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C. R. Chimie 9 (2006) 525–529
COMPTES RENDUS CHIMIE
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Improvement of the inverse-gated-decoupling sequence for a faster quantitative analysis by ^{13}C NMR

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Received 14 March 2005; accepted 13 June 2005

Available online 08 September 2005

Abstract

The inverse-gated-decoupling sequence enables quantitative ^{1}H decoupled ^{13}C spectra to be obtained. We modified this sequence so as to obtain the same result in less time. For that, we determined the optimal ^{13}C longitudinal-magnetisation initial value for a faster relaxation while ^{1}H decoupler is stopped. This value can be calculated via the longitudinal relaxation times and the nuclear Overhauser effects. For a given nucleus, a supplementary delay of ^{1}H decoupling and/or a pulse at the beginning of the recovery delay allows an acceleration of the ^{13}C longitudinal relaxation. We checked this result on the molecule of *N,N*-dimethylacetamide. A simultaneous quantitative analysis of all carbons was carried out with a recovery delay divided by four compared to the usual sequence. ***To cite this article: P. Giraudeau et al., C. R. Chimie 9 (2006)***.

Résumé

La séquence « inverse-gated-decoupling » permet d'obtenir des spectres ^{13}C découplés ^{1}H quantitatifs. Nous avons modifié cette séquence pour que le même résultat soit obtenu en un temps moindre. Pour cela, nous avons déterminé la valeur optimale de l'aimantation longitudinale ^{13}C initiale permettant une relaxation accélérée dès que le découplage est stoppé. Cette valeur peut être calculée à partir des temps de relaxation longitudinale et des coefficients d'effet Overhauser des noyaux considérés. Selon le noyau considéré, l'ajout d'un délai de découplage ^{1}H supplémentaire et/ou d'une impulsion au début du délai de récupération permet une accélération du retour à l'équilibre de l'aimantation ^{13}C. Nous avons vérifié ce résultat sur la molécule de *N,N*-diméthylacétamide. La quantification simultanée de tous les carbones a pu être effectuée avec un délai de récupération divisé par 4 par rapport à la séquence usuelle. ***Pour citer cet article : P. Giraudeau et al., C. R. Chimie 9 (2006)***.

Keywords: Inverse-gated-decoupling; Quantitative NMR; ^{13}C Spectroscopy

Mots clés : Découplage périodique inverse ; RMN quantitative ; Spectroscopie ^{13}C

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doi:10.1016/j.crci.2005.06.030

1. Introduction

^{13}C NMR spectroscopy offers a high potential for the quantitative analysis of complex mixtures, due to the broad spectral range and, usually, an absence of interference between peaks. It suffers, however, from poor sensitivity due to the low abundance and small magnetic moment of the ^{13}C nucleus. This sensitivity is further diminished if the conditions of quantitative NMR – inverse-gated-decoupling procedure [1] and a recycling delay between 5- and 10- times the longest spin–lattice relaxation time [2–4] – are employed. In fact, the use of these acquisition conditions may lead to unacceptably long accumulation times to obtain a spectrum with an acceptable signal/noise ratio for a solute in small concentration. The alternative is to add a paramagnetic relaxation reagent [5], such as triacetylacetonatochromium (III) $Cr(AcAc)_3$, which shortens the relaxation times. The unwanted side-effect of adding $Cr(AcAc)_3$ is that it leads to line-broadening. Furthermore, adding a paramagnetic relaxation reagent may alter the compound under investigation. Alternative methods have been employed, detecting the signal in non-quantitative conditions for comparing concentrations of molecules with similar relaxation properties [6]. Also, the DEPT sequence [7] can be applied for quantitative analysis when optimised [8], but only to compare carbons of the same nature, which resonate in a small spectral range. Finally, when an accurate ^{13}C NMR quantitative spectrum is needed for a sample with different carbons in a 200 ppm range, the inverse-gated-decoupling sequence is still employed with a recycling delay of about seven times the larger T_1, for the different carbons of interest [9]. We propose here to improve the inverse-gated-decoupling sequence so that the recycling delay may be significantly reduced while the results remain quantitative.

