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Pure Shift NMR Approach for Fast and Accurate Extraction of Heteronuclear Couplings

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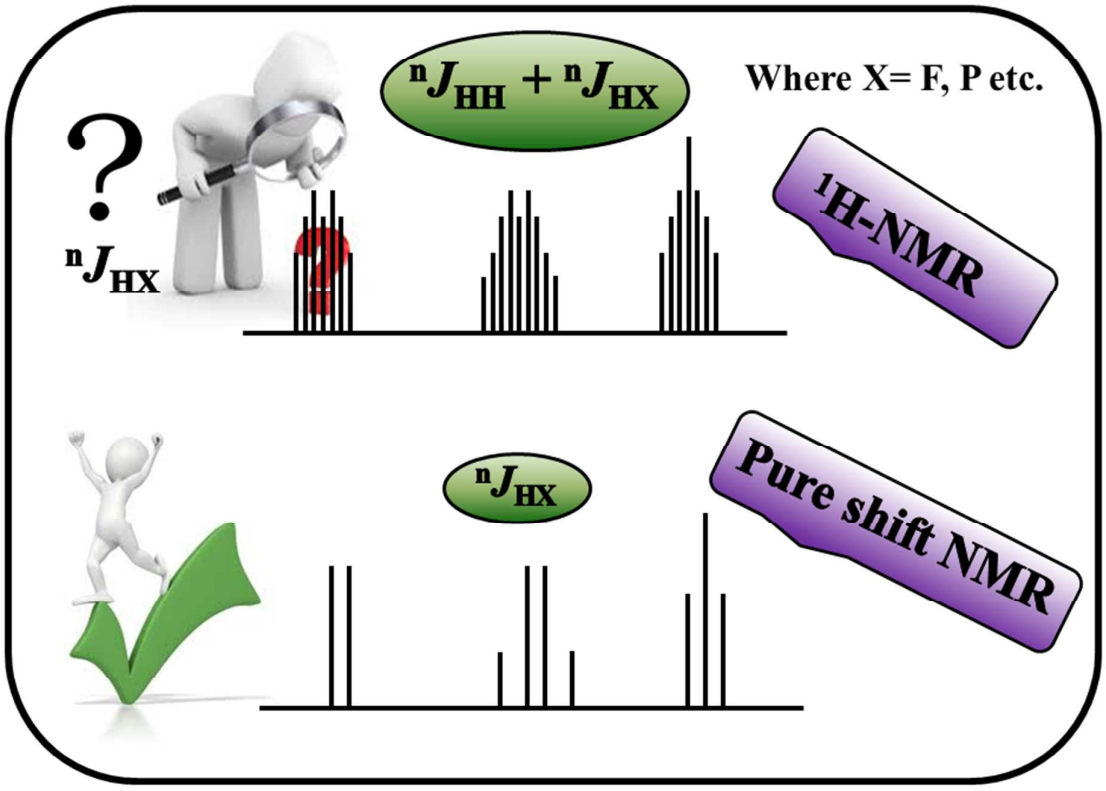
Graphical Abstract



Abstract

The direct and accurate determination of heteronuclear ($^{n}J_{HX,}$ X =^{19}F, ^{31}P) couplings from the one dimensional ^{1}H-NMR spectrum is severely hampered due to the simultaneous presence of large number of $^{n}J_{HH}$. The present study demonstrates the utility of pure shift NMR approach for spectral simplification, precise and direct measurement of heteronuclear couplings. As a consequence of refocusing of homonuclear couplings ($^{n}J_{HH}$) by the pure shift pulse sequence, only heteronuclear couplings ($^{n}J_{HX}$) appear as simple multiplets at the resonance position of each chemically non-equivalent proton, enabling their direct measurement from the 1D-^{1}H spectrum. The experiment is demonstrated on number of molecules containing either ^{19}F or ^{31}P, where $^{n}J_{HF}$ and $^{n}J_{HP}$ could be precisely measured in a straightforward manner. The distinct advantage of the experiment is demonstrated on molecules containing more than one fluorine atom, where most of the available NMR experiments fail or have restricted utility.

