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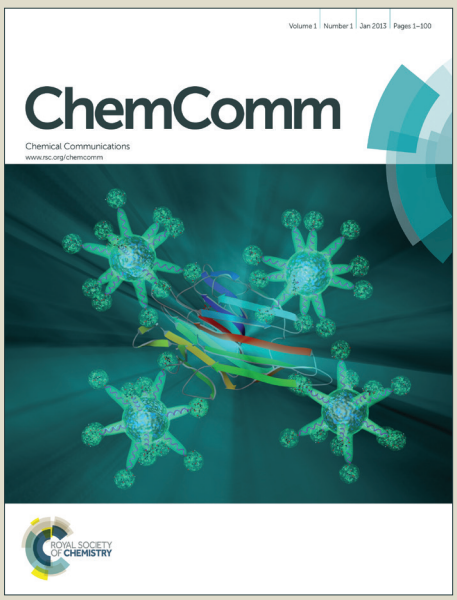

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Sensitivity Enhancement in Slice-Selective NMR Experiments through Polarization Sharing

Cite this: DOI: 10.1039/x0xx00000x

Received ooth April 2014,
Accepted ooth 2014

DOI: 10.1039/x0xx00000x

www.rsc.org/

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Polarization sharing technique is utilized in gradient based slice selective experiments to transfer polarization from unutilized protons to selectively excited protons. This facilitates rapid data acquisition without any customary inter-scan relaxation delay, resulting in average of 2-fold sensitivity enhancement per unit time.

In recent times the spatial encoding NMR technique is employed in number of very exciting applications. The weak gradient field applied along Z-direction during selective pulses, generates slices along sample volume. These slices are used to extract specific information from a specific slice, which in turn results in multiple pieces of information from a single experiment. The increased popularity of the slice selection technique is clearly evident by the number of recently reported novel NMR experiments, such as, ^{1}H broadband homodecoupled experiments,[1] single-scan 2D experiments,[2] pure shift 2D and 3D experiments,[3] diffusion based pure shift NMR experiments,[4] and *J*-resolved type experiments.[5]

The major drawback of all the slice selection based NMR experiments is their low signal to noise ratio (SNR) due to effective utility of very small percentage of total proton spins (1-5%), while the large percentage (≈ 95%) of total spins available are left unutilized.[6] This problem is more severe, especially when one is interested in low concentrated solutions, viz., metabolomic samples, protein samples, natural product extracts, etc. Therefore it is important and also a dire necessity to develop novel methods to enhance the sensitivity of such experiments. In this direction recently few methods have been reported. One is fast pulsing method based on offset frequency shift with each incrementing scan,[7] that allows to select fresh set of protons each time without any inter scan relaxation delay. This in turn helps in acquisition of more number of scans in a given time and consequently enhanced signal intensity. Another method is based on simultaneous multi-slice excitation technique that employs multiple frequency pulses resulting in considerable enhancement in SNR.[6] However, both these methods requires additional optimization in terms of calibration of offset frequency and setting of multiple offsets to avoid distorted multiplets.

The polarization sharing technique cited as "acceleration by sharing adjacent polarization" (ASAP) is recently used to enhance the sensitivity in hetero-nuclear experiments by transferring the unused ^{12}C (98.9 %) attached proton polarization to ^{13}C attached protons (1.1%) via scalar couplings.[8] This aids in acquisition of more number of experiments per unit time without using any customary inter scan relaxation delay, resulting in 3-4 fold signal enhancement per unit time than compared to the spectrum obtained in a conventional way. Further, it is also shown that, the selective excitation of subset of proton spins in a molecule, while leaving other spins unperturbed, shortens spin-lattice (T_1) relaxation time,[9] which often enhances the sensitivity in fast pulsing methods, viz. in peptides, proteins etc.

