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Communication

Ultrafast hetero-nuclear 2D J-resolved spectroscopy

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ABSTRACT

Ultrafast techniques enable the acquisition of 2D NMR spectra in a single scan. In this study, we propose a new ultrafast experiment designed to record hetero-nuclear ^{1}H–^{13}C J-resolved spectra in a fraction of a second. The approach is based on continuous constant–time phase modulated spatial encoding followed by a J-resolved detection scheme. An optional isotopic filter is implemented to remove the signal arising from ^{1}H bound to ^{12}C. While the most evident application of the technique proposed in this paper is the direct measurement of one bond scalar ^{13}C–^{1}H couplings for structural elucidation purposes, it also offers interesting potentialities for measuring ^{13}C isotopic enrichments in metabolic samples. The main features of this methodology are presented, and the analytical performances of the ultrafast hetero-nuclear J-resolved pulse sequence are evaluated on model samples.

1. Introduction

Two-dimensional nuclear magnetic resonance spectroscopy (2D NMR) has revolutionized the utility of NMR as an analytical technique [1,2]. One of the first 2D NMR experiments was the ^{1}H J-resolved (J-res) NMR proposed by Aue et al. [3], which allows the separation of chemical shifts and coupling interactions into two separate dimensions, greatly simplifying data analysis and resolution assignment, while also furnishing information about the homo-nuclear bonds in the sample through the J-couplings. Apart from being a powerful structural elucidation tool, this technique has found applications in bio-chemical studies, including metabolomics [4–6].

In 1976, the hetero-nuclear version of J-res NMR was proposed by Bodenhausen et al. [7]. Analogous to the homo-nuclear case, carbon-proton couplings are observed in the F_1 dimension while carbon chemical shifts are reflected in the F_2 dimension. The ability to probe scalar couplings or Residual Dipolar Couplings (RDCs) between ^{13}C and ^{1}H nuclei is highly helpful for numerous applications, such as molecular identifications or conformational analysis [8–10]. Despite its utility, one of the major disadvantages of this experiment, shared by all multi-dimensional NMR sequences, is the long experiment duration due to the t_1 incremental procedure necessary to sample the indirect F_1 dimension.

Various approaches to shorten the acquisition time of multi-dimensional NMR spectra have been developed [11–16]. The most efficient among these approaches is probably the so-called "ultrafast 2D NMR" approach proposed by Frydman and co-workers [17,18], where the acquisition of 2D NMR data is carried out in a single scan. The usual t_1 encoding is replaced by spatial encoding, followed by a conventional mixing period and by a detection block based on Echo Planar Imaging (EPI) [19]. The principles and features of ultrafast 2D NMR experiments have been described in details in recent literature [20–22]. One of the main advances in ultrafast 2D NMR methods is probably the replacement of the discrete spatial encoding scheme initially proposed by a continuous encoding pattern [23–26], relying on the combination of continuously frequency swept pulses applied during a bipolar gradient. The constant–time phase modulated encoding scheme proposed by Pelupessy [23] was shown to yield an optimal compromise between sensitivity and resolution [27,28], and it progressively became the method of choice to perform spatial encoding necessary for ultrafast experiments [29,30].

Despite its high potential, ultrafast 2D NMR still presents limitations in terms of sensitivity, resolution and spectral width, which have been described in recent papers [27,31–33]. Fortunately, several recent improvements have contributed to increase significantly the performances of ultrafast experiments, such as the introduction of a multi-echo excitation scheme to deal with sensitivity losses caused by molecular diffusion [28,34], or the implementation of various strategies to overcome spectral width and lineshape limitations [31,33,35–37].

In the present study we propose a new ultrafast experiment to record ultrafast hetero-nuclear J-resolved experiments. Its principles and features are described and potential applications are discussed and evaluated.