Introduction

NMR spectroscopy is one of the widely used analytical techniques for the study of small molecules, proteins and peptides [1]. Availability of higher magnetic fields, sophisticated electronics, modern probe design permits easy spectral analyses [2] and quantification at low concentration levels [3]. The multiplicity pattern provides the valuable information about the scalar couplings (*J*). The $^{n}J_{XH}$ (n = 2,3) is extremely useful in the assignment of relative configurations and conformations [4]. The well known Karplus equation is employed to obtain the relative configurations in small molecules using the vicinal coupling constants [4]. Homo- and hetero- nuclear *J*-based analysis have also been proven to be very useful for the analysis of natural products and relative stereochemistry of complex acyclic molecules [5, 6]. Nevertheless the challenging problem of reducing the spectral complexity for the

straightforward determination of couplings continues to persist. The direct extraction of short and long range J_{HX} (X= F, P, etc.) couplings from the one dimensional ^{1}H NMR spectrum is a challenging task due to the paradigm of homonuclear couplings. Although there exist many 2D experiments for the extraction of $^{n}J_{HF}$, such as, 2D ^{1}H-^{13}C [7], ^{13}C-^{19}F [8], ^{1}H-^{19}F [9] correlation, majority of them demand either specially designed ^{19}F probe or require more investment on the instrument time, and also suffer from problem of sensitivity when low abundant nuclei, such as, ^{13}C is involved. Long back the skew projected 2D J-resolved spectrum has been reported for the measurement of heteronuclear couplings, which does not insist on more experimental time and additional equipment [10]. We have also reported spin selective double quantum [11], double quantum J-resolved, spin selective triple quantum, [12] and heteronuclear ^{15}N-^{1}H double quantum-single quantum correlation [12], 2D ^{1}H-^{15}N [13] experiments for the extraction of signs and magnitudes of homo- and hetero- nuclear couplings. The higher quantum detection experiments suffer from both the loss of sensitivity and the linear scaling of inhomogeneity contributions with the higher quantum order [12]. One dimensional 1D-HSQC [14], 1D-HSQMBC-TOCSY experiments [15] and selective TOCSY experiment [16] though available for the extraction of signs and magnitudes of the couplings, they require higher concentrations of sample and cannot be applied in a straightforward manner when more than one fluorine atom is present in the molecule. Furthermore, some of these experiments have limitations due to severe overlap of peaks posing problem for selective excitation. It is always beneficial to devise a simple and convenient one dimensional experiments for such a purpose which is free from such limitations. Thus in the present work we are exploring the utility of a simple NMR experiment, which decouples the entire proton coupled network of spins [17] well known in the literature as pure shift NMR [18] and a number of related experiments have been developed by the group of G.A. Morris [18]. The pulse sequence thus retains only

heteronuclear couplings allowing a fast and reliable method of measuring $^{n}J_{HX}$ (X = ^{19}F, ^{31}P) couplings from the complex ^{1}H spectrum. The usefulness of pure shift experiments is demonstrated on fluorine and phosphorous containing aliphatic and aromatic molecules which exhibit structural and spectral complexity, such as, epifluorohydrin (**a**), 2-fluroacetanilide (**b**), 2-fluoropyridine (**c**), 2,3,4-trifluorophenylcyanide (**d**), 3,4-difluoro-2-nitrophenol (**e**) and Dimethyl vinylphosphonate (**f**) (Fig. 1).

