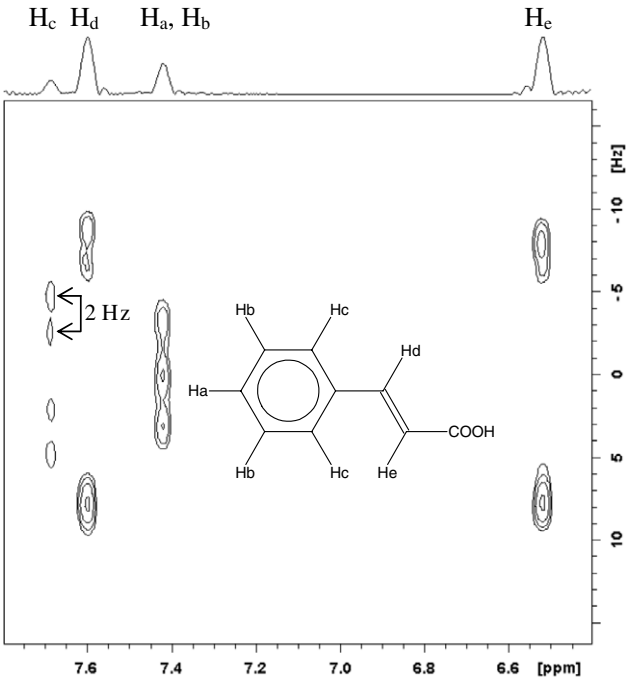

Fig. 2. 2D 500 MHz ultrafast J-resolved spectrum of a 100 mmol L^{-1} cinnamic acid sample in DMSO-d_6 obtained at 303 K. The spectrum, presented in magnitude mode, was recorded in 500 ms using Pelupessy's phase-encoded excitation scheme followed by our new detection block.

the v_2 dimension. The spectrum also reveals a very fine J structure of the aromatic protons (2 Hz) that was not visible with the previous detection scheme.

This new detection scheme offers interesting outlooks for further applications in various domains, from structural analysis to quantitative NMR or *in vivo* spectroscopy. However, for later developments, it seemed necessary to study the resolution and sensitivity aspects of the technique. For this purpose and to illustrate that our method was not limited to a small chemical shift range, we applied it to a 100 mmol L^{-1} solution of 3-ethyl bromopropionate in $CDCl_3$. The ultrafast J-resolved spectrum (Fig. 3b), acquired in 500 ms is compared to the conventional one (Fig. 3a), recorded in 3 h 8 min. Both spectra were obtained with the same acquisition conditions and processed with the same parameters, as described in Section 4. All multiplet structures (3 triplets and 1 quadruplet) are clearly visible on the ultrafast spectrum, and the 2D pattern is the same as on the conventional spectrum. To compare resolution and sensitivity of both methods in the J-coupling dimension, we plotted (Fig. 4a and b) the column corresponding to the quadruplet at 4.16 ppm. The signal-to-noise ratio for the ultrafast method (measured on the highest peak of the quadruplet) is approximately 100, which is still a correct value compared to the one obtained for the conventional method ($S/N = 550$). The resolution in the v_2 dimension for the ultrafast method appears very satisfactory compared to the conventional 2D experiment and should be sufficient for most of J-resolved experiments. However, one should notice (Fig. 3b) the low resolution