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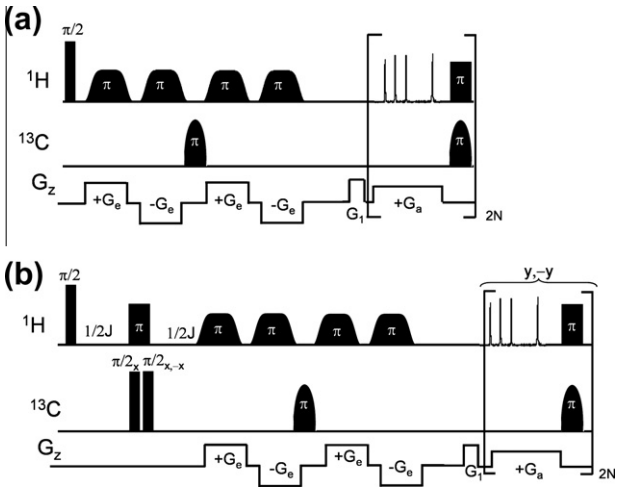

Fig. 1. Pulse sequences for ultrafast hetero-nuclear J-res without (a) and with isotopic filter (b), with ^{13}C decoupling in the ultrafast dimension, using a phase-modulated encoding scheme followed by a J-resolved detection scheme. The π hard pulses on the carbon channel are adiabatic cosine pulses with offset-independent-adiabaticity. The π pulse phase is alternated (y, y, $-y$, $-y$) to avoid undesirable stimulated echoes during detection. For the isotopic filter, two scans are necessary, with the indicated phase cycling on the second $\pi/2$ ^{13}C pulse and on the receiver, as indicated.

2. Method

The ultrafast hetero-nuclear J-resolved pulse sequence is presented in Fig. 1a. The excitation scheme proposed by Pelupessy [23], with a multiecho excitation designed to minimize molecular diffusion effects is employed [34]. A π adiabatic pulse is applied at the ^{13}C frequency in the middle of the excitation period to perform ^{13}C decoupling in the ultrafast dimension. The detection block, initially proposed by Giraudeau and Akoka [38], is modified by separating acquisition gradients with non-selective π pulses applied simultaneously on proton and carbon channels to refocus the effect of internal chemical shifts and proton–proton couplings. However the cumulated effects of the π hard pulse imperfections induce the formation of spurious stimulated echoes [39] for a series of three successive pulses, giving rise to undesirable signals at $v_2 = 0$ Hz. To compensate for it we introduce a y, y, $-y$, $-y$ phase variation on the ^{1}H and ^{13}C π pulses applied during the acquisition. Its effect is to move the artefacts towards the edge of the v_2 coupling range [38].

A notable difference with the homo-nuclear ultrafast J-resolved experiment is the large ^{13}C spectral range which needs to be decoupled. In order to perform homogeneous decoupling of the whole ^{13}C range, all the π hard pulses on the carbon channel are replaced by offset-independent-adiabaticity cosine adiabatic pulses optimized to obtain uniform decoupling [40].

In order to remove the signal arising from ^{1}H bound to ^{12}C, we also propose a modified version of the initial pulse sequence, based on an isotopic filter that can be introduced prior to spatial encoding, as shown on Fig. 1b. Provided that two scans are recorded with appropriate phase cycling on the second ^{13}C $\pi/2$ pulse and the receiver, the unwanted signal is completely filtered out for all protons.

3. Results and discussion

The ultrafast hetero-nuclear J-resolved experiment described in the previous section is first tested on a model mixture of labelled and unlabelled alanine (Fig. 2a). The spectrum is recorded with one scan in 190 ms. Proton-carbon couplings and ^{1}H chemical shifts are observed in the F_1 and the F_2 dimensions, respectively. The spectrum shows signals of ^{1}H attached to ^{12}C (central peaks) and ^{13}C (satellites), respectively. It is important to note that the spectrum is ^{1}H–^{1}H and ^{1}H–^{13}C decoupled in the spatially encoded dimension, with no need to perform the "tilt" operation commonly used in conventional J-resolved experiments.

In addition to the very short experimental duration, the ultrafast hetero-nuclear J-resolved experiment takes advantage of the high sensitivity of ^{1}H detection, which is not the case with the conventional hetero-nuclear J-resolved experiment. Another fundamental difference lies in the capacity of the conventional ^{13}C-detected experiment to detect only the signals arising from ^{1}H–^{13}C pairs, while the ultrafast experiment presented in Fig. 1a also detects resonances arising from ^{1}H bound to ^{12}C. This result can be viewed both as an advantage and a drawback. On the one hand, the possibility of detecting all protons provides an efficient way of measuring specific ^{13}C enrichments when working with partially labelled compounds. For the model alanine mixture, the site-specific ^{13}C enrichments were found identical to those measured by 1D NMR, i.e. 49.6% and 49.3% for the CH and the CH_3, respectively.

