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# Adiabatic Low-Pass J Filters for Artifact Suppression in Heteronuclear NMR

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Adiabatic pulses that consist of frequency-swept radio-frequency (rf) irradiation in the presence of a static magnetic field gradient have many applications in NMR spectroscopy. The prime feature of such pulses is the very effective and uniform inversion of magnetization over large bandwidths at low radio-frequency power—something that is unmatched by other types of conventional or composite pulses.[1,2]

Applications of adiabatic pulses include multipulse decoupling sequences and several multidimensional experiments where broadband inversion is critical in order to refocus evolution under chemical shifts. Another application is for suppression of homonuclear zero-quantum coherence (ZQC) in nuclear Overhauser effect spectroscopy (NOESY) and total correlation spectroscopy (TOCSY) experiments, which is quite successful except for the smallest ZQC frequencies.[3] The ZQC suppression scheme applies a frequency-swept pulse in the presence of a pulsed field gradient. As a result, individual elements of the sample are subject to different effective chemical shift evolution times so that subsequent averaging over the sample results in cancellation of ZQC artifacts.[3]

Herein, we extend the use of adiabatic inversion pulses to the suppression of unwanted correlations or artifacts which obscure heteronuclear multidimensional NMR spectra of medium-size organic molecules or natural products. Such spectra may suffer from complexity and overlap at increasing molecular weight, low purity and low chemical shift dispersion. Indeed, overlap and ambiguities can be particularly severe where chemical-shift dispersion is small, such as in carbohydrates. Low-pass J filters (LPJFs) are crucial for suppression of one-bond correlations in all types of long-range correlation spectra, but unfortunately, they often fail in tightly coupled spin systems.[4,5]

One application of our novel adiabatic filter is as a remedy for the shortcomings of conventional LPJFs in, for example, heteronuclear $^{1}$H–$^{13}$C multiple-bond correlation (HMBC).[5,6] It uses an adiabatic pulse to introduce heterogeneity for evolution under $^{13}$C–$^{1}$H scalar or residual dipolar couplings by means of a frequency-swept 180° pulse on $^{13}$C that inverts $^{13}$C nuclei at different positions in the NMR sample at different times (Figure 1). If the 180° pulse occurs at time zero at the top and at the end of the adiabatic pulse of length $2\tau_2 = 1$ J at the bottom, then the evolution under the coupling varies from

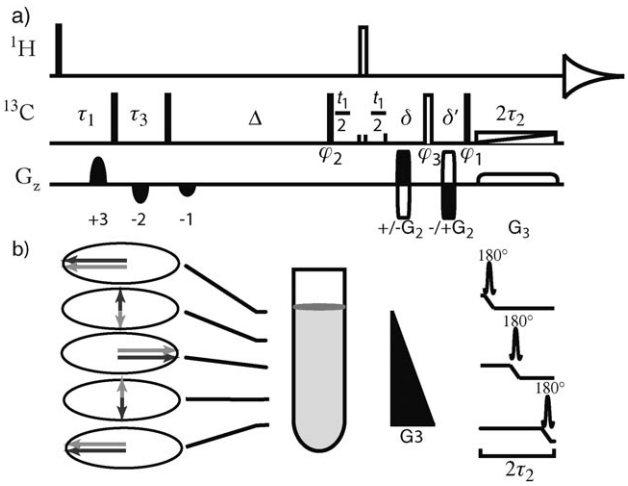


**Figure 1.** Pulse sequence for the *clean* HMBC experiment with an initial second-order and terminal adiabatic low-pass J filter. Recommended delays for the filters are $\tau_1 = 1/2\,[^{1}J^{min} + 0.07\,(^{1}J^{max} - {}^{1}J^{min})]^{-1}$, $\tau_2 = (^{1}J^{max} + {}^{1}J^{min})^{-1}$ and $\tau_3 = 1/2\,[^{1}J^{min} - 0.07(^{1}J^{max} - {}^{1}J^{min})]^{-1}$.[7] $\delta$ and $\delta'$ are gradient delays, where $\delta' = \delta + 6$ µs accounts for the delay of the first point in the $^{13}$C dimension. The integrals over each gradient pulse $G_2$ is $\gamma_H/2\gamma_C$ times the integral over gradient $G_3$ in order to achieve coherence selection. The recommended phase cycle is $\phi_1 = \{x,-x,-x,x\}$, $\phi_2 = \{x,x,4(-x),x,x\}$ and $\phi_3 = \{4(x),4(y),4(-x),4(-y)\}$ with the receiver phase $\{x,-x\}$. The dephasing under one-bond couplings during the adiabatic filter by spatially dependent application of the 180° $^{13}$C pulse is shown in (b).

