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# Indirect determination of chemical shift by coupling evolution during adiabatic pulses

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**Abstract**

The use of adiabatic 180° X-pulses within INEPT refocusing periods results in chemical shift-dependent evolution of *J*-couplings. This has been viewed as a disadvantage and several methods of overcoming it have been suggested. This article shows that there is the potential to use this chemical shift dependence to determine heteronuclear chemical shift without a heteronuclear evolution time. In this way, it possible to estimate heteronuclear chemical shift indirectly from a single one-dimensional proton-observe spectrum and determine it with high accuracy from a extensively-folded two-dimensional proton-observe spectrum.


*Keywords:* NMR; *J*-coupling; Modulation; Proton–carbon correlation; Adiabatic pulse

## 1. Introduction

The increasing sensitivity of NMR spectrometers has prompted the development of a range of methods of speeding up the acquisition of multi-dimensional NMR spectra. These include relaxation optimization [1], minimal sampling in the indirect dimension combined with aliasing or specialised processing methods [2–7] and reduced dimensionality (projection) methods [2]. These methods all rely on time-dependent intensity-modulation of the directly observed signals which is transformed to produce multi-dimensional spectra where chemical shifts can be measured from frequency axes. This makes them ideally suited to NMR spectroscopy of biomolecules where there is extensive overlap of resonances. However, they require acquisition of tens of spectra to give adequate resolution on the indirect frequency axes.

NMR spectroscopy of small organic molecules provides different challenges from spectroscopy of biomolecules. Speed of analysis is a major consideration because of the throughput of modern chemical synthesis, and because the choice of subsequent reaction conditions often depends on the results of an NMR experiment. This need for rapid results is one of the main obstacles to the widespread use of 2D spectra within chemistry research. Compared with spectra of biomolecules, those of small molecules contain fewer resonances so methods which sacrifice peak resolution for faster acquisition would be of potential use. Despite this, only three methods have been proposed which allow chemical shift correlation from a very small number of spectra. The most recently proposed, SPEED, relies on intensity-modulation of the directly observed signals but proposes direct calculation of $^{13}C$ chemical shift from a single complex increment of an indirect $^{13}C$ evolution time [8]. The results of this calculation are combined with a direct-observe one-dimensional $^{13}C$ spectrum to correlate proton and $^{13}C$ chemical shifts, resulting in a pseudo-2D proton–carbon correlation spectrum. Therefore, SPEED requires the acquisition of two proton-observe scans (the real and imaginary or p- and n-type components of the complex increment) and a $^{13}C$ spectrum to produce a proton–carbon correlation spectrum.

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The SITAR [9,10] method was primarily proposed as a way of reducing overlap in NMR spectra of biomolecules, but it also allows chemical shift correlation between protons and an X nucleus. It is radically different from all the previously mentioned methods in that it does not use intensity-modulation as a way of determining chemical shift. Instead, the chemical shift of a heteronucleus is determined simultaneously with proton chemical shift during acquisition by applying continuous-wave decoupling to scale the HX *J*-coupling. This scaling depends on the offset of the X nucleus from the decoupling frequency so, from the true and scaled HX *J*-couplings, the heteronuclear chemical shift can be calculated albeit with twofold degeneracy. This degeneracy can be avoided if the decoupling frequency is placed outside the chemical shift range of the X nucleus, but hardware limitations make this difficult for the full $^{13}C$ chemical shift range [10]. The original proposal of this approach has been subsequently developed [10] to include the use of an S3E element [11] to separate the two components of the HX doublet, so that three scans are required to determine chemical shift; one spectrum without decoupling for measurement of the HX coupling and two S3E scans with decoupling for measurement of the reduced coupling. Although the SITAR approach has the potential to produce pseudo-2D correlation spectra in two or three scans, it has not entered common use. Possible explanations are first, the use of coupled proton spectra which reduces sensitivity and increases overlap and second, twofold ambiguity in X chemical shift.

