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Effective Multiple-Solvent Suppression Scheme using The Excitation Sculpting Principle

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ABSTRACT: An interesting and simple approach to effective multiple-solvent suppression is proposed using the basic principles of the excitation sculpting methodology. Flat baseline and ultra-clean NMR spectra are usually obtained under standard conditions using a modified double pulsed field gradient echo scheme in samples containing mixtures of non-deuterated solvents. The simple performance of such an approach, the high tolerance to experimental mis-settings, the impressive level of solvent suppression and the easy incorporation of the proposed scheme in multi-dimensional NMR experiments make this method suitable for possible applications in liquid chromatography NMR. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: multiple solvent suppression; pulsed field gradient; excitation sculpting; NMR; liquid chromatography

INTRODUCTION

Recently, a powerful scheme known as excitation sculpting has been proposed as a general tool for pure-phase excitation in almost every conceivable experiment. Some applications have been described for effective solvent suppression,[1,2] selective excitation,[3–8] semi-selective excitation[9–12] and isotope filtering[13–15] experiments. The basic pulse sequence is a double pulsed field gradient echo (DPFGE), G_1–S–G_1–G_2–S–G_2, where S can be any inversion element and G_1 and G_2 represent pulsed field gradients (PFG). For solvent suppression applications, a $^{sel}180^{\circ}_{x}$ (solvent)–$^{hard}180^{\circ}_{-x}$ block has been proposed as the refocusing S element, affording a simple way to obtain clean water suppression with excellent flat baseline and without need of phase cycling.[1] In selective excitation schemes, S is simply an inversion soft 180° pulse that affords excellent selectivity, and prevents sidelobe excitation and *J*-coupling evolution, all achieved in a single scan.[6] In contrast to the single pulsed field gradient echo (SPFGE) method, this scheme always works very well, even for rectangular shapes, because its inversion profile is not dependent on the profile of the inversion element. Finally, excellent suppression of unwanted ^{1}H–^{12}C magnetization in inverse experiments has been achieved when S is a BIRD cluster (known as G-BIRD)[13] and, for instance, it has been shown that cross peaks display excellent phase properties in HMQC and HMQC-TOCSY experiments.[14] An important aspect of all these DPFGE approaches is their high tolerance to miscalibrated pulses, gradient strengths, inhomogeneous fields and other experimental parameters.

Recently, we have shown that excellent multiple-site selective excitation can be achieved with a single scan by applying concatenated selective 180° pulses at several frequencies as the inversion element in the DPFGE scheme.[16] Thus, using for each step just the experimental parameters required for single excitation, the whole multi-site selective sequence is very easily set up. The result is a very clean spectrum, displaying only the selected signals. In addition, the phase of each selective signal is simply controlled by the phase of the corresponding selective 180° pulse.

In this paper, we show a simple variant of the DPFGE scheme designed to achieve an effective multiple-solvent suppression in any spectrometer equipped with a single proton channel and even without the need of a shaped pulse modulator unit. Multiple solvent suppression is usually required in liquid chromatography (LC)–NMR applications, in which mixtures of non-deuterated solvents are usually involved.[17] On the other hand, the easy incorporation of the proposed scheme in multi-dimensional NMR experiments affords a powerful way to obtain clean spectra with a very flat baseline and using non-extreme acquisition conditions, a set of characteristics suitable for routine purposes. In principle, multiple-solvent suppression could be also achieved using the conventional DPFGE method proposed originally,[1] but this would require the use of more sophisticated phase-modulated frequency-shifted laminar pulses (SLP) as the inversion element, as recently described for other water-suppression methods such as WATERGATE[18] and WET[19] schemes, or the use of a second proton channel for applying the second

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selective 180° pulse. Applying the pulses sequentially, as opposed to simultaneously, also reduces possible Bloch–Siegert effects that would complicate solvent suppression.