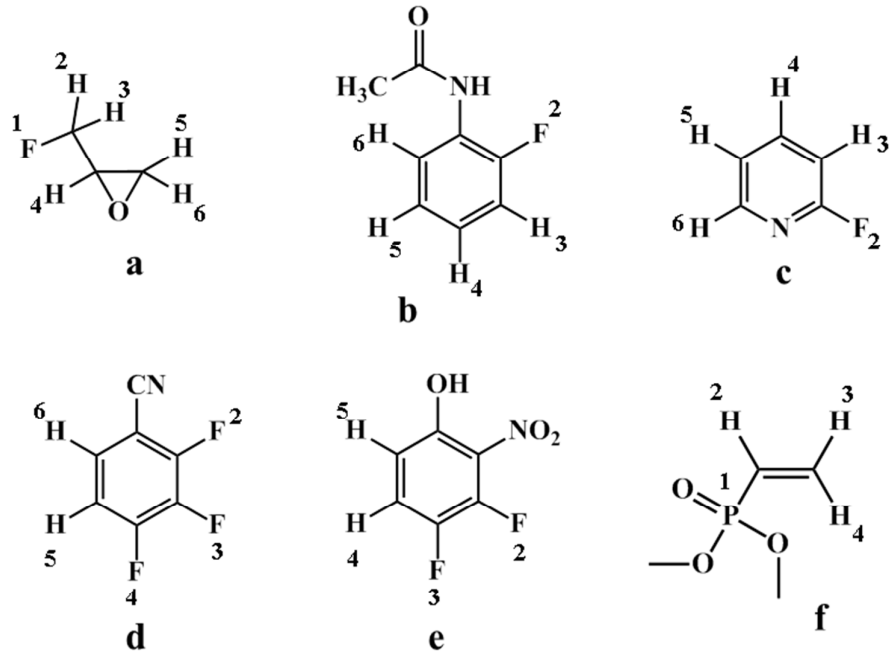


Figure 1: Chemical structures of molecules investigated for the extraction of the $^{n}J_{HX}$ (X= ^{19}F, ^{31}P) couplings using pure shift experiments.

Results and Discussion

Initially the utility of pure shift approach is demonstrated on epiflurohydrin (molecule **a**) whose ^{1}H NMR spectrum is well dispersed but has complex multiplet pattern at each proton site due to both J_{HH} and J_{HF}. The direct extraction of $^{n}J_{HF}$ couplings from this spectrum is

difficult. The application of pure shift sequence removes all the proton-proton couplings and retains only $^{n}J_{HF}$ giving a doublet at the chemical shift position of each chemically non-equivalent proton. The direct measure of doublet separation yields $^{n}J_{HF}$ depicting the immediate advantage of the utility of pure shift. The ^{1}H-NMR spectrum of epiflurohydrin with and without implementation of pure shift is given in Fig.1.