On the other hand, when working with complex mixtures and/or at low ^{13}C enrichments or natural abundance, the ^{1}H–^{12}C resonances may disturb the precise measurement of $^1J_{CH}$ coupling constants, which constitutes one of the main expected applications of the method. The modified version shown in Fig. 1b overcomes this drawback by removing the signal arising from ^{1}H bound to ^{12}C.

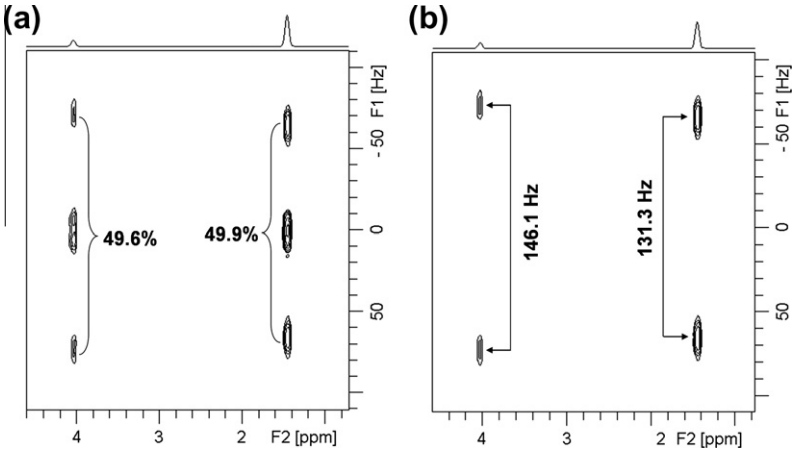

Fig. 2. Ultrafast hetero-nuclear J-res spectra, recorded without (a) and with isotopic filter (b), on a model mixture of labelled and unlabelled alanine. The spectra were acquired with the pulse sequences described in Fig. 1a and b respectively. The isotopic enrichments measured from Fig. 2a and the $^1J_{CH}$ couplings measured from Fig. 2b are indicated.

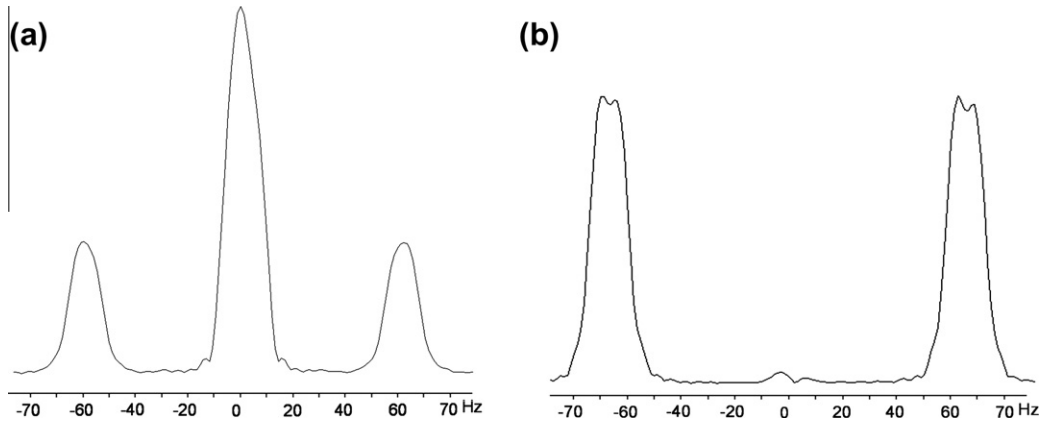

Fig. 3. Projection of columns corresponding to the alanine CH_3 peak at 1.47 ppm, obtained from the 2D spectra in Fig. 2, recorded without (a) and with isotopic filter (b).