2. Theory

2.1. General description of the magnetisation time course during the recovery delay

We consider a simple *IS* spin system (*I* = insensitive: ^{13}C; *S* = sensitive: ^{1}H), coupled by intramolecular dipole–dipole relaxation. All the development will be done for ^{13}C and ^{1}H, but similar results could be deduced for other nuclei.

The standard inverse-gated-decoupling sequence for quantitative measurements has been developed by Freeman et al. [1]. It is $[T - D_{on} - 90° - FID - D_{off}]_n$, where D_{on} and D_{off} symbolise the switching of the proton decoupler, T is a waiting time allowing the system to return to thermal equilibrium, usually chosen between 5 and 10 $T_1(^{13}C)$ [2–4].

During acquisition, *I* magnetisation is detected in the transverse plane while *S* is saturated for obtaining a decoupled spectrum. In the same time, $\langle I_z \rangle$ increases and would reach the steady state value $I_0(1+\eta)$ if the acquisition time was very long. This may increase the delay T necessary for $\langle I_z \rangle$ to go back to I_0 before the next detection. That is why the acquisition time T_{AQ} is usually chosen short and at the end of the acquisition $\langle I_z \rangle = \langle I_z \rangle_{AQ}$ is shorter than I_0, depending on the length of the acquisition time.

At the end of the acquisition, *S* is no longer saturated. The behaviour of *I* and *S* is described by the two differential equations, first derived by Solomon [10]:

$$\begin{aligned} d\langle I_z \rangle/dt &= -R_I(\langle I_z \rangle - I_0) - \sigma_{IS}(\langle S_z \rangle - S_0) \\ d\langle S_z \rangle/dt &= -R_S(\langle S_z \rangle - S_0) - \sigma_{IS}(\langle I_z \rangle - I_0) \end{aligned} \quad (1)$$

where I_z and S_z are the z components of the ^{13}C and ^{1}H magnetisations; R_I and R_S are the inverse of the carbon and ^{1}H longitudinal relaxation times respectively. The quantities σ_{IS} and σ_{SI} are cross-relaxation terms that, in the case of two spins 1/2, are equal: $\sigma_{IS} = \sigma_{SI} = \sigma$. From these coupled equations, it is possible to find I_z magnetisation at any time t during the delay T:

$$\langle I_Z(t) \rangle = I_0 + C_+ \exp[-\lambda_+ t] + C_- \exp[-\lambda_- t] \quad (2)$$

with

$$\lambda_\pm = 1/2 \{ R_I + R_S \pm [(R_S - R_I)^2 + 4\sigma^2]^{1/2} \} \quad (3)$$

$$C_\mp = \pm \left\{ \frac{S_0 \sigma}{\lambda_+ - \lambda_-} \right\} \pm (\langle I_z(0) \rangle - I_0) \left(\frac{\lambda_\pm - R_I}{\lambda_+ - \lambda_-} \right) \quad (4)$$

and $\langle I_z(0) \rangle$ is the I_z value at the beginning of the recycle delay T, when S is no more saturated.

Eq. (3) means that I_z goes back to equilibrium biexponentially. The relaxation rates λ_+ and λ_- are both functions of R_I, R_S and σ, with λ_+ larger than λ_-. This means that the corresponding exponential term $\exp[-\lambda_+ t]$ should vanish more rapidly than $\exp[-\lambda_- t]$.

Then, I_z returns to equilibrium more rapidly if $C_- \ll C_+$. In that case, equilibrium should be reached for $t = 5/\lambda_+$.

It is interesting to determine the value of $\langle I_z(0)\rangle$ for which C_- would become null. It is called $\langle I_z(0)\rangle_{opt}$:

$$\left\langle I_z(0)\right\rangle_{opt} = I_0 - \left(\frac{S_0\,\sigma}{\lambda_+ - R_I}\right) \tag{5}$$

This equation can be simplified in two extreme situations.

If $\sigma >> |R_S - R_I|$, $\lambda_+ - R_I \cong \sigma$ and $\langle I_z(0)\rangle_{opt} \cong I_0 - S_0$.

