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Communication

Purge NMR: Effective and easy solvent suppression

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Abstract

Presaturation utilizing relaxation gradients and echoes is an extremely easy and effective approach to solvent suppression in solution state nuclear magnetic resonance spectroscopy. The experiment produces flat baselines, excellent phase properties, and highly selective suppression that betters that of commonly used sequences. Furthermore, the only parameter that needs adjusting is the presaturation power, making it easy to implement even for non spectroscopists. Considering these factors, we envisage the approach will have wide spread applications.

Keywords: Solvent suppression; Solution state NMR; HR-MAS NMR; Metabonomics; Aqueous systems

1. Introduction

Solvent suppression is used widely in modern NMR especially with the increased popularity of hyphenated techniques such as LC-NMR, and the need to study many chemical and biological systems in non deuterated solvents. Numerous solvent suppression approaches are available today but the classical presaturation experiment is probably still the most commonly employed in routine chemical studies due mainly to its easy implementation. Classical presaturation, however, has numerous drawbacks, which can include poor phase properties and lack of suppression in comparison to more recent gradient based solvent suppression approaches [1]. However, many modern gradient based approaches require careful calibration of power levels, the use of shaped pulses, and often the optimization of parameters in real time to obtain optimal suppression. In this communication, we introduce Presaturation utilizing relaxation gradients and echoes (PURGE) as an alternative approach to solvent suppression. PURGE is a novel approach that is as simple to use as the classical presaturation experiment but performs at least as well as more complex approaches such as WET [2], WATERGATE [3,4], and excitation sculpting [5].

2. Results

Presaturation utilizing relaxation gradients and echoes (PURGE) is an extremely easy and effective approach to solvent suppression in solution state NMR spectroscopy. The basic purge sequence is highlighted in Fig. 1. The initial presaturation period is analogous to that in the classic presaturation experiment and is carried out, on resonance, during the relaxation delay. This is followed by a 90° pulse which flips the magnetization into the XY plane. After a short delay δ (200 μs for routine applications, during which presaturation is applied using the main channel, note power switching delays (4 μs in our case) are not shown) the magnetization is inverted by a 180° pulse, and then refocused by an identical delay δ. During this period (segment A highlighted in Fig. 1) the magnetization is aligned along the XY plane. This block primarily helps suppress exchangeable

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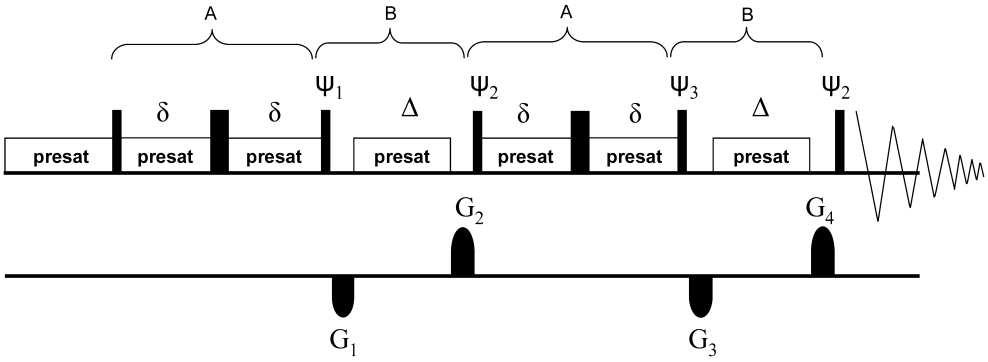