This scheme was tested on the same model mixture (Fig. 2b). The spectrum was recorded with two scans. The 2D spectrum (Fig. 2b) shows the ^{13}C–^{1}H one bond couplings in the F_1 dimension. To evaluate the efficiency of the isotopic filter, we projected the columns of the alanine CH_3 peak at 1.4 ppm (Fig. 3) obtained from the spectra acquired with the two pulse sequences. The perfect suppression of the central peak shows the efficiency of the filter. The $^{1}J_{CH}$ coupling constants measured from the alanine sample (131.3 and 146.1 Hz) do not differ by more than 0.2% than those measured by 1D NMR (131.1 and 146.4 Hz). Moreover, in order to assess the precision of these measurements, ten successive experiments were performed, and the standard deviations on these values were found 0.1 Hz for both sites, indicating the high repeatability of the pulse sequence.

Prospective applications of this experiment include the measurement of coupling constants in non-enriched samples, a situation which is much more challenging regarding the filtering of ^{1}H–^{12}C signals. In order to assess the capacity of our pulse sequence to deal with such a situation, we applied it to a natural abundance ethyl bromopropionate sample. The corresponding spectra are represented in Fig. 4. The spectrum recorded without isotopic filter (Fig. 4a) clearly shows the impossibility to detect signals arising from ^{1}H bound to ^{13}C and to measure the corresponding coupling constants. When the filter is applied (Fig. 4b), these couplings become easily measurable even though the central signal is not totally suppressed. The residual central signal actually depends on the adequacy between the ^{1}H–^{13}C coupling constant of each signal and the delay chosen for the filter from the average coupling constant. Here, coupling constants were between 128 and 155 Hz, and an average J_{CH} of 140.4 Hz was chosen to calculate the filter delay. The resulting relative intensity of the central peak was between 0.4 and 1.7 relatively to the satellites, as a result of the scalar coupling mismatch. Still, the central peak does not affect the precision of the coupling constant measurement, as shown on the vertical projection of the peak at 2.9 ppm. If necessary, additional experiments may be performed while adapting the delay to a particular coupling constant, which could be necessary when the J_{CH} values are spread over a wide range.

While the capacity of the ultrafast method to measure coupling constants is relatively straightforward, its capacity to determine correctly isotopic enrichments in a wide range of values with good accuracy and precision needs to be evaluated. For that, we assessed the analytical performance of the experiment described in Fig. 1a on a series of [1-13C]-glucose mixtures with various isotopic enrichments ranging from 5% to 90%. The corresponding ultrafast 2D spectrum recorded in a single scan is represented in Fig. 5 for the 50% isotopic enrichment mixture. In aqueous solution, glucose undergoes a fast mutarotation reaction leading to a stable equilibrium between α- and β-glucopyranose [41], which explains the number of resonances observed. The isotopic enrichments are measured from the C_1^{α} resonance. For each sample, five 1D NMR and five 2D ultrafast hetero-nuclear J-res spectra are successively recorded to evaluate the precision of the method. For all samples, the isotopic enrichments measured by 2D NMR are plotted versus those obtained by quantitative 1D NMR. The accuracy is evaluated by the slope of the linear regression, and the determination coeffi-

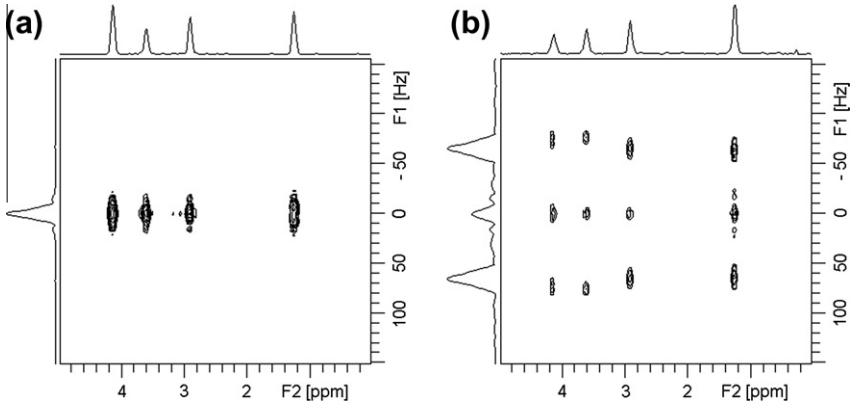

Fig. 4. Ultrafast hetero-nuclear J-res spectra, recorded without (a) and with isotopic filter (b), on a sample of ethyl 3-bromopropionate at natural abundance. The spectra were acquired with the pulse sequences described in Fig. 1a and b respectively. Two scans were necessary to record spectrum (b). The vertical projection of the column corresponding to the signal at 2.9 ppm is represented.