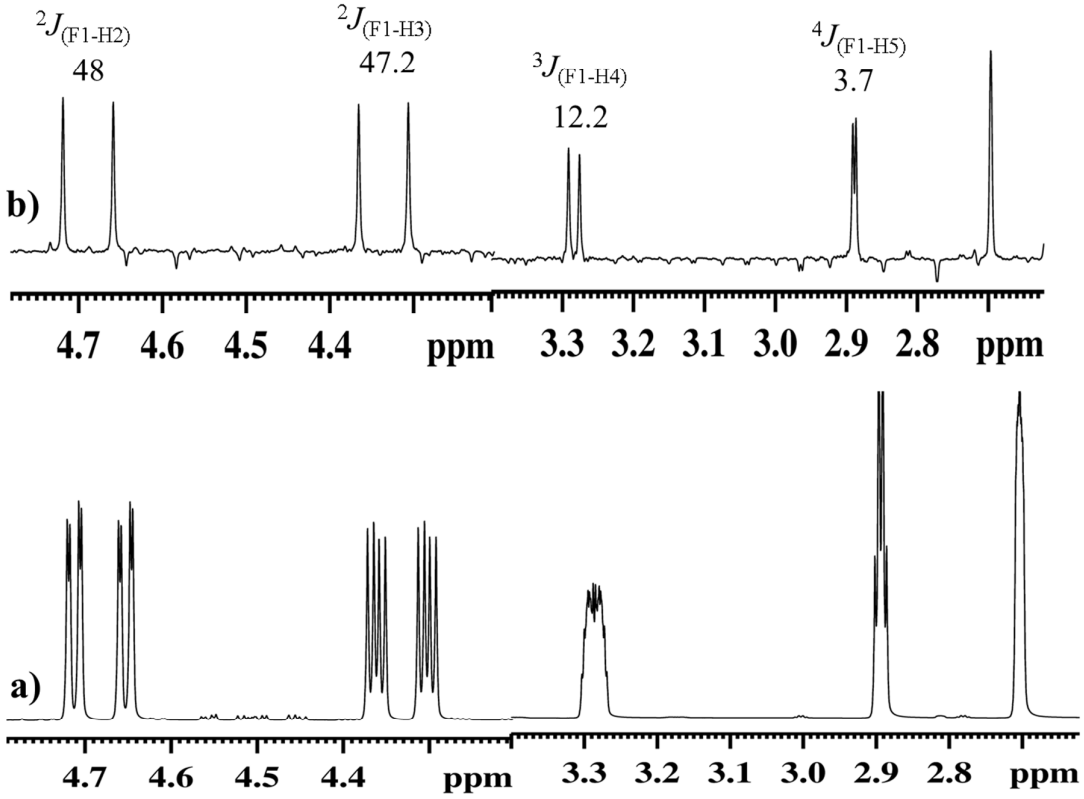


Figure 2: (a) ^{1}H NMR spectrum of epiflurohydrin (molecule a) in $CDCl_3$; (b) Pure shift spectrum of the same molecule, depicting only $^{n}J_{HF}$ couplings.

For wide utility, the experiments were carried out on molecules, such as, 2-fluroacetanilide (**b**) and 2-fluoropyridine (**c**),), 2,3,4-trifluorophenylcyanide **(d)**, 3,4-difluoro-2-nitrophenol, whose spectra are either broader without any coupling fine structures or complex, preventing the direct extraction of $^{n}J_{HF}$. All the corresponding spectra are reported in the supporting information along with the magnitudes of the measured $^{n}J_{HF}$.

The experiment is not restricted to molecules containing single fluorine atom but also can be employed for molecules containing more than one fluorine atoms. For such a purpose the molecule), 2,3,4-trifluorophenylcyanide **(d)** was chosen. The ^{1}H spectrum of this molecule comprises of two well isolated but severely overlapping multiplet pattern in the region δ 7.20-8 ppm, due to the number of proton-proton and proton fluorine couplings, prohibiting the measurements of $^{n}J_{HF}$ (Figure 3a). On other hand the application of pure shift sequence aids in simplifying the spectrum, the analysis of which provides coupling of ^{1}H with all the three fluorines (Figure 3a). Thus the utility of pure shift is fast and reliable for deriving all $^{n}J_{HF}$ from the complex ^{1}H spectrum. The pure shift utility also has distinct advantages over other available methods, such as, 1D-HSQC [14] and 1D-HSQMBC-TOCSY[15] experiments, where the extraction of the couplings fails when the molecules contains more than one fluorine atom. The G-SERF [19] experiment though provides the coupling information, requires multiple experiments to extract all $^{n}J_{HF}$, whereas in the case of the pure shift experiment the coupling can be extracted in one shot from a single experiment. The experiment is also demonstrated on molecule **e** containing two fluorine atoms, the derived spectral parameters are reported in the supporting information.