Fig. 1. The basic PURGE sequence. Narrow rectangles indicate a 90° pulse whereas, wide rectangles indicate 180° pulses. Labels A and B refer to segments discussed in the text. Note all RF pulses are applied on the same channel instrument specific delays (for example delays for power switching, 4 μs in our case) are not shown. The pulsed field gradients were applied along the z-axis with a sine shape, a duration of 1.0 ms and at the following amplitudes as a percentage of 55.5 G cm^{-1}: $G_1 = -13.17$, $G_2 = 52.68$, $G_3 = -17.13$, $G_4 = 68.52$ (values optimized experimentally). The following phase cycles were employed $\psi_1 = x,x,-x,-x$ $\psi_2 = x,x,x,x,-x,-x,-x,-x,-y,y,y,y,-y,-y,-y,-y$ $\psi_3 = x,-x,x,-x,-x,x,-x,x,x,y,-y,y,-y,-y,y,-y,y$ receiver $= x,-x,-x,x,-x,x,x,-x,-y,y,y,-y,y,-y,-y,y$. Unless stated, all other pulses were applied along the X axis. $\Delta = 200$ μs. δ is set at 200 μs for routine chemical applications but it should be noted that this delay can be varied between 3 μs and 15 ms in many samples without any phase distortion and with a only a minor influence on the efficiency of the water suppression. Larger δ values can be used to filter components in mixtures based on their T_2 relaxation, whereas δ may, in extreme cases, need to be reduced for samples exhibiting very broad lines such as membranes or vesicles (note with δ set at 200 μs (routine case), magnetization is only in the transverse plan for 800 μs during the entire sequence and as yet we have not noted any signal loss from any sample due to T_2 relaxation even when very complex semi-solids are studied in HR-MAS).

solvent signals based on a T_2 relaxation process and has been well documented by Connor and Everett [6]. We find the introduction of presaturation during the δ delay significantly reduces residual water. Due to the short duration (200 μs), the presaturation will have little if any "saturation" effect but may act more like a very weak radiofrequency pulse which selectively tilts some of solvent magnetization out of the XY plane preventing its efficient refocusing by the 90–δ–180–δ echo. Next a 90° pulse (segment B highlighted in Fig. 1) inverts the magnetization onto the Z axis and the following spoil gradient (G_1) destroys any residual transverse magnetization. Subsequently, another very short presaturation period Δ (200 μs) follows. This acts much like the previously described presaturation period but this time partially tips some of the main solvent resonance from the Z axis so it is dephased by the gradient G_2 that directly follows. During this period (section B highlighted in Fig. 1) the bulk remains aligned along the Z axis. Depending on the phase ($\psi 1$) the magnetization will have undergone either a 0° or a 180° degree rotation. At this point it is possible to terminate the sequence with an appropriate read pulse. However, we found that by repeating blocks A and B with appropriate phase shifts leads to increased solvent suppression and improved baseline and phase properties in the final spectrum [5]. Finally, at the end of the sequence a final read pulse is applied to tilt the magnetization into the XY plane for detection. The phase cycle shown in Fig. 1 is implemented from Wu et al. [7] and is discussed therein. While the cycling scheme was derived for a different purpose we found it works consistently well here, and we found no advantages by introducing additional cycling of $\psi 1$ or by incrementing the phases of the 180° pulses. However, it is important to note that the full 16 step cycle as shown in Fig. 1 is not required and excellent suppression can be achieved using 4, 8, 12, or 16 scans (note as an example Fig. 3D is obtained using 8 scans), as such the phase cycle can be easily shortened if required.

Fig. 2 compares the PURGE approach with other common solvent suppression techniques. It is clear from Fig. 2 that PURGE provides superior solvent suppression (Fig. 2D) with respect to conventional presaturation (Fig. 2B) and superior suppression and baseline properties in comparison to the commonly employed 3–9–19 WATERGATE [4] experiment (Fig 2C). We tested numerous additional solvent suppression approaches including WET [2], NOESY presaturation [8], FLIPSY [8], excitation sculpting [5], and excitation sculpting using a W5 sequence [9]. In all but the last case we could not achieve the suppression to match PURGE. Using excitation sculpting with a W5 sequence we were able to match the water suppression of PURGE, but as a result of the inversion profile, null points appear at regular intervals in the transformed spectrum, as reported in the original paper [9]. To demonstrate this and further investigate the applicability of the PURGE approach to biological systems we compared some WATERGATE approaches and PURGE on the protein lysozyme (see Fig. 3).