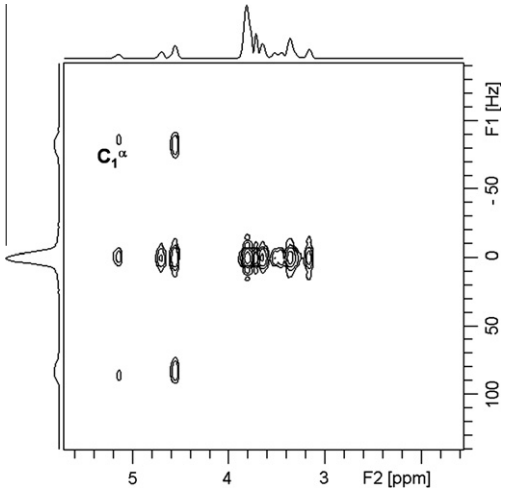

Fig. 5. Ultrafast hetero-nuclear J-resolved spectrum of a solution containing 50% of [1-^{13}C]-glucose and 50% of unlabelled glucose. The C_1^{α} peak was used for measuring ^{13}C isotopic enrichments.

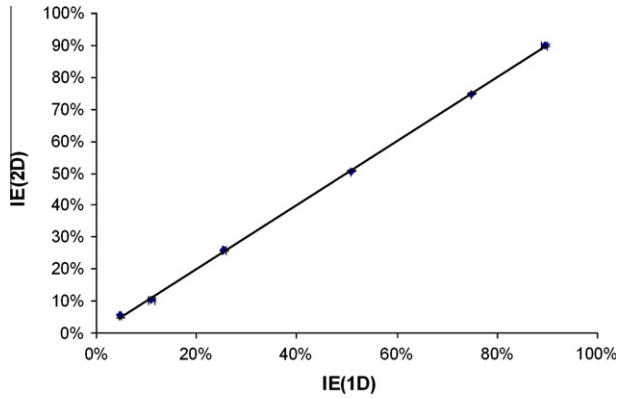

Fig. 6. ^{13}C-enrichments determined from ultrafast hetero-nuclear J-res spectra were plotted versus the values measured from 1D ^{1}H NMR. The [1-^{13}C]-glucose percentages were ranging from 5% to 90%. ^{13}C-enrichments in the C_1^{α} position of glucose were measured from the intensity of satellite signals relatively to the total intensity of the C_1^{α} signals.

cient r^2 is used to assess the linearity (Fig. 6). The values obtained highlight the excellent accuracy (slope close to unity, negligible intercept) and linearity (r^2 close to 1) of the method. Moreover, the small standard deviations (average 2%) highlight its very good repeatability. This high analytical performance opens new application perspectives of the ultrafast methodology as a tool for routine metabolic or fluxomic studies relying on specific ^{13}C-enrichment measurements [42]. The present study adds a new tool to the NMR-based metabolomics toolbox, an area where we had already proved the usefulness of ultrafast experiments [43].

A last point worth dwelling upon is the question of the sensitivity of the experiments described in this paper, especially when natural abundance samples are studied. The answer to this question is not unique, as sensitivity depends on the spectrometer and probe. However an estimation of the limit concentration that can be characterized for a given number of scans can be obtained from the Signal-to-Noise Ratio (SNR) values. For the 6.3 mol L^{-1} natural abundance ethyl bromopropionate sample studied in Fig. 4, an average SNR of 60 was obtained in the J_{CH} coupling dimension (the critical dimension from the SNR point of view) for a 2-scan experiment. Considering that a SNR of 3 corresponds to the limit of detection (LOD) [44], it means that the LOD would be around 0.3 mol L^{-1} with our hardware configuration. This value seems quite high, but lower concentrations are easily reached by signal averaging, a "multi-scan single shot" approach whose potentialities have been recently demonstrated [45]. Moreover, concentration is often not an issue for structural studies of small organic compounds where a sufficient amount of sample is available.