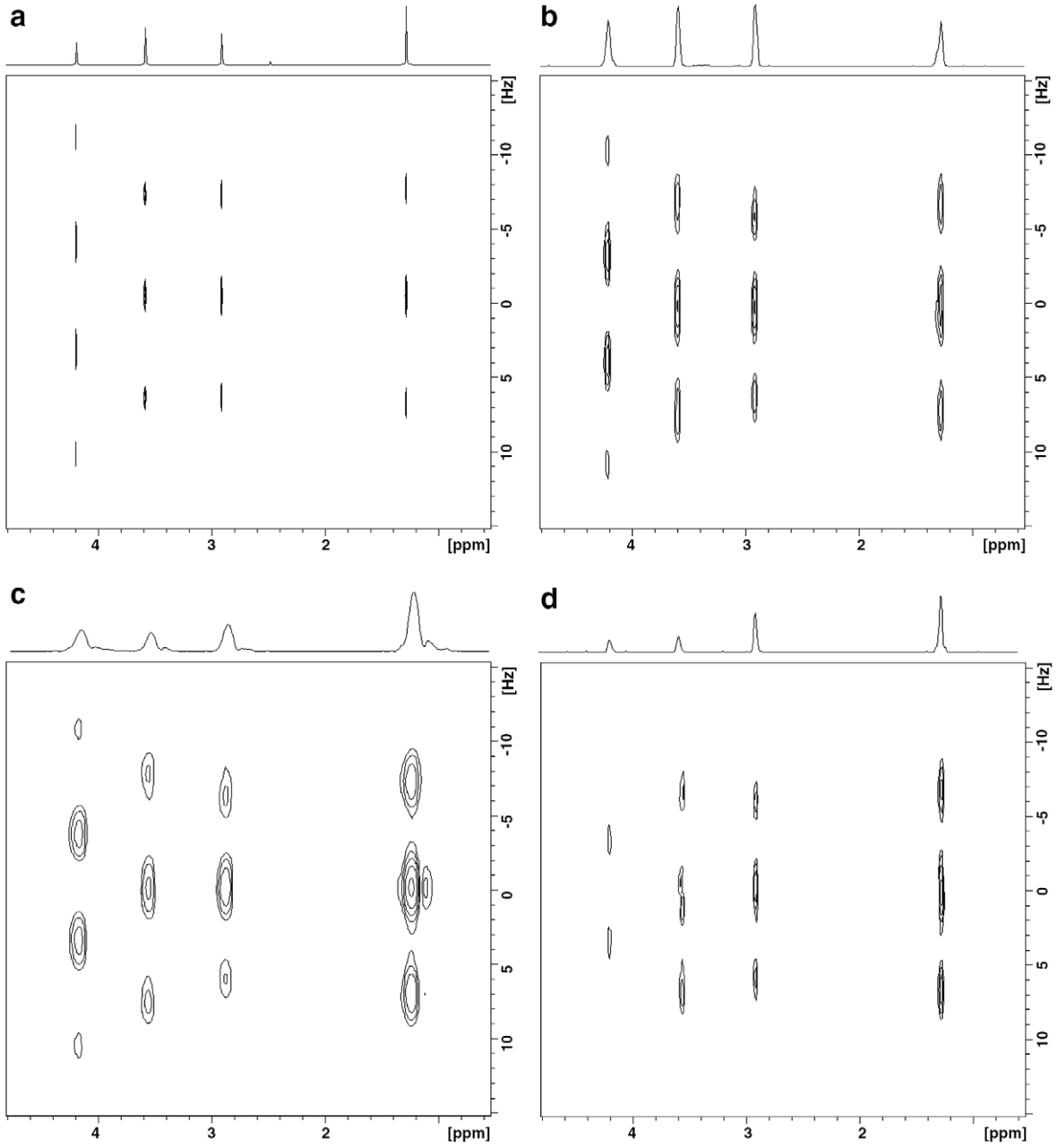

Fig. 3. Comparison between 500 MHz *J*-resolved spectra acquired on a 100 mmol L^{-1} 3-ethyl bromopropionate sample in $CDCl_3$ at 298 K. (a) Conventional spectrum acquired in 3 h 8 min with 32 t_1 increments and 16 scans. (b) Ultrafast spectrum obtained in 500 ms using Pelupessy's (b) or Tal's (c) phase-encoded excitation scheme followed by our new detection block. (d) Ultrafast spectrum acquired in 550 ms by combining the phase-encoded excitation schemes proposed by Tal and Pelupessy and the *J*-resolved detection block. All spectra, presented in magnitude mode, were processed with the same apodization function and post-processing parameters, as described in Section 4. For the mixed encoding scheme (d), the two external peaks of the quadruplet at 4.16 ppm are not visible for signal-to-noise reasons but can be observed on the corresponding column (Fig. 4d).

in the dimension where chemical shifts are expressed. The average half-height width, measured on the 2D ultrafast spectrum projection along k/v_1 axis, is approximately 18.5 Hz. This is due to the limitations of the ultrafast encoding period which should be extended to obtain a better resolution. However, this would decrease the signal-to-noise ratio because of transverse relaxation and translational diffusion. Therefore we chose a total encoding duration of 60 ms, which seemed the best compromise between sensitivity and resolution requirements.