0 to $2\pi$ across the sample volume at the end of $2\tau_2$ and hence cancellation will occur at the end of the filter (Figure 1 b). Explicitly, the attenuation factor for a heteronuclear doublet amounts to $\sin(2\pi J\tau_2)/2\pi J\tau_2$. Thus, suppression of $^{1}$H magnetization one-bond coupled to a $^{13}$C nucleus occurs for $2\tau_2 = \langle {}^{1}J_{CH} \rangle^{-1}$ whilst long-range coupled $^{1}$H magnetization is virtually unaffected by the filter. This filter is robust with respect to pulse miscalibration and operates efficiently without the need to cycle the phases of pulses that otherwise is a common feature of non-destructive LPJFs.[5,6]

We illustrate the novel adiabatic low-pass J filter by an application to HMBC in its recently introduced *clean* version.[5] *Clean* HMBC is designed for suppression of strong-coupling-induced artifacts that can obscure genuine long-range correlations and possibly lead to incorrect spectral assignments. These artifacts arise from incomplete suppression of one-bond $^{1}$H magnetization by conventional initial LPJFs and additional coherence transfers by pulses in the course of the pulse sequence in strongly coupled spin systems. The adiabatic LPJF at the end

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of the pulse sequence suppresses these strong-coupling-induced artifacts.

If the adiabatic LPJF were the only means of suppressing one-bond coupled $^1$H magnetization, the suppression would be inadequate, as this filter is effectively of first order.[4] Therefore, the pulse sequence in Figure 1 is designed with two conventional LPJF steps at the beginning of the sequence and one terminal adiabatic filter in such a way that the three filtering steps together amount to an effective overall third order LPJF.

Figure 2 shows third-order filtered HMBC spectra of raffinose [α-D-galactopyranosyl-(1→6)-α-D-glucopyranosyl-(1→2)-β-D-fructofuranose] recorded without and with the adiabatic LPJF element. 1D sections from the 2D spectra are shown in Figure 2b. Clearly, artifacts are present in the standard HMBC spectrum for all three moieties, fructose, glucose and galactose, whilst they are strongly attenuated (>90%) by the adiabatic LPJF. Due to the low chemical shift dispersion of carbohydrates, resonances for non-anomeric protons and carbons in oligosaccharides are in a highly crowded spectral region for complex oligosaccharides and similar problems are common for a variety of medium-size organic compounds with molecular masses of few kDa. The presence of one-bond artifacts split along the proton dimension by $^1J_{CH}$ exacerbates spectral crowding for these compounds. This is where the adiabatic LPJF comes in handy. Figure 3 shows the application to a mannan preparation from the yeast *Pichia pastoris* (Figure 3).[10] While artifacts clearly render the resonance assignment in the standard HMBC spectrum extremely challenging, the adiabatic filter effectively purges artifacts and thus reduces ambiguity.

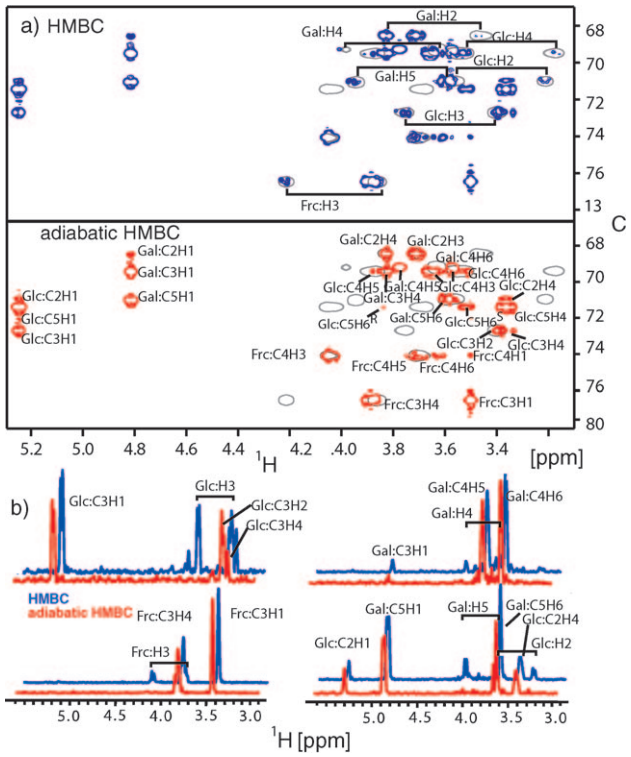


**Figure 2.** a) Third-order LPJF HMBC spectra recorded without (blue) and with (red) the novel adiabatic filter of 50 mM raffinose in $D_2O$ plus an overlaid coupled HSQC spectrum (grey) indicating the positions of strong-coupling-induced artifacts. b) 1D HMBC sections from the $^{13}$C frequencies of $C_4$(galactose), $C_3$(glucose), $C_3$(fructose) and $C_5$(galactose). Use of the adiabatic filter allows the spectra to be recorded with only two scans per increment.