WATERGATE using a W5 train (see Fig. 3A) clearly shows outstanding suppression but at a price. The sequence requires that a delay for binomial water suppression be specified. In Fig. 3A this delay is set at 125 μs such that the null "sidebands" caused by the inversion profile are just at the spectral limits (note how the peaks

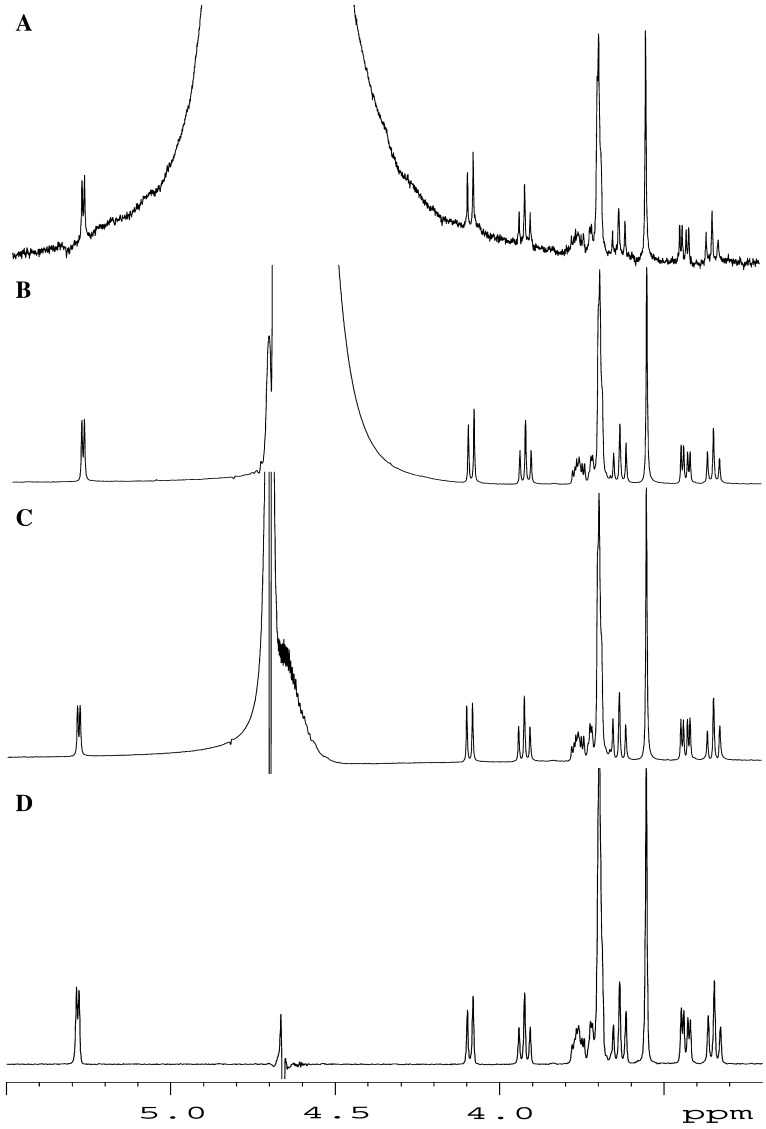

Fig. 2. ^{1}H NMR spectra of a 2 mM solution of sucrose in 90% H_2O/10% D_2O. (A) Without water suppression, (B) using presaturation (C) using 3–9–19 WATERGATE, and (D) using PURGE. ^{1}H NMR experiments were performed with 32 scans, a recycle delay of 3 s, and 32K time domain points. Spectra were apodized by multiplication with an exponential decay corresponding to 0.3 Hz line broadening in the transformed spectrum, and a zero filling factor of 2. Presaturation was applied with a 48 dB attenuation on a 60 W amplifier for both the "classic" presaturation and PURGE experiments.