We also applied our *J*-resolved detection block after Tal's phase encoding scheme [9] for the same sample. The resulting spectrum (Fig. 3c) and the quadruplet column (Fig. 4c) show that the resolution in the v_2 dimension is the same as the one obtained with Pelupessy's excitation scheme, which confirms that our detection block is applicable to both encoding techniques. The signal-to-noise ratio (measured on the highest peak of the quadruplet at 4.16 ppm) is the same as for Peluppessy's technique ($S/N = 100$). However, the resolution in the k/v_1 dimension

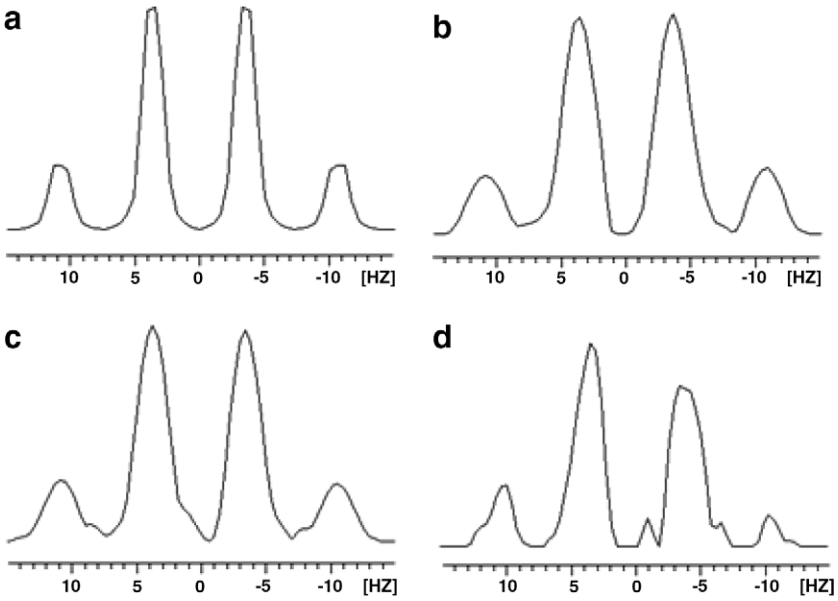

Fig. 4. 2D *J*-resolved spectra columns corresponding to the quadruplet at 4.16 ppm, for a conventional spectrum (a), for ultrafast spectra acquired with Pelupessy's (b) or Tal's (c) excitation schemes followed by the *J*-resolved detection block, and for an ultrafast spectrum obtained by combining both excitation schemes followed by the *J*-resolved detection block (d). All spectra were processed in the same way, as described in Section 4.

is significatively lower than in the precedent experiment, for an almost identical duration of the encoding period (52.5 ms). The average half-height width is 52 Hz. We tried to circumvent this drawback by using a longer $\pi/2$ chirp pulse, but it leaded to an important decrease of the signal-to-noise ratio.

After testing the *J*-resolved detection block with both encoding patterns, it seemed interesting to combine both excitation schemes to enhance the resolution in the k/v_1 dimension. To perform this combination, the $\pi/2$–π encoding block must be used first as it includes the excitation of spins in the transverse plane. It is immediately followed by the two identical 180° adiabatic pulses originating from the π–π scheme.

This mixed phase-encoding scheme was applied to the 3-ethyl bromopropionate sample with the same detection block. Nevertheless, the acquisition gradient G_a was increased as the spreading of the echo signals along the indirect domain *k*-axis was extended, due to a longer encoding period. The resultant spectrum (Fig. 3d) shows a better resolution in the indirect k/v_1 dimension. The half-height width was decreased to 17 Hz. The resolution along the v_2 axis is not significatively modified (Fig. 4d) as the T_a period remains unchanged. However, the long encoding period (112.5 ms) results in an important loss of sensitivity which is clearly visible for high chemical shifts. Its effect is a significant decrease of the signal-to-noise ratio ($S/N = 15$ for the highest peak of the quadruplet at 4.16 ppm).

Considering the results presented above, Pelupessy's scheme coupled to our new detection block could appear as the best compromise between sensitivity and resolution. However, Tal's scheme should be carried out under similar conditions before its comparison with Pelupessy's pattern. For this purpose, experiments will be performed in further analysis to compare both techniques using the same encoding level. As suggested by Andersen and Kockenberger [10], the use of opposite-sign gradients during the $\pi/2$ and π chirp pulses will be considered to increase the spatial encoding in Tal's scheme. Finally, the mixed scheme should be useful for spectra with many peaks in a small chemical shift range. This aspect should be developed in later works.

