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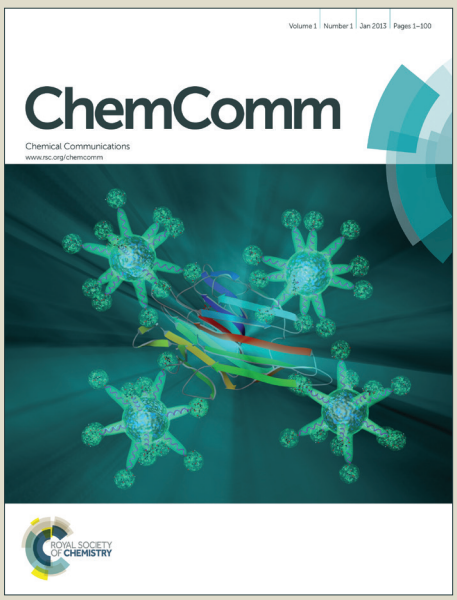

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Quick Re-introduction of Selective Scalar Interactions in Pure-Shift NMR Spectrum

Cite this: DOI: 10.1039/x0xx00000x

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Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/x0xx00000x

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A new 1D NMR experiment cited as 'Quick G-SERF' which re-introduces selective proton-proton scalar interactions in pure shift spectrum during real time data acquisition, is reported. The method provides information on multiple proton-proton couplings from a single experiment, analogous to 2D G-SERF technique, while significantly shortening the experimental time by 1-2 orders of magnitude due to reduced dimension and enhanced sensitivity.

Ever since the report on the dihedral angular dependence of $^3J_{HH}$ (vicinal couplings) by Karplus,[1] the magnitude of proton-proton couplings continue to be important NMR constraints in obtaining the spatial configuration of organic molecules.[2] There are number of reports on the utility of $^3J_{HH}$ for the conformational and configuration analysis of wide range of molecules, such as, small organic molecules,[3] cyclic compounds,[4] natural products,[5] multiple chiral centered molecules[6] and also biomolecules (peptides, proteins, DNA).[7] The efficient utility of $^3J_{HH}$ values in structural analysis depends on the accuracy of their measurement and the number of such independent $^3J_{HH}$ determined for a given site or for a molecule. More often the complex multiplicity pattern, large line widths and small proton chemical shift dispersion in 1D ^{1}H NMR spectrum, hinders the measurement of J_{HH}. Over the years the technological advances in NMR instrumentation and skillful design of new experimental techniques have partially succeeded in addressing these problems. The combined utility of higher magnetic fields and the multidimensional experimental methods have achieved larger chemical shift dispersion of peaks, reduced spectral complexity and enhanced resolution. The noted classical methods in this direction are 2D COSY and *J*-resolved type experiments.[8,9] The resolved type experiments separate the chemical shifts and couplings in two dimensions. In addition, the application of selective or smaller flip angle pulses reduces the number of proton spin-spin interactions, which in turn minimizes the spectral overlap and aids in the accurate measurement of J_{HH}. Such 2D experiments are applicable even to larger molecules with complex coupled spin systems, but demand more experimental time to achieve higher resolution. Recently few 1D NMR experiments, based on multi-frequency decoupling approaches, have also been reported for the measurement of J_{HH}.[10] These experiments reduce the size of the coupled spin system by selective removal of one or more scalar couplings, giving peaks with simplified multiplets, facilitating easy analyses and faster measurement of J_{HH} compared to two dimensional approaches. However, for the measurement of all J_{HH}, one has to carry out large number of experiments and hence insists on longer experimental time.

On the other hand the spatial encoding technique facilitates generation of slices along the sample volume by the application of weak gradient field.[11] From a single slice selective experiment multiple pieces of information can be derived by cleverly and independently manipulating spins in each slice. Based on spatial encoding technique, couple of experiments has been reported recently for the measurement of inter-proton couplings, viz., G-SERF[12] and PCR-COSY[13]. From the slice selective experiment it is possible to extract different couplings from different slices. Thus a single experiment suffices for the determination of multiple couplings. However, these methods are low sensitive 2D based approaches and demand more time (few hours) to obtain well resolved spectra.