4. Conclusion

Ultrafast hetero-nuclear 2D J-resolved spectroscopy appears to be an efficient and versatile analytical tool to measure the proton-carbon couplings in a single scan or to access isotopic information. When an isotopic filter is employed, the most evident application of this technique is probably the direct measurement of one bond scalar ^{13}C–^{1}H couplings for structural elucidation purposes. Moreover, it should also form an interesting tool for measuring Residual Dipolar Couplings in oriented media [46]. When considering the non-filtered experiment, it opens the way towards applications in the field of metabolic and fluxomic studies by the measurement of isotopic enrichments in metabolic samples. The use of ultrafast J-resolved spectroscopy for such purposes would allow considerable time-saving that could significantly improve repeatability on successive measurements. Finally we are considering the extension of the ultrafast methodology to 3D NMR techniques in order to reach a higher level of discrimination between metabolite signals.

5. Experimental

A 1.16 mol L^{-1} mixture of various alanine isotopomers in D_2O was prepared as described in ref [42]. A 6.3 mol L^{-1} sample of ethyl 3-bromopromionate was prepared by adding 900 μL of this compound with 200 μL of acetone-d_6. Six samples containing unlabelled (natural abundance) and [1-^{13}C]-glucose in various ratios were prepared as described in Ref. [42]. The total amount of glucose was 50 mg, and the carbon-13 enrichment in position one was 5%, 10%, 25%, 50%, 75%, and 90%, respectively. The final glucose concentration was 0.46 mol L^{-1}. All the labelled compounds were purchased from Eurisotop (France).

All the NMR spectra were recorded at 298 K on a Bruker Avance III 500 spectrometer, at a frequency of 500.13 MHz with a cryogenically cooled probe including z-axis gradients and $\pi/2$ pulse of duration (PW_{90}) = 9.9 μs (except for the glucose sample where PW_{90} = 6.6 μs).

For all the ultrafast experiments, spatial encoding was performed using a constant-time spatial encoding scheme with four successive 15 ms smoothed chirp encoding pulses. The sweep range for the encoding pulses (60 kHz) was set to be significantly larger than the chemical shift range, and the amplitude of the encoding gradients was adapted to obtain a frequency dispersion equivalent to the frequency range of the pulses (G_e = 8.2 G/cm). A cosine adiabatic pulse with offset independent adiabaticity [40] of 300 μs with a sweep range of 60 kHz was applied at the ^{13}C frequency in the middle of the excitation period to perform ^{13}C decoupling in the ultrafast dimension.

The J-resolved detection block was formed of 128 detection gradients of duration T_a = 1.3 ms each. The acquisition gradient amplitude G_a was 40 G/cm. A non-selective π pulse and a cosine adiabatic pulse were applied simultaneously on proton and carbon channels, respectively.

The amplitude and duration of the pre-acquisition gradient were adapted to adjust the peak position in the ultrafast dimension. All the spectra were acquired and analyzed using the Bruker program Topspin 2.1. The specific processing for the ultrafast spectra was performed using our home-written routine in Topspin. The processing included an optimized gaussian apodization in the

ultrafast dimension to improve the line width and sensitivity, while conventional processing ($\pi/8$ shifted sinebell function and zero-filling) was performed in the conventional dimension. An automatic polynomial ($n = 5$) base line correction was applied in the conventional dimension.