RESULTS AND DISCUSSION

The proposed inversion element is constructed by a simple extended modification of the original sequence. Thus, for a two-site suppression scheme, the block $^{sel}180^{\circ}_{x}$(solvent A)–$^{hard}180^{\circ}_{-x}$–$^{sel}180^{\circ}_{x}$(solvent B) [Fig. 1(a)] can be defined. This block, in fact, does not affect the two solvent signals and, therefore, they will be efficiently dephased by the effect of PFGs. In this way, each solvent-selective 180° pulse can be defined which its own duration, shape, frequency and phase as a function of the required selectivity and the corresponding solvent proportion. All these values should match exactly those used in a conventional single-signal suppression experiment.

We tested the proposed experiment on a standard sample of 50 mM sucrose dissolved in H_2O–CH_3CN–D_2O (60:30:10). The conventional ^1H spectrum shows essentially nothing but the intense singlets of both solvents [Fig. 2(a)]. As a reference, note the signal intensity of the satellites of the acetonitrile resonance compared with the sucrose resonances [Fig. 2(b)]. Both solvent resonances are efficiently suppressed at an excellent level [Fig. 2(c)] by applying the sequence of Fig. 1 and using the same experimental parameters as described in the original sequence. In our case, we used a 1.9 ms rectangular pulse for each selective inversion and, owing to its poor selectivity, the nearest resonances are partially distorted. In this case, the receiver gain setting was experimentally improved by a factor of 70 and thus the typical dynamic range limitations of very dilute samples can be overcome. Note the excellent level of both solvent signal suppressions in Fig. 2(d). On the other hand, unwanted satellite signals from the acetonitrile resonance could be optionally removed by applying carbon decoupling during the corresponding selective 180° proton pulse.

This scheme can be easily incorporated into multidimensional experiments [Fig. 1(b)] in the same way as known for the WATERGATE block, just prior to acquisition.[21,22] A similar approach has been also proposed using the DPFGE scheme.[2] Figure 3(a)–(c) show the first increment of 2D TOCSY, NOESY and ROESY spectra using the same experimental conditions as described in Fig. 2(c). It can be noted that excellent suppression for both signals is achieved in all cases. If desired, the same method could be applied for some of the water flip-back versions of these experiments.[22–24]

As an example, Fig. 4 shows the corresponding pure-adsorption 2D TOCSY spectrum of the H_2O–CH_3CN solution of sucrose, in which all cross peaks can be clearly observed without the presence of any residual solvent signal. No post-processing was used. Similar results were obtained for the 2D NOESY and 2D ROESY experiments (data not shown).

Finally, as demonstrated for multiple selective excitation,[16] it should be possible to add extra selective pulses if more solvent signals were present. The phase properties of the proposed approach should not be affected but the sequence could become too long and, therefore, the effects due to transverse relaxation and *J*-coupling evolution could affect the overall sensitivity of the