If $\sigma << |R_S - R_I|$, $\lambda_+ \cong R_S$. The nuclear Overhauser enhancement factor η is defined by the relationship:

$$\eta = \frac{S_0\,\sigma}{I_0\,R_I} \tag{6}$$

In that case, $\langle I_z(0)\rangle_{opt}$ can be written:

$$\left\langle I_z(0)\right\rangle_{opt} = I_0\left(1 - \frac{R_I}{\lambda_+ - R_I}\,\eta\right) \tag{7}$$

$$\approx I_0\left(1 - \frac{R_I}{R_S - R_I}\,\eta\right) \approx I_0 \tag{8}$$

This shows that $\langle I_z(0)\rangle_{opt}$ can vary in a wide range.

In most cases, it should be possible to adapt the NMR sequence so that at the beginning of the delay T, $\langle I_z\rangle = \langle I_z(0)\rangle_{opt}$ instead of $\langle I_z\rangle_{AQ}$. This would enable $\langle I_z\rangle$ to go back to equilibrium faster. The modification will depend on the value of $\langle I_z(0)\rangle_{opt}$ compared to $\langle I_z\rangle_{AQ}$.

2.2. Optimisation of the sequence

Three cases can be considered.

(*i*) If $\langle I_z\rangle_{AQ} < \langle I_z(0)\rangle_{opt} < I_0$, it is possible to saturate S during a delay T_{sat} after the acquisition until $\langle I_z\rangle = \langle I_z(0)\rangle_{opt}$. In that case, T_{sat} is adjusted so that:

$$I_0(1+\eta)\left[1 - \exp\left(-R_I\,(T_{AQ} + T_{sat})\right)\right] = \left\langle I_z(0)\right\rangle_{opt} \tag{9}$$

(*ii*) If $-\langle I_z\rangle_{AQ} < \langle I_z(0)\rangle_{opt} < \langle I_z\rangle_{AQ}$, $\langle I_z\rangle$ can be reduced to the desired value by applying a RF pulse of angle θ.

(*iii*) If $-I_0(1+\eta) < \langle I_z(0)\rangle_{opt} < -\langle I_z\rangle_{AQ}$, $\langle I_z\rangle$ could be amplified by saturating S after the acquisition until $\langle I_z\rangle = -\langle I_z(0)\rangle_{opt}$. An inversion pulse would then enable one to obtain the desired value for $\langle I_z\rangle$.

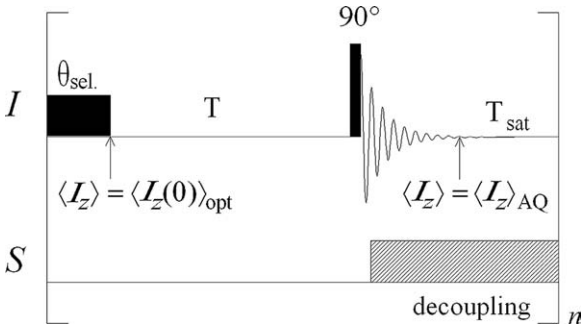

Fig. 1. Inverse-gated-decoupling sequence optimised for a faster quantitative analysis. A supplementary delay T_{sat} and a selective pulse θ_{sel} are added at the end of the acquisition. The values of T_{sat} and θ_{sel} *are adjusted so that* $\langle I_z\rangle = \langle I_z(0)\rangle_{opt}$ at the beginning of the recovery delay T.

Carbons with different relaxation parameters may be present in a same sample. In that case, a global optimisation could still be obtained by saturating S during a delay T_{sat} sufficient for increasing ^{13}C magnetisation with the larger value of $\langle I_z(0)\rangle_{opt}$, then replacing the RF pulse of angle θ by series of selective pulses with adequate flip angles for each carbon.

The corresponding sequence is represented in Fig. 1 for two types of carbons. As this sequence consists in Inverse-Gated Optimised Decoupling, we propose to call it 'InGOD'.

3. Material and methods

N,N-Dimethylacetamide, obtained from Sigma Aldrich, was diluted to 10% (v/v) in deuterated water and analysed in a 5-mm sample tube. All NMR spectra were recorded at 303 K, on a Bruker Avance 400 DPX spectrometer operating at 9.4 Teslas. ^{13}C spectra were recorded at a spectrometer frequency of 100.62 MHz with a 5-mm dual probe. Free induction decays (FIDs) were accumulated in 64-K channels, with a spectral width of 19.38 kHz, an acquisition time of 1.69 s, using 90° read pulses of length 6.8 μs, and eight scans. Proton decoupling was obtained with the WALTZ16 mode. Selective pulses were obtained by applying an attenuated RF field during 133 μs. An exponential apodisation function of 5.0 Hz was applied to the FID prior to Fourier transformation. Phase and baseline were corrected manually. The areas of the peaks were measured