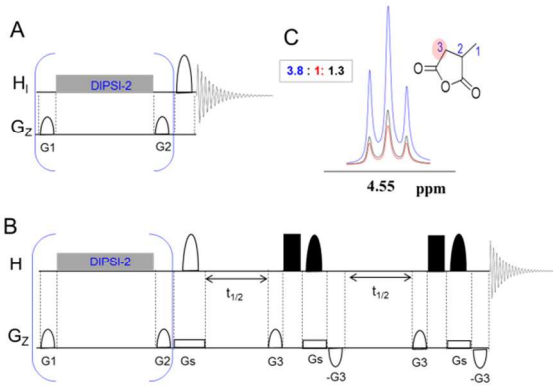

Fig 1: Pulse sequence of ASAP-Sel1d **(A),** and pure-shift ASAP-ZS **(B).** The unfilled and filled shaped bars on ^{1}H channel are selective 90^{0} and 180^{0} pulses respectively; filled rectangular bars are 180^{0} hard pulses; G3 and -G3 are the field gradient pulses used for coherence selection; Gs is slice selection gradient; Initial block in both **A** and **B** consisting of DIPSI-2 flanked by two gradients G1 and G2 constitute isotropic mixing block for polarization transfer. **(C)** Intensity comparison of selectively excited ^{1}H peak (proton at 3 in propylene carbonate) from Sel1d with inter-scan delay 2 s (red) and 75 ms (black, T_1 enhanced relaxation), and ASAP-Sel1d (blue) with minimum inter-scan delay of 35 ms obtained in identical experimental time.

As discussed earlier, only 1-5 % of total proton spins are used in slice selection based experiments and remaining nearly 95% are left unutilized. In the present method we have used ASAP technique to enhance sensitivity by transferring such unused protons spin

polarization partially to detectable 1-5 % of protons via scalar couplings. The polarization transfer is achieved by isotropic mixing of magnetization prior to slice selection.[8] The method helps in acquiring more number of scans in a given time without any inter-scan relaxation delay (fast pulsing) and results in an average of 2-fold sensitivity enhancement per unit time, which also includes contribution from the enhancement due to shortened T_1-relaxation time. The biggest advantage of this technique is, it does not need any additional calibration unlike in earlier reports[6,7] and can be implemented in most of the slice selection based experiments without much modification in the existing pulse sequences. Since the method works based on fast acquisition of data points and hence might also find utility in study of fast reaction kinetics and dynamics.[7a] However, the sensitivity gain due to ASAP module will not be achieved for isolated protons. The utility of the technique is illustrated in different examples and the sensitivity enhancement due to ASAP and enhanced T_1 relaxation is quantified.

Initially the effectiveness of ASAP for sensitivity enhancement is tested in a simple selective excitation experiment by selectively exciting the proton at '3' in propylene carbonate molecule (Fig. 1C). The polarization shared selective excitation pulse sequence cited as ASAP-Sel1d, is given in Fig. 1A. The well-known DIPSI-2,[10] isotropic mixing block, with optimised duration of 40 ms is used prior to selective pulse to achieve polarization transfer. The ASAP-Sel1d with minimum inter scan relaxation delay of 35 ms is recorded for 4 scans in 4.5 sec. Two normal 1D selective excitation experiments (Sel1d) with inter scan relaxation delays of 75 ms (= relaxation delay + DIPSI-2 duration in ASAP) and 2 sec were acquired respectively for 4 and 2 scans in identical time to that of ASAP-Sel1d. Other experimental parameters were kept identical in all the experiments and are given in ESI.† The intensities of the peaks from the experiments are compared in Fig. 1C. A gain in signal intensity approximately by a factor of 2-4 per unit time in ASAP-Sel1d experiment (blue) and 1.2 per unit time due to T_1 enhanced relaxation (black, Sel1d with inter-scan delay of 75 ms) than compared to normal Sel1d (red, inter-scan delay of 2 sec) is obtained.