3. Conclusion

The new detection scheme presented in this paper appears very efficient to obtain ultrafast 2D *J*-resolved spectra in a single scan. It can be applied to either Pelupessy's or Tal's excitation schemes. The combination of both encoding patterns offers an interesting outlook for resolution improvement in the k/v_1 domain, and should be detailed later.

The most evident applications of this new technique are the fields where coupling constant information is necessary such as structural analysis. Moreover, *J*-resolved spectroscopy has been proved to be an efficient tool to separate 1D overlapping signals for quantitative analysis [14]. The use of ultrafast *J*-resolved spectroscopy for such purposes would allow considerable time-saving that could significantly improve repeatability on successive measurements. Finally, this method could be used for *in vivo* spectroscopy on whole body MR systems.

Some improvements will be evaluated in later works: the use of 180 degree pulses during acquisition could be applied to the new constant acquisition gradient scheme recently proposed by Shrot and Frydman [12].

The replacement of the 180° hard pulses by composite or adiabatic pulses could also be considered.

4. Experimental

In order to obtain 100 mmol L^{-1} solutions, 14.8 mg of *trans*-3-phenylacrylic acid (cinnamic acid), purchased from Sigma–Aldrich, were dissolved in 1 mL of DMSO-d_6, and 13.0 μL of 3-ethyl bromopropionate, purchased from Sigma–Aldrich, were dissolved in 1 mL of $CDCl_3$. After homogenization, each sample was filtered and analyzed in a 5 mm tube.

NMR spectra were recorded at 298 K for 3-ethyl bromopropionate and 303 K for cinnamic acid, on a Bruker Avance 500 DRX spectrometer, at a frequency of 500.13 MHz with a triple resonance TBI probe including *z*-axis gradient.

The conventional *J*-resolved spectrum (Fig. 3a) was acquired with 16 transients and 32 t_1 increments separated by 13.9 ms, leading to a spectral width of 72 Hz in F_1 dimension. FIDs in F_2 dimension were accumulated in 4.9 K channels, with a spectral width of 2500 Hz and a sampling period of 1.0 s. A $5{\cdot}T_1 = 20$ s repetition time was applied to avoid partial saturation.

For Pelupessy's encoding scheme, the following parameters were used: bipolar excitation gradient with gradients G_e of 30 ms and 3.2 G cm^{-1} each; 30 ms Wurst-8 adiabatic pulses [15] with a sweep range of 28.3 kHz. For Tal's scheme, a π/2 Wurst-8 chirp pulse with a 20.8 kHz sweep range was applied during 50 ms with a $G_e^{\pi/2}$ gradient of 2.0 G cm^{-1}. The π/2 pulse was carefully calibrated to obtain an accurate 90° excitation with an adiabaticity parameter of 0.068 [16]. It was followed by a π Wurst-8 adiabatic pulse with a 208 kHz sweep range, applied during 2.5 ms with a G_e^{π} gradient of 20 G cm^{-1}. The mixed encoding scheme was obtained by combining the pulses described above with corresponding gradients.

The *J*-resolved detection block was formed of 64 detection gradients of duration $T_a = 6.9$ ms each and a strength G_a adapted to observe the relevant chemical shift range during T_a (for cinnamic acid, $G_a = 1.6$ G cm^{-1}; for 3-ethyl bromopropionate, $G_a = 4.7$ G cm^{-1} for Pelupessy's scheme, 1.4 G cm^{-1} for Tal's scheme and 6.5 G cm^{-1} for the mixed scheme). For each experiment, the gradient G_c, applied during 500 ms, was set to adjust the centre of the chemical shift range in the middle of the acquisition window.

All spectra were processed in the same way in the *J*-coupling dimension (F_1 for the conventional spectrum, v_2 for ultrafast spectra): zero-filling once and identical apodization functions. All ultrafast spectra were subjected to noise subtraction along the k/v_1 axis.

All spectra were analyzed using the Bruker program Topspin 2.0. The specific processing for ultrafast spectra was performed using our home-written routine in Topspin.

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