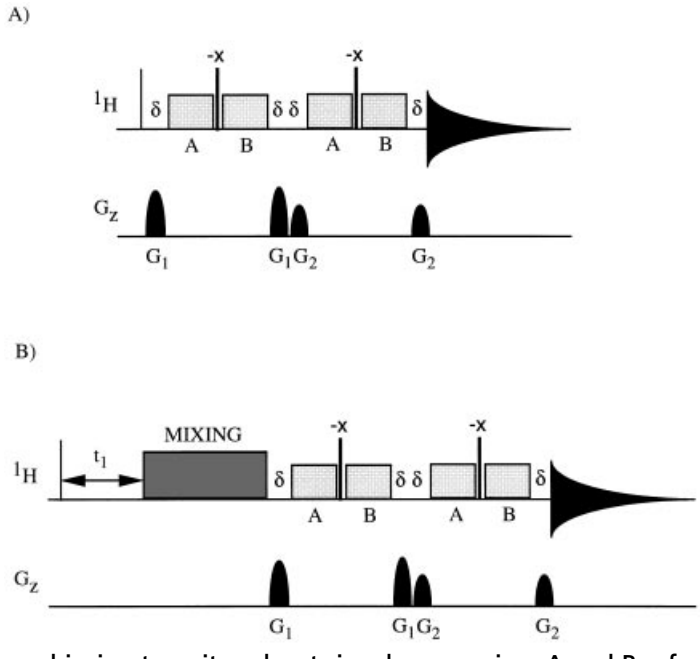

Figure 1. (a) Basic scheme for achieving two-site solvent signal suppression. A and B refer to individual resonances. G_1 and G_2 represent PFG and have equal duration (δ) but different strengths. (b) General scheme for recording 2D homonuclear experiments with multiple-solvent suppression. Phase-sensitive 2D TOCSY, ROESY and NOESY spectra can be recorded depending of the mixing process used.

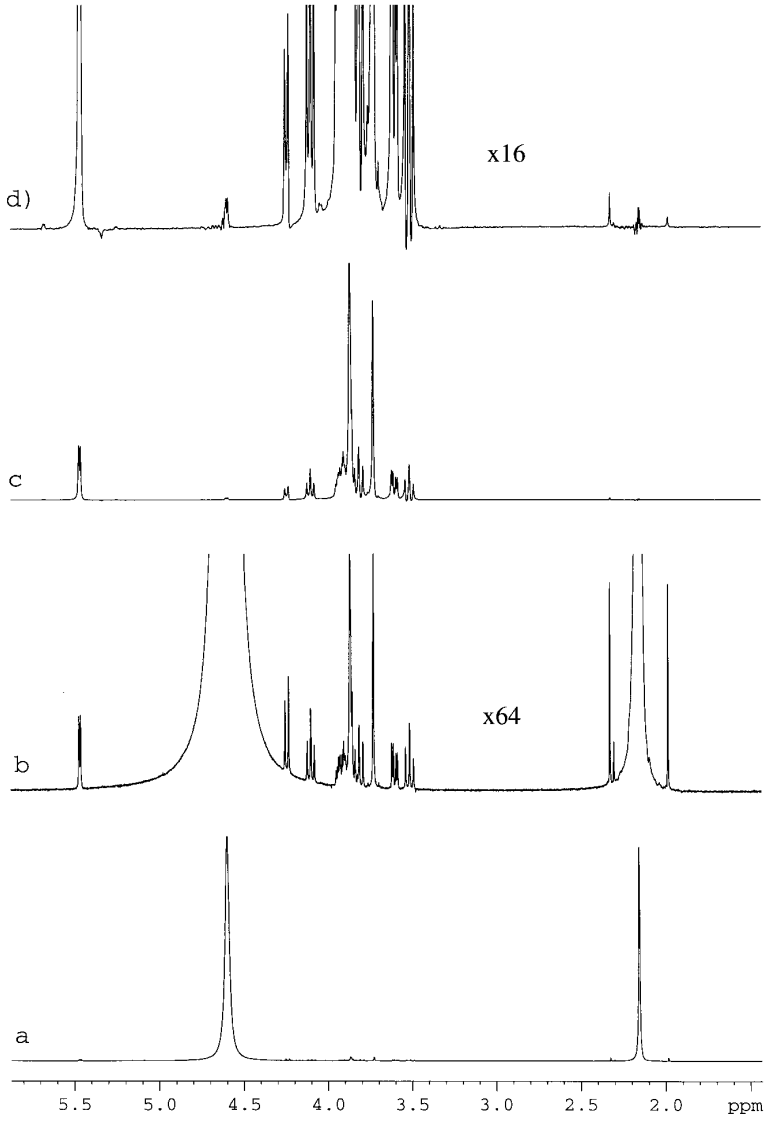

Figure 2. (a) Conventional ^{1}H 400 MHz spectrum of a sample of sucrose dissolved in H_2O–CH_3CN–D_2O (65:25:10); (b) same as (a) but vertically scaled by a factor of 64; (c) ^{1}H spectrum after applying the pulse sequence of Fig. 1; (d) same as (c) but vertically scaled by a factor of 16. A rectangular 1.9 ms pulse was applied as a selective inversion pulse on each solvent resonance.

experiment. However, this sensitivity loss might be partially recovered by applying a strong and short spin-lock prior to acquisition in order to remove antiphase dispersion contributions.[18]

CONCLUSIONS

A powerful and very simple method has been proposed for suppressing multiple signals in conventional proton spectra. This approach can be generally useful for samples containing multiple non-deuterated solvents. In addition, it can be easily incorporated into multi-dimensional experiments, thus allowing the analysis of complex samples and the observation of peaks buried under the intense solvent signals.