A final method of acquiring a proton–carbon correlation spectrum relies on pulse-field gradients to encode indirect chemical shift within a single scan via a spatially-dependent phase [12]. This method will not be considered further here.

This article proposes an alternative method to determine chemical shift in an indirect dimension: chemical shift-dependent coupling modulation. This has the potential to measure chemical shift in an indirect dimension from a single scan. The method relies on the inversion profile of a frequency-swept (adiabatic) pulse to introduce coupling modulation. As the use of adiabatic inversion pulses has become widespread, a number of articles have highlighted the problems associated with their use during refocusing delays [13–15]. Because adiabatic pulses are frequency-swept, the time during a pulse when a spin is inverted depends on its chemical shift. This leads to chemical shift-dependent evolution of *J*-couplings. Almost all articles considering this have suggested methods of overcoming the chemical shift dependence.

Instead, however, chemical shift-dependent coupling evolution can be used to determine chemical shift. This concept is demonstrated by the modified proton–carbon HSQC pulse sequence shown in Fig. 1. At point **a**, proton magnetization is anti-phase with respect to the CH coupling. The adiabatic pulses cause chemical shift-selective inversion of $^{13}C$ magnetization so the adiabatic $^{13}C$ inversion/proton inversion pulse-set results in chemical shift-dependent evolution of the CH coupling and the phase of the CH doublet will depend on the $^{13}C$ chemical shift. If we assume that the adiabatic pulse is linear, then CH doublets with a $^{13}C$ chemical shift inverted at points **b**/**b**′ will experience CH coupling evolution for $2T$, CH doublets inverted at **c**/**c**′ will be refocused, while CH doublets inverted at **d**/**d**′ will experience CH coupling evolution for $-2T$. This results in differing phases for the doublets at different $^{13}C$ chemical shifts. CH doublets with a $^{13}C$ chemical shift inverted at **c**/**c**′ will be anti-phase, while those inverted at **b**/**b**′ or **d**/**d**′ will be in phase (but with opposite signs). Peaks inverted at times in between will have phases between these extremes. Therefore, from the phase, the $^{13}C$ chemical shift can be determined. This can be expressed as:

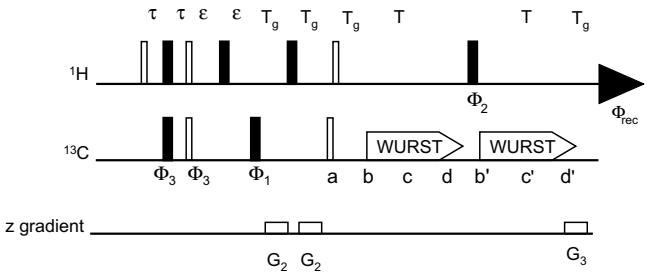


Fig. 1. Pulse sequence used in the work described here. Up to point **a** the experiment is identical to a gradient-selected HSQC [13] except for the addition of an extra proton Hahn Echo to refocus and return magnetization from protons not attached to $^{13}C$ to the *z*-axis prior to acquisition. The final refocusing period is explained in the introduction. 90° pulses are indicated by empty bars, 180° pulses by filled bars and the 180° adiabatic pulses by arrowed shapes. Note that the two adiabatic pulses have identical sweep direction. All spectra shown here were acquired with a single scan, but spectral quality can be improved with phase cycling of $\Phi_1 = x, y, -x, -y$, $\Phi_2 = x, x, -x, -x$ and $\Phi_{rec} = x, -x$. Other pulses were phase $x$, except the proton 180° pulses and the second proton 90° pulse which were phase $y$. The final adiabatic pulses were 1.9 ms 30 kHz WURST-20 pulses ($B_{1,max} = 3.8$ kHz). The $^{13}C$ transmitter was centred at 75.75 ppm. The delays indicated are $\tau = 1.8$ ms, $\varepsilon = T_g + t_1/2$, $T_g = 0.6$ ms and $T = 1.9$ ms. Gradients $G_1$ and $G_2$ were applied at 15.6 G/cm for 0.5 ms and 30.1 G/cm for 0.5 ms. For the 2D experiment, phase discrimination was obtained by acquiring p- and n-type spectra for each value of $t_1$ and axial peak displacement was carried out by inverting $\Phi_3$ and $\Phi_{rec}$ for alternate values of $t_1$.