by Lorentzian deconvolution with the Bruker program X-WinNMR 2.6. Nuclear Overhauser enhancement factors (η) for the different carbon sites were obtained by comparison of the corresponding peak areas I_{sat} and I_0 when ^{1}H were decoupled and inverse-gated decoupled respectively [1], waiting 320 s between the end of the acquisition and the read pulse. They are defined as: $I_{sat}/I_0 = 1 + \eta$. The recovery of ^{13}C magnetisation was studied by applying either an inverse-gated-decoupling sequence or the optimised sequence (Fig. 1) and waiting delays from 3 μs to 320 s before the read pulse.

Longitudinal relaxation times of ^{13}C were determined by using an inversion–recovery sequence with the decoupler on continuously. An inversion–recovery sequence was employed for ^{1}H longitudinal relaxation times determination. There, the baseline was corrected automatically and the areas of the peaks were analysed within the Bruker software.

4. Results and discussion

At the temperature considered, *N,N*-dimethylacetamide is composed of three non-equivalent CH_3 groups and of a carbonyl group. As the relaxation properties of the two N–CH_3 groups are quite close, we considered only three different spin systems: CH_3–C, CH_3–N and C=O. Their relaxation times are given in Table 1, together with their nuclear Overhauser enhancement η at steady state while ^{1}H were saturated. For each carbon, the longitudinal relaxation time of the closer ^{1}H group was also reported. The cross-relaxation coefficients σ were deduced from these values via Eq. (6). This enabled the calculation of λ_+ via Eq. (3). Finally, the value of $\langle I_z(0)\rangle_{opt}$ was calculated via Eq. (7) and is reported in Table 1. The value of $\langle I_z\rangle$ at the end of the acquisition was measured by applying an inverse-gated-decoupling sequence where the delay T is null. It is called $\langle I_z\rangle_{AQ}$ and is also reported. From these results, and in order to increase the carbon relaxation, we have chosen to enhance all ^{13}C magnetisation during a delay T_{sat} so that for C = O $\langle I_z\rangle = \langle I_z(0)\rangle_{opt}$ at the end of the delay T_{sat}. The length of T_{sat} was deducted from Eq. (9). It is 17.7 s. Then, the ^{13}C magnetisation of the CH_3 was significantly reduced by applying a selective 90° pulse at their average frequency. Fig. 2 represents the ^{13}C magnetisation time course as a function of T for the optimised pulse sequence (■) compared to the standard inverse-gated-decoupling sequence (○) for the carbonyl group (Fig. 2a) and for a CH_3 carbon bound to the carbonyl (Fig. 2b). Similar curves as in Fig. 2b were obtained for the other carbons CH_3. One observes that, in both cases, magnetisation recovers faster during the delay T when the InGOD sequence is employed. For a given carbon, if one compares this delay T to the T_1 of the corresponding carbon, it is in a range from 0.17 T_1, for the carbonyl, to 3.7 T_1, for the carbon C-2, which is much lower than the delay usually recommended for the inverse-gated-decoupling sequence. As a matter of fact, the additional delay T_{sat} makes the experiment longer. In order to assess the efficiency of our optimised version against the standard inverse-gated-decoupling sequence, we compared the global delay $T_{sat} + T$ necessary for magnetisation relaxation to the larger relaxation time T_1^{max} (Table 2). Finally, the delay needed for complete recovery of the magnetisation of all carbons is 50.7 s, which is less than 1.8 T_1^{max}, thus much less than the delay of 7 T_1^{max}, usually recommended for allowing magnetisation of quaternary carbons to return to equilibrium after the inverse-gated-decoupling sequence [9].