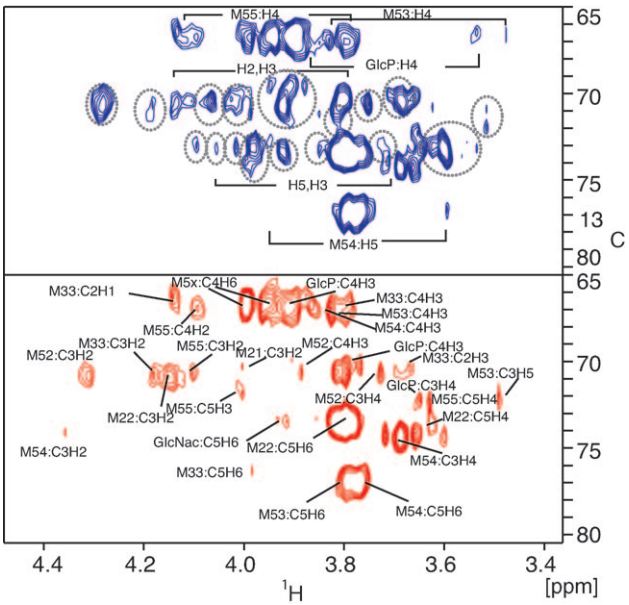


**Figure 3.** HMBC spectra without (blue) and with (red) the adiabatic LPJF of 3 mg *Pichia pastoris* mannan dissolved in 600 mL $^2H_2O$. Artifacts are encircled with grey dotted lines.

In summary, we have introduced a novel adiabatic low-pass J filter and shown an application for artifact suppression in heteronuclear multiple-bond correlation NMR. This purging of artifacts reduces ambiguity and is crucial in crowded spectra. The minimum instrument time compared to the earlier *clean* HMBC scheme is reduced by a factor of four by the adiabatic LPJF.[5]

Earlier works have made use of schemes to generate spatially dependent shift evolution for fairly different applications.[3,8,9] Our novel adiabatic element might also find application in this realm of ultrafast multidimensional NMR spectra[8] as a means of generating a coupling dimension in a time-effective manner.

## Experimental Section

All NMR experiments were performed on a Bruker DRX 400 spectrometer. Raffinose was dissolved in $D_2O$ (600 μL) to a final concentration of 50 mM without further adjustment of pH or ionic strength. Mannan from the yeast *Pichia pastoris* was purified in $^{13}$C isotope enriched form as decribed previously.[10] The mannan (3 mg) was dissolved in $D_2O$ (600 μL) without further adjustments. NMR spectra of raffinose and *Pichia pastoris* mannan were recorded at 30 °C and 50 °C, respectively. Both chirp[1] and WURST (wideband, uniform rate, and smooth truncation)[2] pulses were tested for use in the swept 180° pulse/gradient pair. Results were comparable and spectra shown in Figures 2 and 3 were recorded with 180° chirp pulses. Adiabatic pulses were swept through 25 kHz in 6.78 ms $[2(J_{min}^{CH}+J_{max}^{CH})^{-1}$, with $J_{min}^{CH}=125$ Hz, $J_{max}^{CH}=170$ Hz], with a maximum field strength $\gamma B_1$ of 1.7 kHz. The gradient pulse that is applied simultaneously with the adiabatic pulse was applied

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at 20% of the maximum gradient strength (60 G cm$^{-1}$) as a rectangular shape. $G_2$ was applied as a rectangular pulse of 3 ms duration with 90.25% of the maximum gradient strength. The strong gradients for coherence selection and adiabatic low-pass J filter were followed by recovery delays of 200 μs to minimize eddy-current-induced artifacts. The heteronuclear multiple-bond correlation spectra of raffinose shown in Figure 2 were recorded as data matrices of 1024 ($^1$H)×128 ($^{13}$C) complex points with total acquisition times of 256 ($^1$H) and 25 ($^{13}$C) ms, while HMBC spectra of the mannan as shown in Figure 3 were recorded as data matrices of 2048 ($^1$H)×32 ($^{13}$C) complex points with total acquisition times of 512 ($^1$H) and 6 ($^{13}$C) ms. The spectra without the adiabatic LPJF employed an initial 3rd order LPJF using the same $\tau_1$, $\tau_2$, $\tau_3$ delays.

**Keywords:** analytical methods · artifact suppression · heteronuclear multiple-bond correlation · low-pass J filter · NMR spectroscopy

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