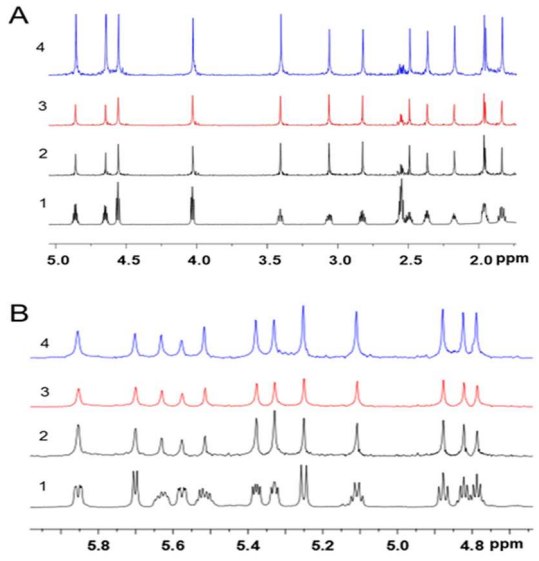

Fig 2. **(A)** Mixture of propylene carbonate, γ-valerolactone, 1-indanol and *L*-menthol ^{1}H 1D spectrum (1, black). **(B)** Expanded Hα region of cyclosporin-A ^{1}H 1D spectrum (1, black); In both A and B, 2 is pure-shift ZS ^{1}H spectrum with inter scan delay of 135 ms (black), 3 is pure-shift ZS ^{1}H spectrum with inter scan delay of 2 s (red) and 4 is pure-shift ASAP-ZS ^{1}H spectrum (blue). By ASAP a sensitivity enhancement of 100-110% in A and 5-10% in B are observed. Due to enhanced T_1-relaxation a sensitivity enhancement of 5-10% in A and 90-100% in B are observed (2, black).

After successfully establishing the gain in sensitivity in a simple selective excitation experiment, the potential utility of ASAP module was subsequently explored in slice selective experiments. Initially the method is illustrated in pure-shift pseudo 2D Zangger-Sterk (ZS) experiment,[1a] which is used to obtain broadband homo-decoupling spectrum based on slice selection. The experiment has wide applications in simplifying complex spectra and the extraction of hetero-nuclear couplings.[11]

The pulse sequence for pure-shift ZS-method with polarization sharing cited as ASAP-ZS is given in Fig. 1B. It consists of initial DIPSI-2 isotropic mixing block flanked by two gradients for polarization transfer, followed by conventional pure-shift ZS sequence and a hard and soft 180^0 pulses before acquisition to bring back the unused magnetization to z-direction. The experiment is demonstrated on two different examples, one is a mixture of organic molecules (propylene carbonate, γ-valerolactone, 1-indanol and L-menthol), which have longer proton T_1 relaxation times (≈ 2.5-6 sec) and another example of cyclosporin-A (50 mM in C_6D_6), whose protons have smaller T_1 relaxation time (≈ 1-2 sec). The individual spectra of conventional pure-shift ZS-method with inter scan relaxation delay of 2 sec and 135 ms for acquisition of 4 and 16 scans respectively and ASAP-ZS (polarization sharing) with minimum inter scan relaxation delay of 35 ms for 16 scans at each data point were in almost identical time. In ASAP-ZS, the polarization transfer is achieved by DIPSI-2 isotropic mixing block with optimized duration of 40 ms prior to pure shift ZS pulse sequence. The 2D data of ASAP-ZS and ZS are processed to 1D spectra by using macro [available from Manchester University Website of G. A. Morris group],[12] which are shown in Fig. 2A (mixture) and Fig 2B (cyclosporin A). It is observed that in case of mixture of molecules the sensitivity gain of 100% per unit time is achieved by ASAP (Fig 2A, blue, spectrum number 4) and 5-10 % per unit time is achieved due to shortened T_1 relaxation (Fig 2A, black, spectrum number 2) compared to normal ZS (Fig. 2A, red, spectrum number 3). Conversely in the case of cyclosporine-A the sensitivity gain of 5-10 % by ASAP (Fig 2B, blue, spectrum number 4) and a gain of 100 % by enhanced relaxation (Fig 2B, black, spectrum number 2) are observed. This difference in the intensity behavior in both the cases is due to the effect of T_1 relaxation. The other experimental parameters are kept constant in all cases and are given in ESI. The sensitivity enhancement by ASAP module is illustrated in another important slice selection based experiment, namely G-SERF. It is ^{1}H detected 2D experiment[5], developed based on the SERF[13] (selective refocusing) experiment to extract all the spin-spin couplings experienced by a particular spin from a single experiment, which are useful in obtaining the structure and the conformation analysis of complex molecules.