$$\phi = 360°J\ (2T_i - T), \tag{1}$$

where $\Phi$ is the observed phase (with anti-phase defined as 0°), $J$ the CH coupling and $T$ the pulse-length. $T_i$, the time when a given chemical shift is inverted by the adiabatic pulse, can be calculated using a Bloch simulator and the C–H coupling constant can be measured relatively accurately from the experiment shown in Fig. 1. There is no need for additional spectral data. Previous work [16] suggested that the effective coupling constant during the adiabatic pulse is reduced by the pulse's spin-locking effect. However, better agreement with observations was obtained using the un-reduced coupling constant (results not shown).

This approach is similar to SITAR in that it does not rely on intensity-modulation in the acquisition dimension to determine $^{13}C$ chemical shift. Therefore, it can be combined with intensity-dependent methods of frequency determination such as incrementation of an evolution time combined with Fourier-transformation or other processing methods. This can be achieved with the experiment shown in Fig. 1 by incrementing the delays $\varepsilon$.

## 2. Results

The WURST-20 pulse used in this work is a linear frequency sweep, making calculation of $T_i$ relatively straightforward [17]. Simulation of the pulse used in this work showed that:

$$T_i = 0.876 \times T \times \left(\frac{\varpi}{\Delta\varpi} + 0.07\right), \quad (2)$$

where $\omega$ is the frequency offset of the coupled $^{13}C$ nucleus and $\Delta\omega$ is the sweep-width of the WURST-20 pulse. Combining Eqs. (1) and (2) with the experimental parameters used here and scaling to chemical shift (ppm) gives:

$$\sigma = 75.094 - 165.788 \times \frac{\phi}{J}. \quad (3)$$

Eq. (3) allows the estimation of likely accuracy of the method. Errors in determination of $J$ introduce an error which is chemical shift-dependent; a 5 Hz error would introduce an error of up to 3.6 ppm at 5 ppm and 145 ppm, but zero error on-resonance. In contrast, errors in phase measurement results in errors independent of chemical shift. A 5° error in phase results in an error of up to 6 ppm in chemical shift, depending on the value of $J$.

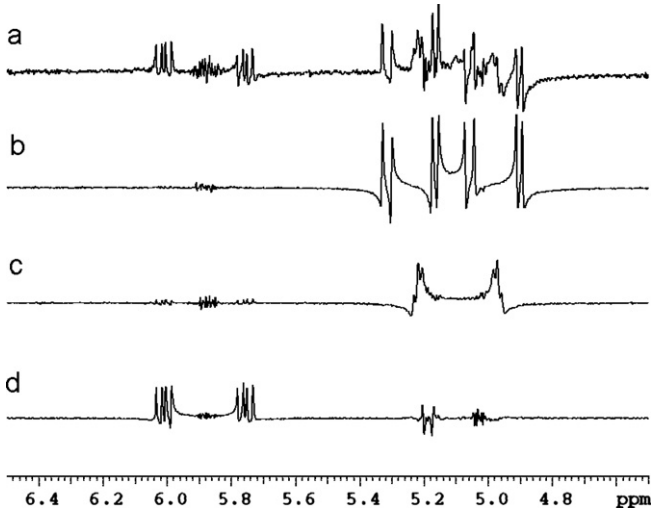


Fig. 2. (a) Low-field region of a 5% solution of linalool in deuterochloroform, acquired using the experiment shown in Fig. 1. A single scan was acquired, taking less than 3 s. (b) Row extracted from the 2D spectrum shown in Fig. 3 at a chemical shift of 5.1 ppm. Two doublets are observed from the alkenyl methylene at a $^{13}C$ chemical shift of 111.9 ppm. (c) Row extracted from the 2D spectrum shown in Fig. 3 at a chemical shift of 4.4 ppm. The doublet is from the alkenyl methine at a $^{13}C$ chemical shift of 124.5 ppm. (d) Row extracted from the 2D spectrum shown in Fig. 3 at a chemical shift of 1.9 ppm. The doublet is from the alkenyl methine at a $^{13}C$ chemical shift of 145.2 ppm.