at ~11 and −1 ppm are slightly attenuated in the Fig. 3A). With a delay of 125 μs the inversion profile is very wide and many of the peaks close to the water resonance are completely suppressed. These peaks can be recovered by using a narrower inversion profile (hence a longer binomial delay) but result in the introduction of sidebands into other spectral regions. In our hands we could not match the selective water suppression offered by PURGE (Fig. 3D) without introducing at least four sidebands into the spectral region using the W5 approach. Fig. 3B shows an example of such sidebands produced by a WATERGATE sequence (binomial delay 250 μs, 3–9–19 sequence). In addition to the sidebands it is clear the suppression of the water cannot match that obtained with PURGE using 3–9–19 WATERGATE. Fig. 3C demonstrates WATERGATE employing water selective 90° pulses. In this experiment sidebands are not an issue, however, the water suppression is less effective in comparison with PURGE and in addition information from peaks close to the water resonance are lost. While PURGE clearly produces the best combination of water selectivity and suppression it is important to note that PURGE employs presaturation, and thus saturation transfer which can lead to the reduction of exchangeable signals such as amides is a potential issue.

To further test PURGE with a "real world" sample, we applied the approach to study an aqueous metabolite extract from the earthworm *Eisenia fetida*. Fig. 4C clearly shows the outstanding suppression that can be achieved and the excellent baseline and phase properties.

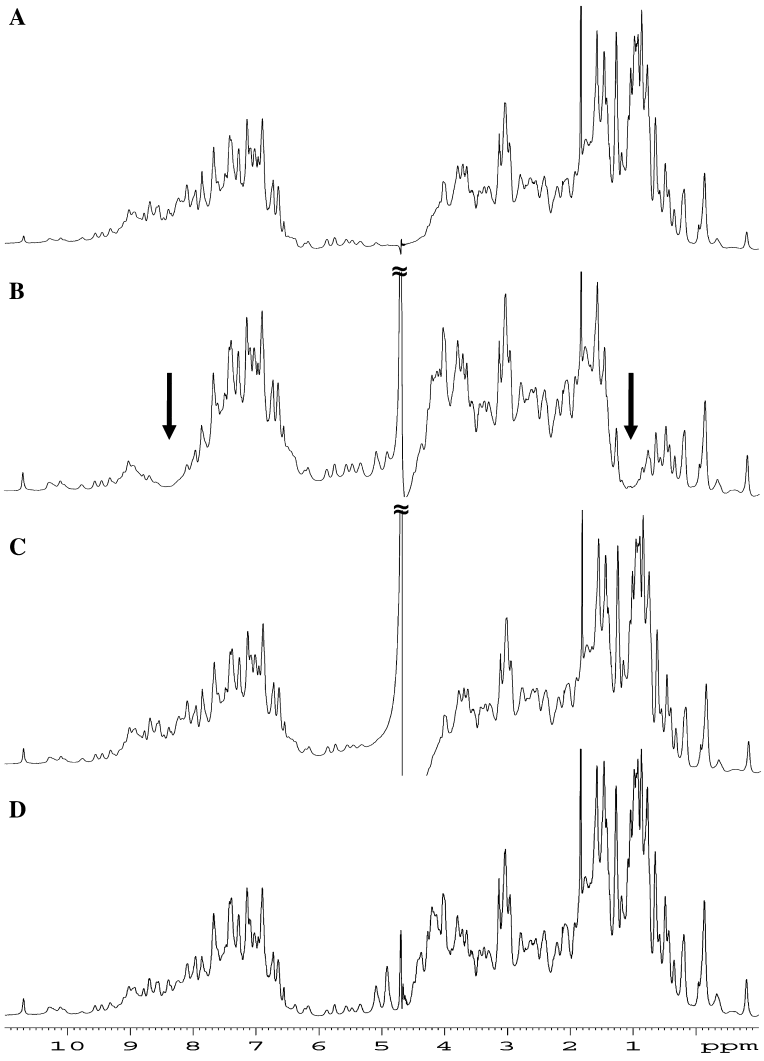

Fig. 3. ^{1}H NMR spectra of a 5 mM solution of lysozyme in 90% H_2O/10% D_2O. (A) WATERGATE using a W5 pulse train (binomial delay set at 125 μs so sidebands are just outside the spectral region), (B) WATERGATE using a 3–9–19 pulse train binomial delay set at 250 μs so sidebands are apparent (indicated by arrows), (C) WATERGATE using 90° water selective pulses, and (D) using PURGE with a presaturation corresponding to a 52 dB attenuation on a 60 W amplifier. ^{1}H NMR experiments were performed with 8 scans, a recycle delay of 3 s, and 16K time domain points. Spectra were apodized by multiplication with an exponential decay corresponding to 2 Hz line broadening in the transformed spectrum, and a zero filling factor of 2.