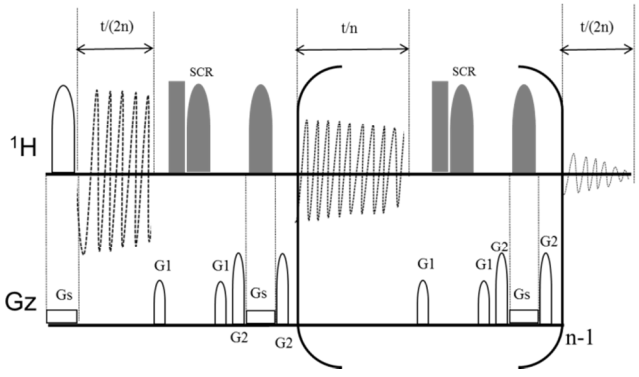

Figure 1: The pulse sequence of QG-SERF. Unfilled and filled shaped bars on ^{1}H channel represent 90^0 selective EBurp shaped and 180^0 Gauss shaped pulses respectively; SCR represents the selective couplings re-introductory

180^0 pulse. The filled rectangular bars represent 180^0 hard pulses. G_Z refers to gradients along Z-axis. Gradient Gs is used for slice selection. Gradients G1 and G2 are used for coherence selection; t is acquisition time and (t/n) ≈ $1/3(J_{HH})$.

Recent advances in NMR instrumentation enable the application of gradient and RF pulses during real-time data acquisition by interrupting the free induction decay at regular intervals. This facilitates the reduction in the dimensionality of the experiment from 2D to 1D, while still retaining the identical information, through clever manipulation of spins during real-time acquisition, thereby drastically reducing the time scale of an experiment. One such example is the instant broadband homo-decoupled Zangger-Sterk (ZS) experiment,[14] in which decoupling blocks are introduced approximately at $1/3(^3J_{HH})$ during real time data acquisition to get pure shift ^{1}H spectrum. This sequence resulted in saving of experimental time by 1-2 orders of magnitude as a consequence of greater sensitivity and resolution, compared to conventional pseudo 2D ZS approach.[11]

In this communication, we are reporting a novel 1D NMR experiment cited as 'Quick G-SERF' (QG-SERF) for the measurement of J_{HH} from the 1D ^{1}H spectrum, whose pulse sequence is given in Figure 1. Very shortly before the submission of this manuscript, Zangger *et. al.,* published a similar work carried out independently by using an identical approach.[15] The QG-SERF experiment provides multiple couplings information analogous to that of G-SERF technique, but in few minutes which otherwise would have taken few hours (1-4 hours) by 2D G-SERF.[12] The present method is designed by applying combined ZS decoupled block and additional 180^0 selective pulse (SCR - selective couplings re-introductory pulse) at regular intervals during the real time data acquisition for n times, for the acquisition time of 't', where t/n ≈ $1/3(J_{HH})$. The ZS block removes scalar interactions and yields broadband decoupled pure shift 1D spectrum, whereas application of additional selective 180^0 (SCR in Figure 1) pulse without any slice selection retains all the couplings to the selected proton. Hence this designed sequence results in 1D spectrum with simplified scalar coupled multiplet pattern at each chemical shift position of coupled partners of the selected proton, while the remaining protons are completely broadband decoupled. In short, QG-SERF gives pure shift 1D spectrum, with the retention of J_{HH} of the selected proton.