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References

[1] J. Jeener, Lecture Presented at Ampere International Summer School II, Basko Polje, Yugoslavia, 1971.
[2] W.P. Aue, E. Bartholdi, R.R. Ernst, Two-dimensional spectroscopy. Application to nuclear magnetic resonance, J. Chem. Phys. 64 (1976) 2229–2246.
[3] W.P. Aue, J. Karhan, R.R. Ernst, Homonuclear broad band decoupling and two-dimensional J-resolved NMR spectroscopy, J. Chem. Phys. 64 (1976) 4226–4227.
[4] F. Rastrelli, A. Bagno, Selective J-resolved spectra: a double pulsed field gradient spin-echo approach, J. Magn. Reson. 182 (2006) 29–37.
[5] C. Ludwig, M.R. Viant, Two-dimensional J-resolved NMR spectroscopy: review of a key methodology in the metabolomics toolbox, Phytochem. Anal. 21 (2010) 22–32.
[6] A. Khatib, E.G. Wilson, H.K. Kim, A.W.M. Lefeber, C. Erkelens, Y.H. Choi, R. Verpoorte, Application of two-dimensional J-resolved nuclear magnetic resonance spectroscopy to differentiation of beer, Anal. Chim. Acta 559 (2006) 264–270.
[7] G. Bodenhausen, R. Freeman, D.L. Turner, Two-dimensional J spectroscopy: proton-coupled Carbon-13 NMR, J. Chem. Phys. 65 (1976) 839–840.
[8] J.S. Taylor, D.S. Garrett, M.P. Cohrs, Solution-state structure of the Dewar pyrimidinone photoproduct of thymidylyl-(3′ .fwdarw. 5′)-thymidine, Biochemistry 27 (1988) 7206–7215.
[9] U.R. Prabhu, S.R. Chaudhari, N. Suryaprakash, Visualization of enantiomers and determination of homo- and hetero-nuclear residual dipolar and scalar couplings: the natural abundant 13C edited J/D-resolved NMR techniques, Chem. Phys. Lett. 500 (2010) 334–341.
[10] J. Farjon, J.-P. Baltaze, P. Lesot, D. Merlet, J. Courtieu, Heteronuclear selective refocusing 2D NMR experiments for the spectral analysis of enantiomers in chiral oriented solvents, Magn. Reson. Chem. 42 (2004) 594–599.
[11] V.A. Mandelshtam, H.S. Taylor, A.J. Shaka, Application of the filter diagonalization method to one- and two-dimensional NMR spectra, J. Magn. Reson. 133 (1998) 304–312.
[12] R. Brüschweiler, F. Zhang, Covariance nuclear magnetic resonance spectroscopy, J. Chem. Phys. 120 (2004) 5253–5260.
[13] E. Kupce, R. Freeman, Two-dimensional Hadamard spectroscopy, J. Magn. Reson. 162 (2003) 300–310.
[14] E. Kupce, R. Freeman, Projection-reconstruction technique for speeding up multidimensional NMR spectroscopy, J. Am. Chem. Soc. 126 (2004) 6429–6440.
[15] P. Schanda, B. Brutscher, Very fast two-dimensional NMR spectroscopy for real-time investigation of dynamic events in proteins on the time scale of seconds, J. Am. Chem. Soc. 127 (2005) 8014–8015.
[16] B. Vitorge, G. Bodenhausen, P. Pelupessy, Speeding up nuclear magnetic resonance spectroscopy by the use of SMAll Recovery Times – SMART NMR, J. Magn. Reson. 207 (2010) 149–152.
[17] L. Frydman, A. Lupulescu, T. Scherf, Principles and features of single-scan two-dimensional NMR spectroscopy, J. Am. Chem. Soc. 125 (2003) 9204–9217.
[18] L. Frydman, T. Scherf, A. Lupulescu, The acquisition of multidimensional NMR spectra within a single scan, Prod. Natl. Acad. Sci. USA 99 (2002) 15858–15862.
[19] P. Mansfield, Spatial mapping of the chemical shift in NMR, Magn. Reson. Med. 1 (1984) 370–386.
[20] M. Gal, L. Frydman, Ultrafast multidimensional NMR: principles and practice of single-scan methods, in: D.M. Grant, R.K. Harris (Eds.), Encyclopedia of NMR, Wiley, New-York, 2009.
[21] M. Mishkovsky, L. Frydman, Principles and progress in ultrafast multidimensional nuclear magnetic resonance, Annu. Rev. Phys. Chem. 60 (2009) 429–448.
[22] A. Tal, L. Frydman, Single-scan multidimensional magnetic resonance, Prog. Nucl. Magn. Reson. Spectrosc. 57 (2010) 241–292.