In addition we have expanded the 3–4 ppm region, the most complex part of the spectra, to demonstrate the quantitative nature of the approach see Fig. 5. Despite the complexity of the spectrum it is clear from Fig. 5 that the spectrum obtained using purge is near identical to the signals in the original sample. Considering this and the lack of distortions introduced into the sample we feel PURGE may be especially useful in statistical NMR studies, which are growing in popularity as fields such as metabonomics expand. In statistical studies, even baseline distortions, poor phase properties or inconsistent solvent suppression can, if not appropriately handled, seriously skew statistical results. Thus PURGE, with its flat baselines, excellent phase properties, and highly selective suppression may help at least reduce some of these artifacts influencing large statistical studies.

Finally, it is important to consider the potential implementation of PURGE in multidimensional experiments. In theory any 90° pulse can be replaced by the PURGE block, shown in Fig. 1. To test this we replaced the first pulse of a TOCSY experiment and employed the first eight increments of the phase cycle shown in Fig. 1. Fig. 6 shows the results. The PURGE-TOCSY shows excellent suppression (Fig. 6A) that betters that of normal presaturation (Fig. 6B) and that of the WET approach (Fig. 6C). However, it is important to note that unlike WET, PURGE can only suppress one resonance per spectrometer channel and thus is not as versatile for samples with numerous solvent signals.