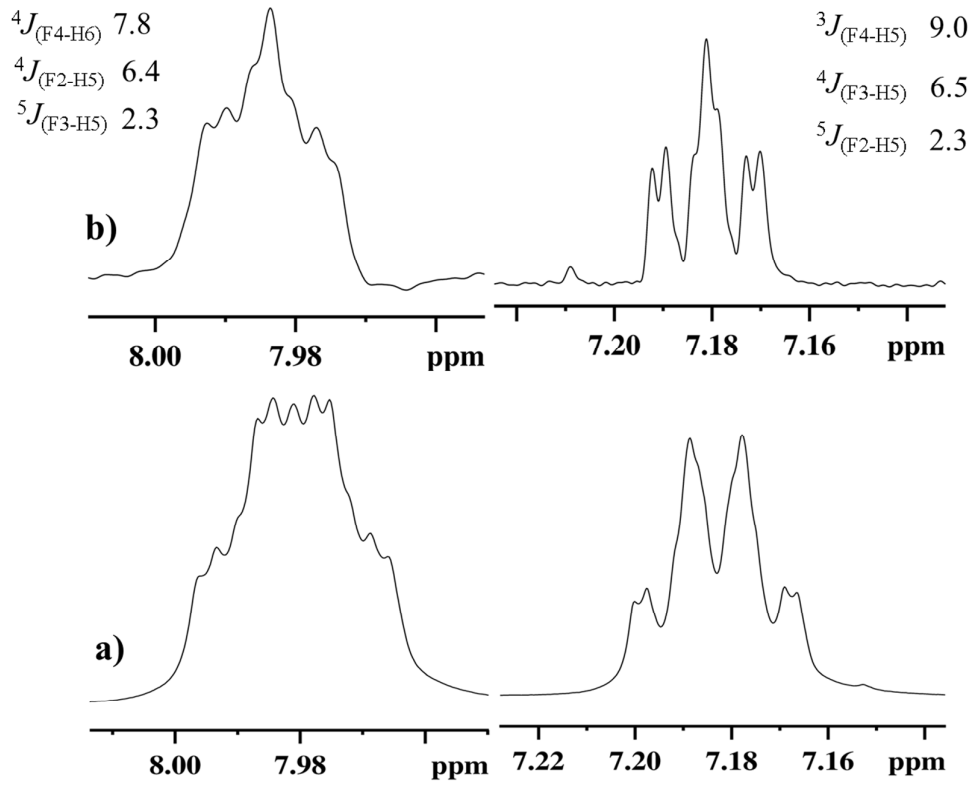


Figure 3: (a) ^{1}H NMR spectrum of 2,3,4-Trifluoronitrobenzene in $CDCl_3$; (b) Pure shift spectrum of the same molecule, depicting only $^nJ_{HF}$ couplings.

The Pure shift experiment also finds immediate application when other abundant heteronuclei, such as, phosphorous is present in the molecule, where we are able to extract $^nJ_{HP}$ couplings from the simple 1D-^{1}H pure shift NMR spectrum. This is demonstrated on dimethyl vinylphosphonate (f) whose chemical structure is reported Fig. 1 and the corresponding ^{1}H spectrum is given in Fig. 4. The ^{1}H spectrum exhibits a complex pattern due to the presence of large number of $^nJ_{HH}$ and $^nJ_{HP}$ couplings. As a consequence the spectrum is severely overlapped and does not display any coupling fine structures. In such situations the extraction of $^nJ_{HP}$ is severely hampered and peaks are overlapped posing a challenge for the assignment of peaks. Recently reported selective 1D–TOCSY experiment [16] also has restricted utility in situations since the selective excitation of any of the peaks is

not possible due to overlap. However, the pure shift NMR has an immediate advantage and $^{n}J_{HP}$ could easily be extracted without any ambiguity. The extracted couplings and the pure shift spectrum are given in Fig. 4.