One should note that for the sample studied, the quaternary carbon is well separated from the CH_3 groups, which have similar relaxation properties and are in a small range. Therefore, it is very easy to apply a selective pulse on the CH_3 groups without disturbing the carbonyl magnetisation. If protonated carbons would

Table 1
Relaxation properties for the different carbons and for the protons dipolar coupled to them. Comparison of the magnetisation $\langle I_z\rangle_{AQ}$ at the end of the acquisition to the optimal value $\langle I_z(0)\rangle_{opt}$ deduced from the relaxation parameters

Carbon	$T_1(^{13}C)$	$T_1(^1H)$	η	$\frac{\langle I_z\rangle_{AQ}}{I_0}$	$\frac{\langle I_z(0)\rangle_{opt}}{I_0}$
C-3 (C=O)	28.8 s	2.81 s	0.85	0.18	0.91
C-2 (CH_3–N)	9.04 s	2.99 s	1.63	0.59	0.22
C-1 (CH_3–C)	8.62 s	2.81 s	1.70	0.63	0.21

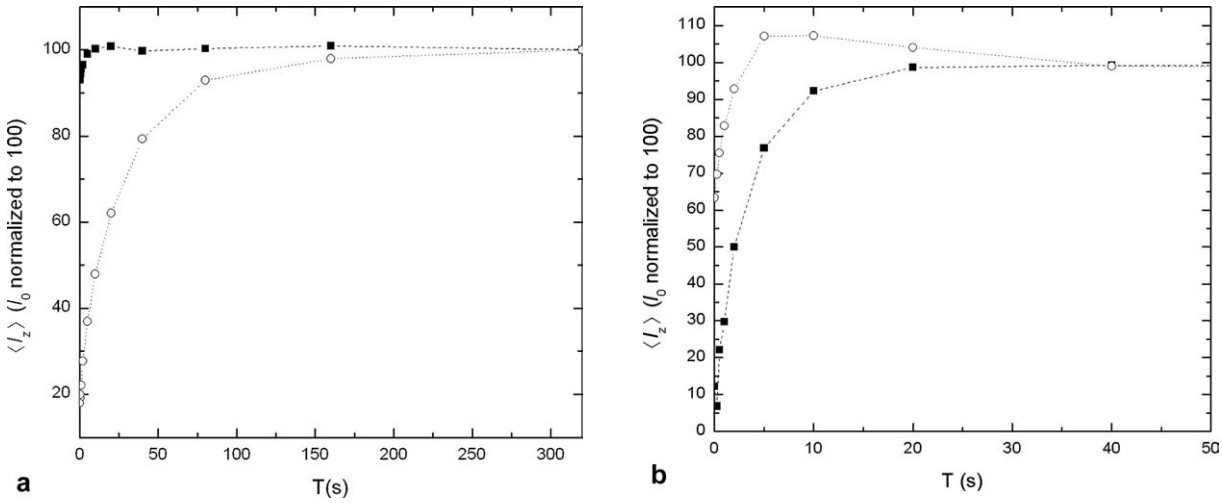

Fig. 2. ^{13}C longitudinal-magnetisation time course as a function of the recycle delay T, for the carbonyl group (**a**) and for a CH_3 carbon bound to the carbonyl (**b**). In the two graphs, the signals detected after the optimised sequence (■) are compared to those detected after the standard inverse-gated-decoupling sequence (○).

Table 2
Recovery delay of *N,N*-dimethylacetamide ^{13}C magnetisation after the saturating delay and the selective pulse for which $\langle I_z \rangle = I_0$ with a 1% precision

Carbon	T	$T_{sat} + T$
C-3 (C=O)	5 s	22.7 s (0.79 T_1^{max})
C-2 (CH_3–N)	33 s	50.7 s (1.8 T_1^{max})
C-1 (CH_3–C)	21.5 s	39.2 s (1.4 T_1^{max})

be in a more wide range, it should be possible to apply a series of selective pulses with optimal angles at the desired frequencies. For a better selectivity, shaped pulses as gaussians should then be used instead of low power rectangular pulses.

5. Conclusion

We have enhanced the inverse-gated-decoupling sequence for quantitative analysis to be performed faster. This optimisation can be performed accurately if the longitudinal relaxation times of the cross-relaxing nuclei are known, together with the NOE coefficients. It is particularly efficient in a sample containing quaternary carbons. The quantitative analysis of other carbons can be achieved provided that a selective pulse is applied at their resonance frequency. If this cannot be done, quantitative analysis could be performed in two separate experiments optimised for quaternary and fast relaxing carbons respectively. This method may be applied each time precise quantitative NMR is needed despite the presence of dipolar couplings.

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