Fig. 2 shows the chemical shift-dependent nature of refocusing observed using the pulse sequence shown in Fig. 1. The spectrum was acquired on linalool, which is an especially challenging molecule for the approach because it contains a wide range of $^{13}C$ chemical shifts, strong proton–proton couplings and extensive overlap. Fig. 2a demonstrates that the four different alkenyl doublets of linalool have different phases owing to their three different $^{13}C$ chemical shifts.

Table 1 shows the results of applying Eq. (3) to the spectra shown in Figs. 2 and 3. For each chemical shift, the observed multiplet phase and the calculated chemical shift are given. From a single scan (Fig. 2a), calculated $^{13}C$ chemical shifts are generally around 5 ppm from the actual value, but one is 10 ppm from the actual value. Such a large error is on the limit of acceptability for routine application to small organic molecules. Increasing the number of scans does reduce the RMS error slightly, suggesting that signal-to-noise and residual $^{12}C$ magnetization contribute to the

Table 1
Observed phases, coupling constants and calculated chemical shifts from the spectra shown in Figs. 2 and 3[a]

| $\sigma$ | $J$ | 1D (1 scan) | | 2D (32 scans) | | SITAR | |
|---|---|---|---|---|---|---|---|
| | | Phase | $\sigma$ (calc) | Phase | $\sigma$ (calc) | $J_r$ | $\sigma$ (calc) |
| 17.9 | 125.8 | 41.2 | 20.8 | 42.9 | 18.5 | 116.7 | 18.6 |
| 23.0 | 126.1 | 35.4 | **28.5** | 36.1 | 27.6 | 114.6 | 23.5 |
| 25.9 | 125.7 | 40.6 | 21.6 | 36.3 | 27.3 | 113.8 | 26.1 |
| 28.0 | 125.6 | 35.6 | 28.1 | 34.8 | 29.1 | 114.9 | **21.3** |
| 42.2 | 125.2 | ND | ND | 27.0 | 39.4 | 103.6 | 39.5 |
| 111.9 | 158.6 | −42.6 | **119.6** | −32.9 | 109.5 | 136.5 | 106.9 |
| 111.9 | 154.5 | −24.3 | **101.1** | −33.3 | 110.8 | 133.5 | 107.3 |
| 124.5 | 150.5 | −42.4 | 121.7 | −42.1 | 121.4 | 136.8 | **117.2** |
| 145.2 | 152.4 | −56.8 | **136.8** | −61.6 | 142.1 | 144.5 | **135.7** |
| RMSD | | | 6.6 | | 2.7 | | 5.5 |

[a] Calculated values shown in bold are more than 5 ppm from the correct value. ND indicates one chemical shift which could not be determined owing to overlap with intense methyl peaks. Note that SITAR gives two possible solutions for any observed coupling constant; the value closest to the correct one was selected.