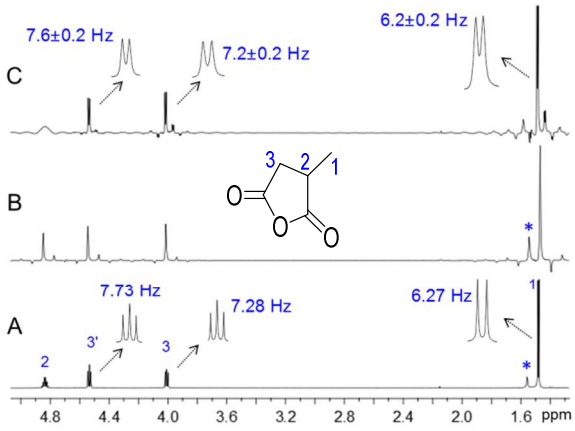

Figure 2: The chemical structure with numbering of protons of propylene carbonate; conventional ^{1}H 1D spectrum (A), 1D pure-shift ZS-spectrum (B) and 1D QG-SERF spectrum (C). Peaks 3 and 3' in A are B respectively are the multiplets due to pairwise scalar interactions among coupled network of protons, and singlets due to broadband proton-proton decoupling. Doublet for protons 1, 3 and 3' in spectrum C arise only due to couplings with proton 2. Water peak is marked with *

Initially to illustrate the concept of designed sequence, 'QG-SERF' spectrum is obtained on a simple molecule propylene carbonate (Figure 2) for the measurement of all J_{HH} pertaining to proton 2. Experimentally the reintroduction of inter-proton couplings of proton 2 is achieved by applying 180^0 selective pulse (SCR in Fig. 1) on proton 2. Total of 80 transients were acquired in 5 minutes. For the purpose of comparison, the corresponding 1D instant ZS pure-shift spectrum is also reported in Fig. 2B. The other experimental details are given ESI.

The conventional 1D proton spectrum of propylene carbonate is given in Figure 2A, which depicts multiplets for protons 2, 3 and 3' and a doublet for proton 1. Even in this simple example there will be ambiguity in the magnitudes of inter-proton couplings of protons; 3' and 2, or 3 and 2, or 3' and 3, when measured from this 1D spectrum. On the other hand the 'QG-SERF' 1D spectrum obtained by selectively re-introducing couplings of proton 2 reported in Figure 2C, gives pure shift 1D spectrum while retaining only couplings to proton 2. The doublet separations at the chemical shift positions of protons 1, 3 and 3' gave magnitudes of the couplings without any ambiguity (given on top of the spectrum). In order to evaluate the accuracy of the measured couplings from QG-SERF, we have also reported coupling values derived from the conventional 1D spectrum (Figure 2A). Both these values are in good agreement with each other, within the experimental error. It may be noted that a maximum deviation of ±0.2 Hz is observed in QG-SERF due to loss of signal during RF and gradient pulses in real-time acquisition.

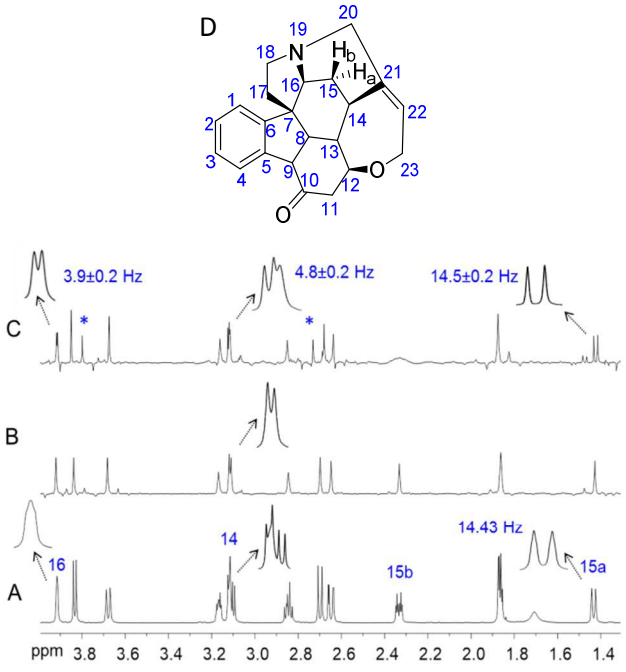

Figure 3. The chemical structure and numbering of protons in strychnine (D); conventional ^{1}H 1D spectrum (A), 1D pure-shift ZS-spectrum (B) and 1D 'QG-SERF' spectrum (C). Spectrum C gives only couplings of protons with proton 15b. Artefact peaks are marked with *.