EXPERIMENTAL

All experiments were performed at 400 MHz on a Bruker ARX400 instrument at 300 K using an inverse broadband probehead incorporating a Z-gradient. All 1D spectra were acquired with four scans without dummy scans. The gradient length was $\delta = 1$ ms and the gradient values were $G_1 = G_2 = 7.5$ G cm^{-1} and $G_3 = G_4 = 4.5$ G cm^{-1}. The selective 180° pulses were of 1.9 ms duration with a rectangular shape applied on resonance on each solvent signal. TOCSY experiments were recorded applying a 7.6 kHz MLEV-17 pulse train for 65 ms. NOESY experiments were recorded using a mixing time of 500 ms. In ROESY experiments, a 2.5 kHz continuous-wave period was applied for 500 ms centered at 4.0 ppm. EXORXYCLE was applied in all experiments for all components on every other inversion element in the DPFGE scheme and the detector. Two-dimensional experiments were acquired applying TPPI on the 90° pulse preceding the variable t_1 period. Two-dimensional data were recorded with a size of 256 K, accumulating four scans for each of the 128 increments, and processed using zero-filling up to 512K and pure-cosine window multiplication in both dimensions.

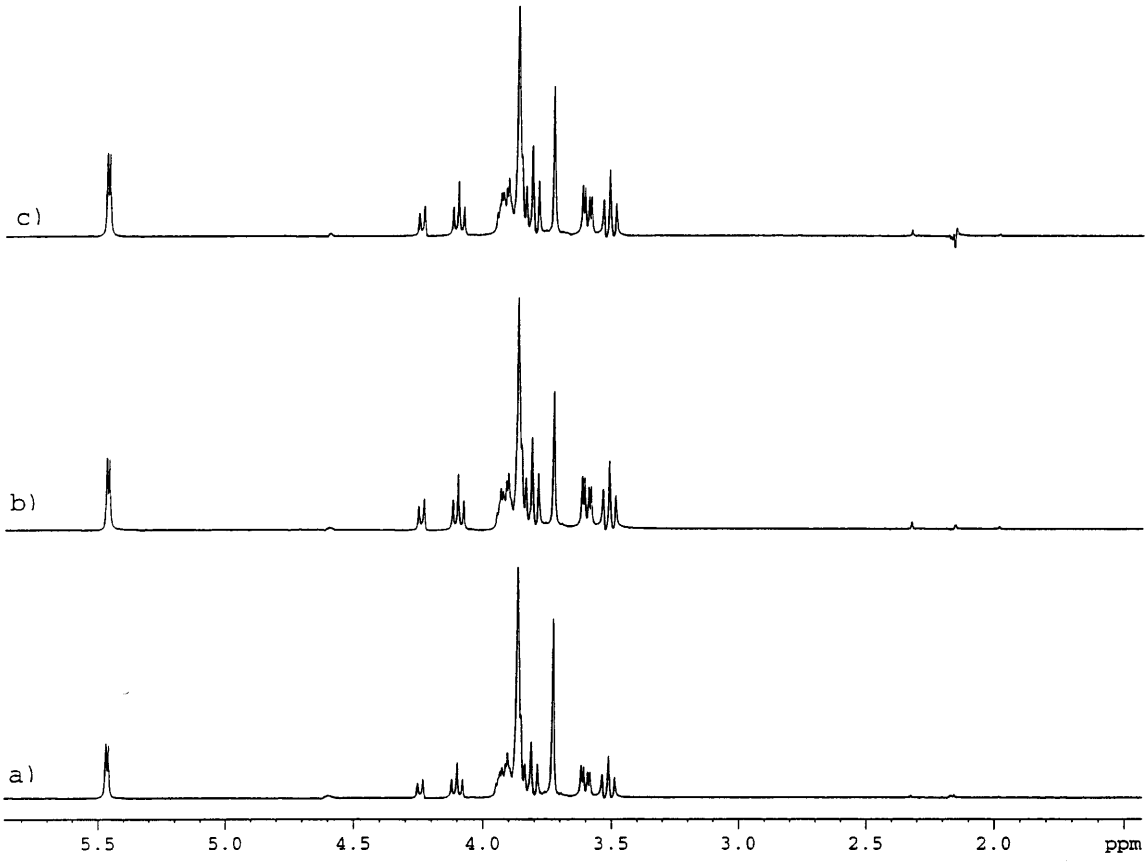

Figure 3. 1D spectra corresponding to the first increment of the (a) TOCSY, (b) ROESY and (c) NOESY experiments acquired using the pulse sequence of Fig. 1(b) (t_1 fixed at a minimum value of 3 μs) under the same experimental conditions as in Fig. 2(c).

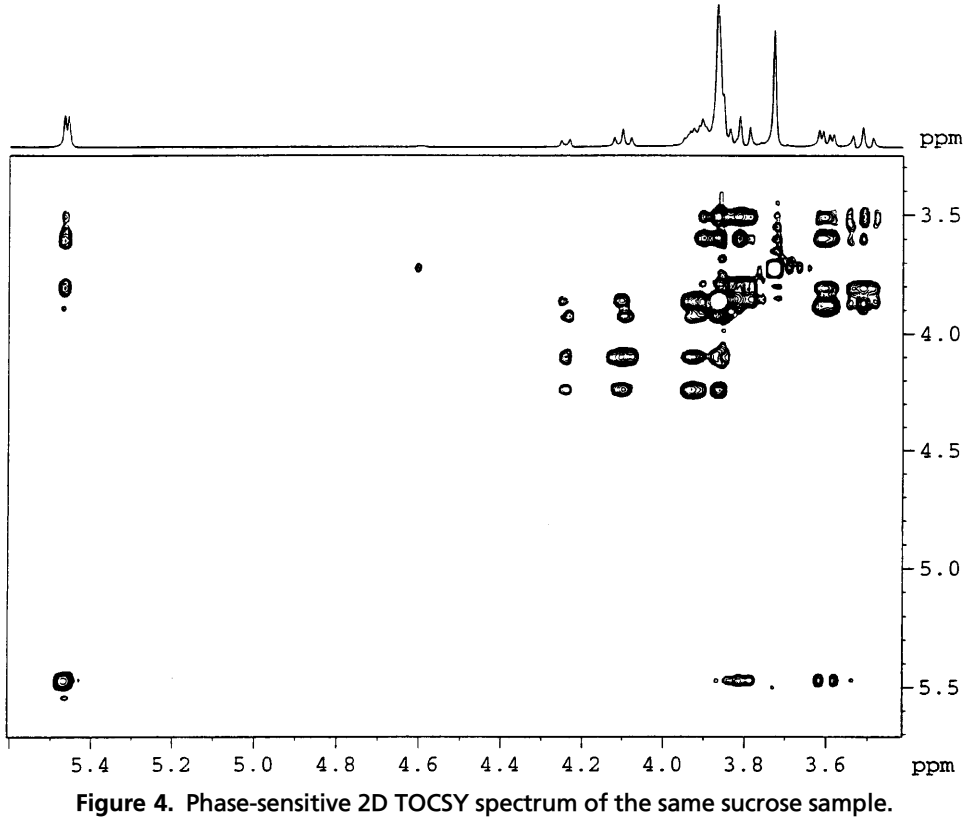

Figure 4. Phase-sensitive 2D TOCSY spectrum of the same sucrose sample.

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