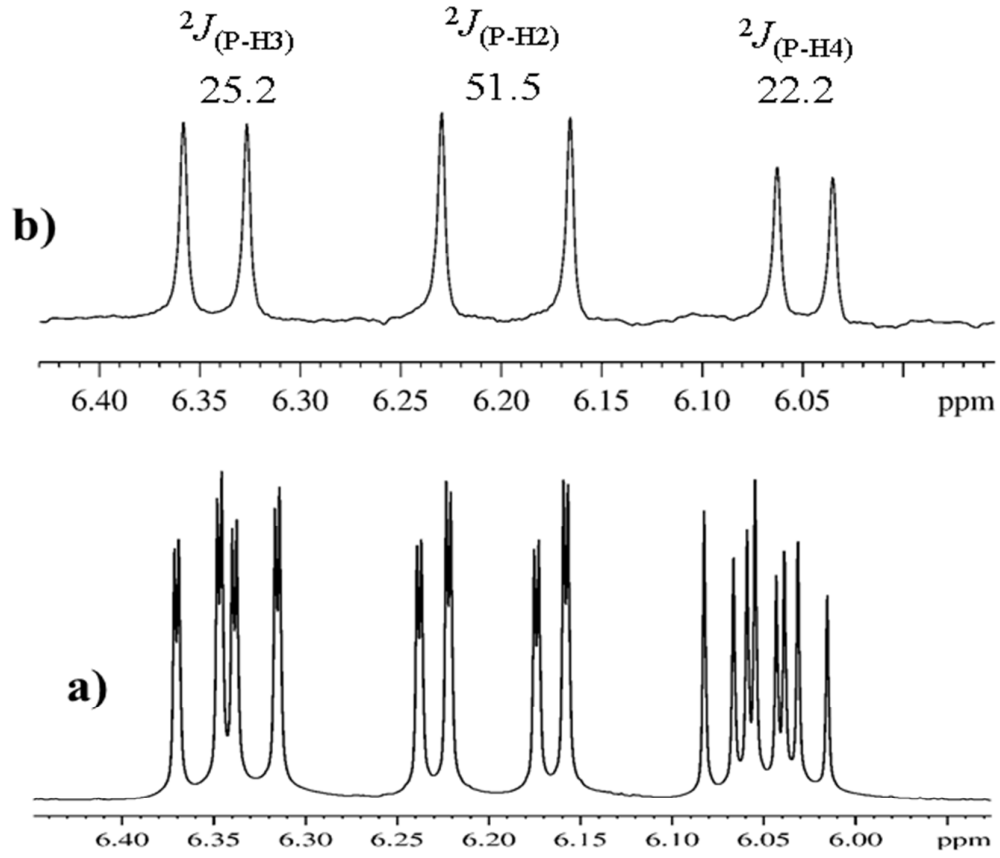


Figure 4: (a) ^{1}H NMR spectrum of dimethyl vinylphosphonate in $CDCl_3$; (b) Pure shift spectrum of the same molecule, depicting only $^{n}J_{HP}$ couplings.

The studies clearly reveal that pure shift approach has enormous advantages as the heteronuclear coupling information between two abundant spins is directly derived from the simplified one dimensional spectrum. It is fast, effective and can be easily implemented. However, one limitation of the present approach is that the relative signs of the couplings cannot be derived. Nevertheless this problem can be circumvented by the blend of pure shift

with two dimensional sequence such as HSQC [pureshift HSQC], which demands more investment on the instrument time.

Conclusions:

In summary, the pure shift NMR approach offers the enormous advantages as it provides all the couplings between proton and other abundant heteronuclei for a given molecule in a single experiment there by filling the lacuna in the current available methods as far as the measurement of $^{n}J_{HP}$ and $^{n}J_{HF}$ coupling constants from conventional the 1D-NMR is concerned. The experiment can be performed in a matter of few minutes and the complex multiplets gets simplified to an easily interpretable doublet patterns in cases when single fluorine or phosphorous is present. This is the biggest advantage. Compared to 1D-HSQC, 1D-selecitve TOCSY and 1D-HSQMBC-TOCSY experiments, the utility of pure shift is faster, more sensitive, and easier to interpret and does not demand more than one experiment.

Experimental

The commercially available samples were purchased and used as received. The samples were prepared directly in the NMR sample tube with nearly 20 mM concentration in the solvent $CDCl_3$. All the spectra were recorded on Bruker AV-III 800 MHz NMR spectrometer. For carrying out pure shift experiments the pulse program "push1dzs" available in the public domain of the Manchester NMR methodology group website (http://nmr.chemistry.manchester.ac.uk) was used. The protocol provided on the same website was followed for carrying out the experiment. The refocusing step was carried out using rsnob (shape pulse) of duration 22 ms, combined with slice selection gradient strength of 0.6 to 0.9 $G.cm^{-1}$. Each of the 32 increments in t1 was acquired with 4 scans with a recycle delay of 2 s between two successive fids. The total time domain points in t_2 dimension are 8K. The time dome data was zero filled to 1024k points before processing

and was processed with sinebell window function. Data was processed automatically with the AU program named *pshift* provided at the same website (http://nmr.chemistry.manchester.ac.uk). The AU program converts the raw data to pure shift FID. The FID was subsequently Fourier transformed with line broadening window function of 0.3 Hz.

Acknowledgements:

We are very much grateful to Prof. G.A. Morris for permitting us to download and use the relevant pulse program and macros from their website (http://nmr.chemistry.manchester.ac.uk). SRC would like to thank UGC 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).

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