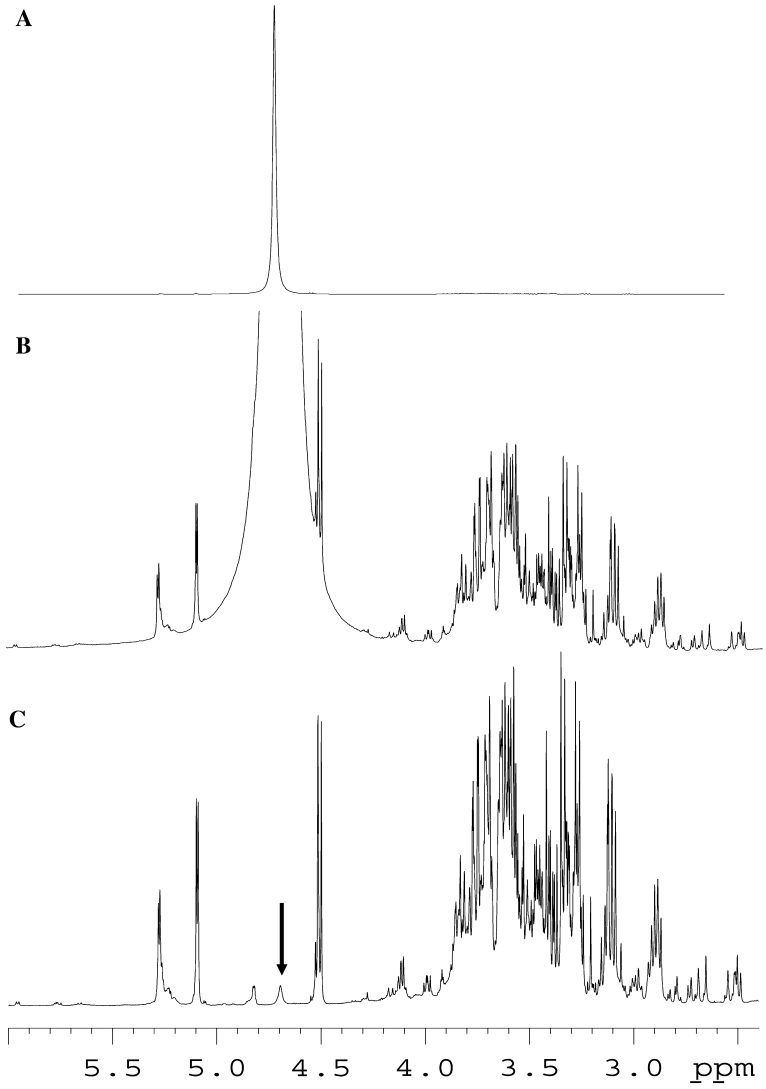

Fig. 4. ^{1}H NMR spectra of an aqueous extract from an earthworm. (A) Without water suppression, (B) same as A but with a vertical expansion of × 256, (C) using the PURGE sequence shown in Fig. 1. The arrow identifies the residual water. See supplementary information for details. Presaturation was applied with a 55 dB attenuation of a 60 W amplifier in the PURGE experiment. ^{1}H NMR experiments were performed with 32 scans, a recycle delay of 3 s, and 32K time domain points. Spectra were apodized by multiplication with an exponential decay corresponding to 0.3 Hz line broadening in the transformed spectrum, and a zero filling factor of 2.

Considering the success of the PURGE-TOCSY it seem likely PURGE may be useful building block in many experiments, although its incorporation into sequences that already employ extensive phase cycling may be somewhat laborious.

3. Conclusions

PURGE is an extremely easy and effective approach to solvent suppression in solution state nuclear magnetic resonance spectroscopy. The experiment produces flat baselines, excellent phase properties, and highly selective suppression that betters that of commonly used sequences. Furthermore, the only parameter that needs adjusting is the presaturation power, making it easy to implement even for non spectroscopists. For routine chemical applications we envisage PURGE should be a very effective substitute for conventional presaturation and may prove to be a useful building block for use in a number of multidimensional applications.

4. Experimental

4.1. Sample preparation

Three samples were used in this study. The first two samples were 2 mM sucrose, and 5 mM lysozyme, respectively, both were dissolved in 90% H_2O/10% D_2O and were purchased as 5 mm standards from Wilmad Glass, New Jersey.