In the subsequent step, the potential application of the 'QG-SERF' is demonstrated on the strychnine molecule (with more structural complexity, Figure 3D), for the measurement of all possible J_{HH} with proton 15b. The selected region of conventional 1D ^{1}H spectrum is

reported in Figure 3A, the proton 15b experiences scalar interactions with three other protons 15a, 14 and 16. The peaks in 1D spectrum of protons labeled as 14 and 16 depicts the complex multiplet structures, which severely hampers the accurate measurement of couplings. On the other hand the recorded QG-SERF spectrum given in Figure 3C, exhibits complete removal of multiplicity pattern of peaks and retains only couplings of proton 15b with the other protons 15a, 14 and 16, giving doublet at each chemical shift position, permitting the easy and accurate measurement of coupling strengths. The measured coupling values are reported adjacent to the expanded peaks, which are in agreement with the values reported earlier.[12] The 1D QG-SERF spectrum was obtained with 80 transients acquired in 5 minutes. Comparatively the same information with good accuracy is obtained in 4 hours by G-SERF.[12] Thus the present method demonstrates enormous reduction in the experimental time. The reintroduction of couplings to 15b is achieved by pulsing selective 180^0 pulse (SCR in Fig. 1) on 15b proton. Other experimental details and the application of QG-SERF to another molecule menthol are reported in ESI.

The QG-SERF experiment gave some unwanted artifact peaks in the spectrum. There are various sources for such artefacts as discussed below. In the present study we observed periodic and less intense artefacts for the simple molecule (Figure 2). For more structurally complex molecule (Figure 3) and in situations when there is a possibility of two different peaks have very closely resonating frequencies, additional aperiodic artefacts with little higher intensity (marked with *) were observed. However, they can easily be identified by comparing with corresponding ZS pure shift spectrum. An interesting observation is that the aperiodic artefacts are offset dependent (Figure 3C).

The various sources for such artefacts have been extensively discussed in the literature.[10,14] The experiments of this class are critically reliant on sizes of the FID chunks (t/n in Figure 1), width of the selective pulse, and size of slice selected (i.e, strength of weak slice selective gradient). The optimal size of chunk should be t/n ≈1/3 (J_{HH}), where t is total length of FID. More the number of interruptions of the FID, greater are the chances of signal loss due to transverse relaxation. The larger size of the FID chunks also results in unwanted residual couplings and more periodic artefacts. The utility of long soft pulses to select slice of thin frequency band causes loss of sensitivity due to transverse relaxation. On the other hand if the soft pulses are shorter in length, it ends up in selecting more than one desired resonances, again resulting in more artefacts in the spectrum. The choice of smaller size of the slice also results in sensitivity loss. On the other hand with the choice of larger slice size, there is higher chance of selecting more than one desired resonances, which again yields unwanted peaks in the spectrum. The utility of additional 180^0 pulse (SCR in Figure 1) in the new QG-SERF sequence also causes more signal loss and transverse relaxation causing broadening of peaks. The implication of the above discussion is that there is a compromise among sensitivity, experimental time, artifacts free spectrum and achievable resolution. Therefore utmost care has to be exercised in optimizing the above mentioned parameters while running experiments of this type. Furthermore the average line broadening observed in QG-SERF is ≈ 3 Hz, which sets the limit for the size of the couplings that could be measured in the present study.

In summary, the novel one dimensional QG-SERF reported in the present communication provides an easy and efficient approach for the measurement of multiple scalar couplings from a single experiment. In comparison with the 2D G-SERF, the present experiment is an order of magnitude faster in time, permits unambiguous measurement of multiple J_{HH} in complex organic molecules. Hence, we strongly believe that the present experiment significantly helps in speeding up structural elucidation process.

Lokesh would like to thank Indian Institute of Science for SRF. NS gratefully acknowledges the generous financial support by the Science and Engineering Research Board, Department of Science and Technology, New Delhi (grant No. SR/S1/PC-42/2011). Authors are grateful to Prof. K.V. Ramanathan for helpful discussions.

Notes and references

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Electronic Supplementary Information (ESI) available: [Experimental details, pulse program code, table of couplings value and sample preparation]. See DOI: 10.1039/c000000x/

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