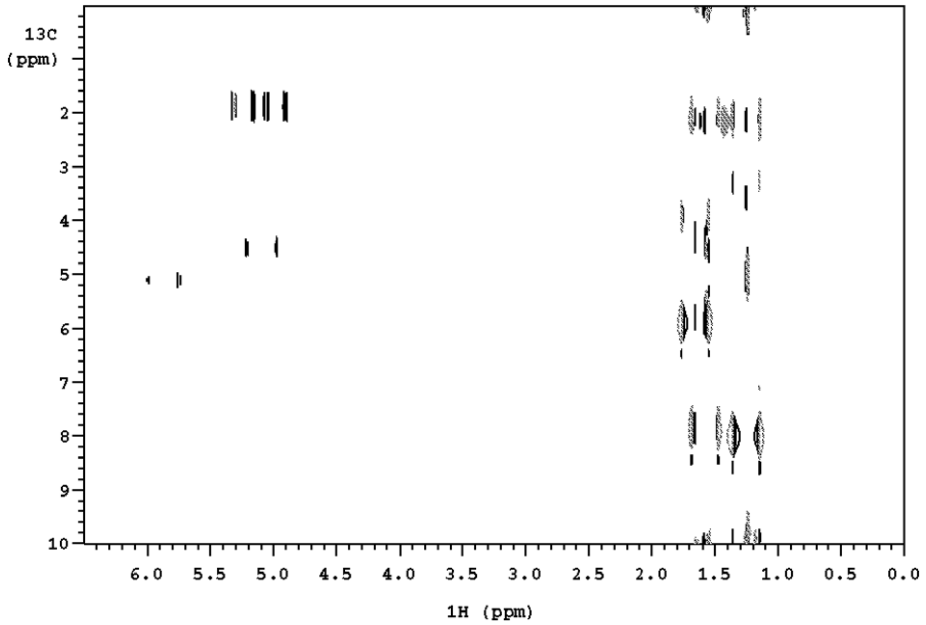


Fig. 3. 2D spectrum acquired using the experiment shown in Fig. 1. The $^{13}C$ spectral width was 10 ppm (1506 Hz), resulting in extensive folding. Sixteen complex $t_1$ increments were acquired with one scan per element, giving a total acquisition time of 90 s. Negative levels are shown with a single, dashed contour. The $^{13}C$ axis shows frequency offset from the high-field spectral edge.

relatively large errors (results not shown). Despite this improvement, some errors greater than 5 ppm are still observed.

As explained in Section 1, the method proposed here can be combined with conventional evolution times and this provides one way of resolving resonances from one another and improving accuracy. Shown in Fig. 2b–d and Fig. 3, is an example of this approach with an indirect $^{13}C$ spectral width of 10 ppm. The $^{13}C$ axis is folded 13 times, but chemical shift can be determined by measuring the doublet phase in the proton dimension and applying Eq. (3). All chemical shifts calculated in this way are within 5 ppm of the correct value. This is sufficient for routine applications but the accuracy can be improved even further by combining the result from Eq. (3) with chemical shifts measured on the highly-folded axis. This gives an RMS error of less than 1 ppm.

Table 1 also shows the reduced coupling constant ($J_r$) and calculated chemical shift obtained by applying off-resonance CW decoupling, as used in SITAR. Note that an S3E element was not used, because this requires an extra scan and because residual magnetization from protons attached to $^{12}C$ cannot be returned to the $z$-axis prior to acquisition resulting in poorer quality spectra. The accuracy is slightly better than that obtained by applying the coupling evolution method to a single scan spectrum. However, this overlooks the requirement with SITAR to acquire a control spectrum as well. Calculations based on a coupling evolution spectrum acquired with two scans have similar accuracy to the SITAR results acquired with one control and one decoupled scan (results not shown).

## 3. Discussion

The approach described here has the main advantage that $^{13}C$ chemical shift can be estimated using a single scan proton-observed spectrum; no other information (such as a one-dimensional $^{13}C$ spectrum) is required. However, the accuracy of the method needs to be improved for routine application. Assessment of the likely sources of error suggests that the most significant is that from determining doublet phase. There are two obvious ways of improving the accuracy of phase determination. The first would be to develop a fitting program to calculate phase rather than relying on manual phasing as was done here. This has the advantage that there would be no increase in the number of scans needed to obtain a spectrum. The second approach would be to use an S3E element to separate out the two doublet components, as is done in SITAR. The reduction in overlap would simplify phase determination, but would double the number of scans needed and would also reduce the effectiveness of suppression of residual magnetization from protons attached to $^{12}C$.