The pulse sequence for G-SERF with polarization sharing cited as ASAP-G-SERF is given in ESI.† The experiment was initially demonstrated on *L*-menthol (5 mg in 500 µl $CDCl_3$). The individual spectra of G-SERF with inter scan relaxation delays of 2 sec and 75 ms and ASAP-G-SERF (polarization sharing) were recorded in identical time with acquisition of 4, 8 and 8 transients at each data point respectively. In ASAP-G-SERF a minimum delay of 35 ms is used between the scans and the polarization sharing is achieved using DIPSI-2 isotropic mixing block with duration of 40 ms. All other experimental parameters were kept identical in all experiments. The recorded ASAP-G-SERF and G-SERF (inter-scan

delay of 2 sec) spectra are reported in Figs. 3A and 3B respectively, which yield all the ^{1}H-^{1}H scalar couplings of proton 6 in menthol molecule. The intensity of two cross peaks from each experiment are compared and shown as inset in Fig. 3A. The blue, black and red colored peaks are respectively from ASAP-G-SERF, and two G-SERF experiments with inter-scan delays of 35 ms and 2 sec. A minimum of 2-fold sensitivity enhancement per unit time is obtained in ASAP-G-SERF (blue) than compared to normal G-SERF (red), with better quality spectrum. The G-SERF with inter-scan relaxation delay of 75 ms resulted in least sensitivity due to longer T_1 relaxation time (2.5-4.5 sec) of menthol protons. The complete details of the experiment are given in the ESI. †

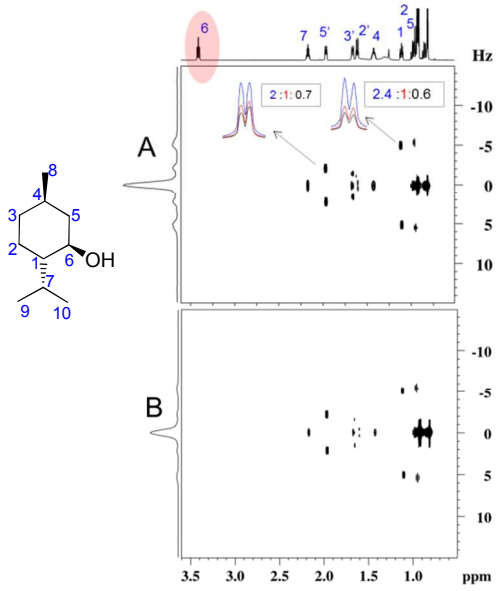

Fig 3. Chemical structure of *L*-menthol and comparison of 2D ^{1}H spectrum of ASAP-G-SERF **(A)** with G-SERF **(B)** spectrum of *L*-menthol. The intensities of cross peaks are compared from each experiments in **A**, ASAP-G-SERF (blue) and G-SERF, each with 75 ms (black) and 2 sec inter-scan delay (red). It is clearly evident that there is average of 2-fold sensitivity enhancement per unit time in ASAP-G-SERF.

The wide utility of the experiment is demonstrated on another complex molecule, strychnine (5 mg in 500 µl). Two G-SERF experiments with inter-scan delay of 2 sec and 95 ms and ASAP-G-SERF spectra with minimum inter-scan of 35 ms were recorded in identical time with acquisition of 4, 8 and 8 transients at each data point respectively. The spectrum gives information on all the ^{1}H-^{1}H couplings of 15b proton (Fig S2, ESI†). The intensity of cross peaks obtained from each of the experiment, blue one from ASAP-G-SERF and red one and black one from G-SERF with inter-scan delays of 2 sec and 95 ms are compared. It is observed that there is nearly 2-fold signal enhancement per unit time by the ASAP-G-SERF technique. All the experiments were carried out on a 800 MHz Bruker NMR spectrometer which is facilitated with a cryo probe. All the experiment details are given in the ESI.†

The results of the study clearly establish that the blend of polarization sharing technique and enhanced T_1 relaxation to the slice selective experiments yielded the sensitivity gain approximately by a factor of 2-fold per unit time. The method is easy to implement, does not insist on additional calibration or optimization and modification of existed pulse sequence. The polarization sharing can be combined with other sensitivity enhancing techniques like multiple slice selection, instantaneous homo-decoupling method[1b] to further push the sensitivity limit. Thus the present method promise to be potentially useful in extending applicability and effectiveness of slice selection based NMR experiments.

Acknowledgement

We thank Prof. G. A. Morris group for permitting us to use the relevant macros from their website. Lokesh would like to thank Sachin R Chaudhari and K V Ramanathan for useful discussions and IISc for scholarship. 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).

Notes and references

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†Electronic Supplementary Information (ESI) available: Experimental details, pulse sequences and 2D ^{1}H G-SERF spectrum of strychnine.
See DOI: 10.1039/c000000x/

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