[23] P. Pelupessy, Adiabatic single scan two-dimensional NMR spectroscopy, J. Am. Chem. Soc. 125 (2003) 12345–12350.
[24] B. Shapira, Y. Shrot, L. Frydman, Symmetric spatial encoding in ultrafast 2D NMR spectroscopy, J. Magn. Reson 178 (2006) 33–41.
[25] Y. Shrot, L. Frydman, Spatial encoding strategies for ultrafast multidimensional nuclear magnetic resonance, J. Chem. Phys. 128 (2008) 052209.
[26] Y. Shrot, B. Shapira, L. Frydman, Ultrafast 2D NMR spectroscopy using a continuous spatial encoding of the spin interactions, J. Magn. Reson 171 (2004) 163–170.
[27] P. Giraudeau, S. Akoka, Sources of sensitivity losses in ultrafast 2D NMR, J. Magn. Reson. 192 (2008) 151–158.
[28] P. Giraudeau, S. Akoka, Sensitivity losses and line shape modifications due to molecular diffusion in continuous encoding ultrafast 2D NMR experiments, J. Magn. Reson. 195 (2008) 9–16.
[29] P. Giraudeau, G.S. Remaud, S. Akoka, Evaluation of Ultrafast 2D NMR for Quantitative Analysis, Anal. Chem. 81 (2009) 479–484.
[30] C. Wu, M. Zhao, S. Cai, Y. Lin, Z. Chen, Ultrafast 2D COSY with constant-time phase-modulated spatial encoding, J. Magn. Reson. 204 (2010) 82–90.
[31] P. Pelupessy, L. Duma, G. Bodenhausen, Improving resolution in single-scan 2D spectroscopy, J. Magn. Reson. 194 (2008) 169–174.
[32] Y. Shrot, L. Frydman, The effects of molecular diffusion in ultrafast two-dimensional nuclear magnetic resonance, J. Chem. Phys. 128 (2008) 164513.
[33] Y. Shrot, L. Frydman, Spatial/spectral encoding of the spin interactions in ultrafast multidimensional NMR, J. Chem. Phys. 131 (2009) 224516.
[34] P. Giraudeau, S. Akoka, Resolution and sensitivity aspects of ultrafast J-resolved 2D NMR spectra, J. Magn. Reson. 190 (2008) 339–345.
[35] P. Giraudeau, S. Akoka, A new gradient-controlled method for improving the spectral width of ultrafast 2D NMR experiments, J. Magn. Reson. 205 (2010) 171–176.
[36] P. Giraudeau, Y. Shrot, L. Frydman, Multiple Ultrafast, Broadband 2D NMR spectra of hyperpolarized natural products, J. Am. Chem. Soc. 131 (2009) 13902–13903.
[37] P. Giraudeau, S. Akoka, Sensitivity and lineshape improvement in ultrafast 2D NMR by optimized apodization in the spatially encoded dimension, Magn. Reson. Chem. 49 (2011) 307–313.
[38] P. Giraudeau, S. Akoka, A new detection scheme for ultrafast J-resolved spectroscopy, J. Magn. Reson 186 (2007) 352–357.
[39] E.L. Hahn, Spin echoes, Phys. Rev. 80 (1950) 580–594.
[40] E. Tenailleau, S. Akoka, Adiabatic 1H decoupling scheme for very accurate intensity measurements in 13C NMR, J. Magne. Reson. 185 (2007) 50–58.
[41] N. Le Barc'H, J.M. Grossel, P. Looten, M. Mathlouthi, Kinetic study of the mutarotation of D-glucose in concentrated aqueous solution by gas–liquid chromatography, Food Chem. 74 (2001) 119–124.
[42] S. Massou, C. Nicolas, F. Letisse, J.-C. Portais, NMR-based fluoxomics: quantitative 2D NMR methods for isotopomers analysis, Phytochemistry 68 (2007) 2330–2340.
[43] P. Giraudeau, S. Massou, Y. Robin, E. Cahoreau, J.-C. Portais, S. Akoka, Ultrafast quantitative 2D NMR: an efficient tool for the measurement of specific isotopic enrichments in complex biological mixtures, Anal. Chem. 83 (2011) 3112–3119.
[44] ICH-Q2B, Validation of analytical procedures: methodology, 1995. <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm073384.pdf>.
[45] M. Pathan, S. Akoka, I. Tea, B. Charrier, P. Giraudeau, Multi-scan single shot quantitative 2D NMR: a valuable alternative to fast conventional quantitative 2D NMR, Analyst 136 (2011) 3157–3163.
[46] C.M. Thiele, Residual dipolar couplings (RDCs) in organic structure determination, Eur. J. Org. Chem. 34 (2008) 5673–5695.