Compared with the approach described here, SITAR gives slightly more accurate results. If the heteronuclear chemical shift range of interest is narrow, then SITAR will be even more accurate and the twofold ambiguity in chemical shift can be avoided by CW decoupling at the edge of the chemical shift range, rather than in the centre. Furthermore, if HX coupling constants can be accurately predicted, then no control scan is required. This makes SITAR ideal for a heteronucleus which has a narrow frequency distribution of chemical shifts and a predictable range of coupling constants. However, $^{13}C$ has a wide fre-

quency distribution of chemical shifts and difficult-to-predict coupling constants. In this case, the approach described here has the advantages that only one scan is required, and it gives a unique chemical shift rather than two possible values.

The SITAR results reported in Table 1 are considerably less accurate that those in the original descriptions of the SITAR method. Four factors are likely to have contributed to this reduced accuracy. First, these results were obtained without using an S3E element. Second, the sample used was at natural abundance not 100% $^{13}C$ enriched. Third, the molecule studied contains a much more challenging range of chemical shifts, proton–proton couplings and proton–carbon couplings than the original publication. Fourth, a 1D spectrum was used rather than multi-dimensional spectra resulting in increased overlap.

It should be noted that the method described in this article could be combined with SITAR if a control spectrum without decoupling is required. If the control spectrum were acquired using the coupling evolution approach described here, it would not only give the coupling constant but could also be used to calculate a chemical shift to resolve the twofold ambiguity in chemical shift of the SITAR method. If this approach is applied to the data shown in Table 1, the RMS deviation in $^{13}C$ chemical shift is less than either approach used alone so coupling evolution is a useful complement to SITAR.

Compared with SPEED, the approach described here has the disadvantage that coupled spectra are acquired resulting in increased overlap and halved sensitivity. Against this, it has the advantages that it requires only one scan rather than two, and does not require a one-dimensional $^{13}C$ spectrum. SPEED has the additional disadvantage that it relies on intensity, so is not independent of other methods of frequency determination which rely on intensity such as Fourier-transformation. However, this does mean that the SPEED approach could be combined with the approach described in this article with no increase in the time taken to acquire data. This would provide two independent estimates of $^{13}C$ chemical shift from the same data set.

This article demonstrates the potential of using coupling evolution during adiabatic pulses to determine chemical shift indirectly. Although it uses proton–carbon correlation, it could be applied to any experiment where one nucleus is correlated with one other coupled nucleus. It will be of most use in experiments where the coupling constant between the nuclei is large, and the chemical shift range of the nucleus observed indirectly is large. The experiments which would benefit most from rapid acquisition are those with the highest sensitivity such as proton–proton DQF-COSY and TOCSY. These involve multiple coupling partners, relatively small couplings, and narrower chemical shift ranges so it is unlikely that coupling evolution during adiabatic pulses could be exploited to speed up the acquisition of these experiments.

## 4. Conclusion

This article has demonstrated the potential of coupling evolution during an adiabatic pulse to allow estimation of $^{13}C$ chemical shift from a single scan proton-detected experiment. Combination of this approach with a conventional $t_1$ evolution time and a narrow $t_1$ spectral width allows acquisition of a proton–carbon HSQC with 1 ppm $^{13}C$ resolution in 32 scans.

## 5. Experimental

The experiments reported here were acquired using a 5% solution of linalool in $CDCl_3$ on a Varian Unity Inova 600 MHz spectrometer fitted with a 5 mm H{CN} PFG Cold Probe. $^{13}C$ chemical shifts were measured from a 1D direct-observe $^{13}C$ spectrum. Bloch simulations of the WURST-20 pulse were performed using the program 'pulsetool' supplied with the VNMR program (Varian Inc., Palo Alto, CA). SITAR measurements were acquired using the HSQC sequence of [14] except for the addition of an extra proton Hahn Echo to refocus and return magnetization from protons not attached to $^{13}C$ to the $z$-axis prior to acquisition. CW decoupling was applied during acquisition at 75.75 ppm with $B_1 = 3209$ Hz.

All coupling constants and phases were determined manually in duplicate. Chemical shifts were calculated within Microsoft Excel 2003 (version 11) using Eq. (3) or Eq. (6) of [9] as appropriate.

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