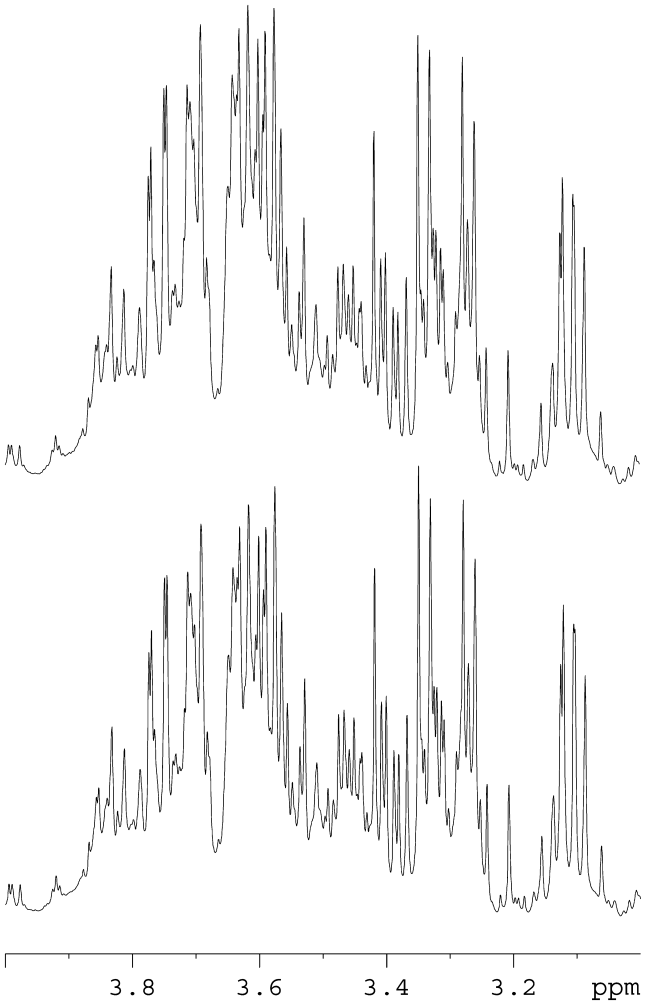

Fig. 5. An expansion of the ^{1}H NMR spectrum for an aqueous earthworm extract. This region has been expanded as it is the most complex part of the spectrum, and contains many carbohydrate signals with exchangeable groups. If any sample signals were being attenuated by the PURGE approach they should be most apparent in this region. There is near perfect agreement between the top spectrum (no suppression) and the bottom spectrum (using PURGE). All other spectral regions showed the same excellent correlation.

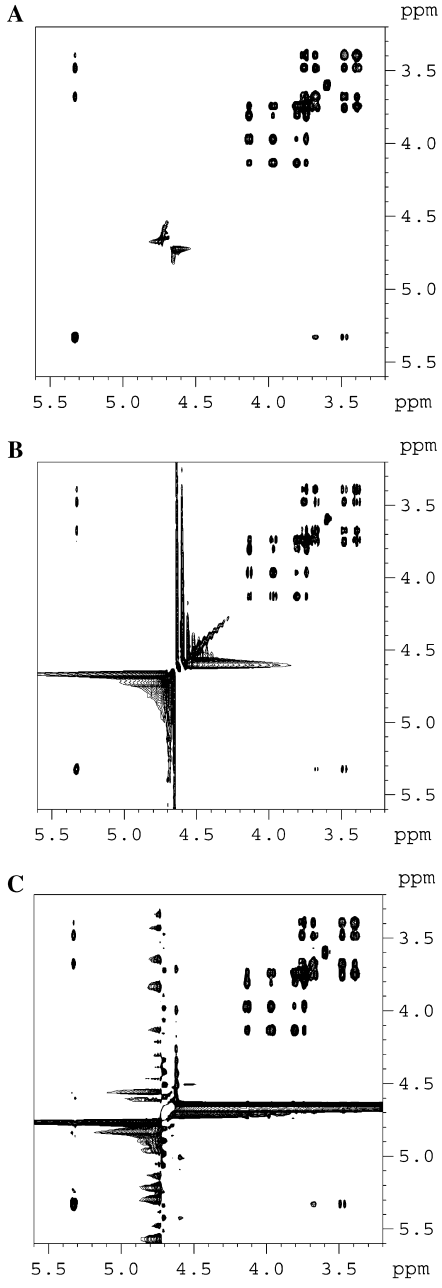

Fig. 6. (A) PURGE-TOCSY, (B) TOCSY with presaturation. (C) TOCSY acquired using WET [2], for a 2 mM solution of sucrose in 90% H_2O/10% D_2O. All spectra were acquired with 8 scans, a 60 ms mixing time, and processed using a sine-squared function with phase shifts of 90° and a zero filling factor of 2. For the WET spectrum 20 ms Gaussian pulses were employed and optimization of the power levels was carried out in real time on the 1D FID to optimize suppression. PURGE and presaturation were carried out using a presaturation power of 48 dB attenuation on a 60 W amplifier without any real time optimization.

The third sample was an aqueous extract from an earth worm (*E. fetida*). In brief the extract was prepared by homogenizing a living earthworm, using a stainless steel hand mixer. The slurry was centrifuged and supernatant collected. D_2O was added as a lock. The final solution was ~75% H_2O/25% D_2O.

4.2. NMR experiments

All NMR experiments were carried out on an Bruker Avance 500 MHz spectrometer equipped with a 5 mm ^{1}H–BB–^{13}C TBI probe with an actively shielded *Z*-gradient. Where a direct comparison of spectra is involved all experiments where carried out on the exact same sample, it was not removed, adjusted or shimmed in any way between measurements. Important experimental parameters are supplied in the appropriate figure captions.

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