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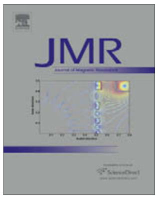

Improvements to selective refocusing phased (SERFph) experiments

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ABSTRACT

Selective refocusing experiments are very powerful for extracting proton–proton couplings one by one. However we demonstrate in the present work that various spectral artefacts are produced by the initial sequence and we show that the combined addition of a refocusing π pulse and a zero-quantum filter greatly improves the experimental sensitivity, and moreover leads to observation of pure absorption lineshapes in the resulting phased 2D spectrum. These developments are applied to the differentiation of enantiomers dissolved in a chiral liquid crystal.

1. Introduction

The differentiation of enantiomers by NMR is a challenge in organic chemistry [1]. It has been shown how this may be achieved by using a chiral liquid crystal as a solvent, and the most efficient solvents for this purpose are lyotropic liquid crystals obtained by dissolving a synthetic homopolypeptide such as poly-(γ-benzyl)-L-glutamate (PBLG) or poly-(ε-carbobenzoxy)-L-lysin (PCBLL) in a non-denaturing organic co-solvent ($CHCl_3$, CH_2Cl_2, 1,4-dioxane, *N*,*N*-dimethylformamide etc.) [2–8]. In these chiral anisotropic solvents there is a difference in the diastereomeric interactions between the chiral solute and the oriented polypeptide fibers, which produces a difference in their orientational order [9]. Consequently, any anisotropic NMR interaction, such as the dipolar coupling, the chemical shift or the quadrupolar splitting for nuclear spins $I > 1/2$, are different for enantiomers [2–7]. Deuterium NMR has proved to be particularly effective in measuring enantiomeric excess by this method because of the simplicity of the spectra, which are dominated by the large quadrupolar interaction. However, deuterium at natural abundance is an insensitive nucleus for NMR detection, which limits its applications even when using a cryoprobe. Proton NMR has high sensitivity but the spectra of enantiomers in a chiral liquid crystal are often poorly resolved and cannot be used to measure the excess.

Recently, we have developed a new method which simplifies the ^1H NMR spectra of molecules dissolved in chiral liquid crystals. This approach, which has been successfully applied to the determination of enantiomeric purity in optically enriched mixtures, uses a homonuclear, selective refocusing, 2D phased experiment (SERFph) [10,11]. But, under certain experimental conditions, the signals in the phased 2D spectrum have a mixed dispersion–absorption line shape which limits the accuracy on the enantiomeric excess determination. In this work, we present different methodological improvements of this sequence to suppress every source of signal distortion.

2. Results and discussion

The SERF sequence, which was initially developed by Fäcke and Berger, is based on the well-known 2D J-resolved experiment [12]. To measure a single proton–proton coupling, the different radiofrequency pulses are semi-selective. The experiment is useful only when the chemical shifts between protons are large compared to the spin–spin total, residual couplings, T_{ij}. Consider the simplest case of two such protons A and X, the first semi-selective, $\pi/2$ pulse is applied at ν_A, the resonance frequency of the only nucleus to be detected in the t_2 acquisition time. After an evolution time $t_1/2$ both A and X nuclei receive semi-selective π pulses and only the desired coupling T_{AX} evolves during the t_1 delay, whereas all the other couplings and the chemical shift are refocused. Unfortunately the phase of the signal is modulated by the coupling during t_1 and thus after a double Fourier transform, the resulting peak shapes are in 'phase-twist'. For this reason, the 2D spectrum is generally processed in magnitude mode. Recently, Nuzillard has shown that a phaseable 2D spectrum can be obtained by using linear prediction toward the negative values of t_1 [13].

The SERFph pulse sequence, which is shown in Fig. 1a, starts as a basic 2D J-resolve sequence, and a *z*-gradient filter is added before t_2, the acquisition [11]. The *z*-filter allows every antiphase single-quantum coherence to defocus, and only retains the in-phase coherences which have evolved during t_1, leading to an improvement in

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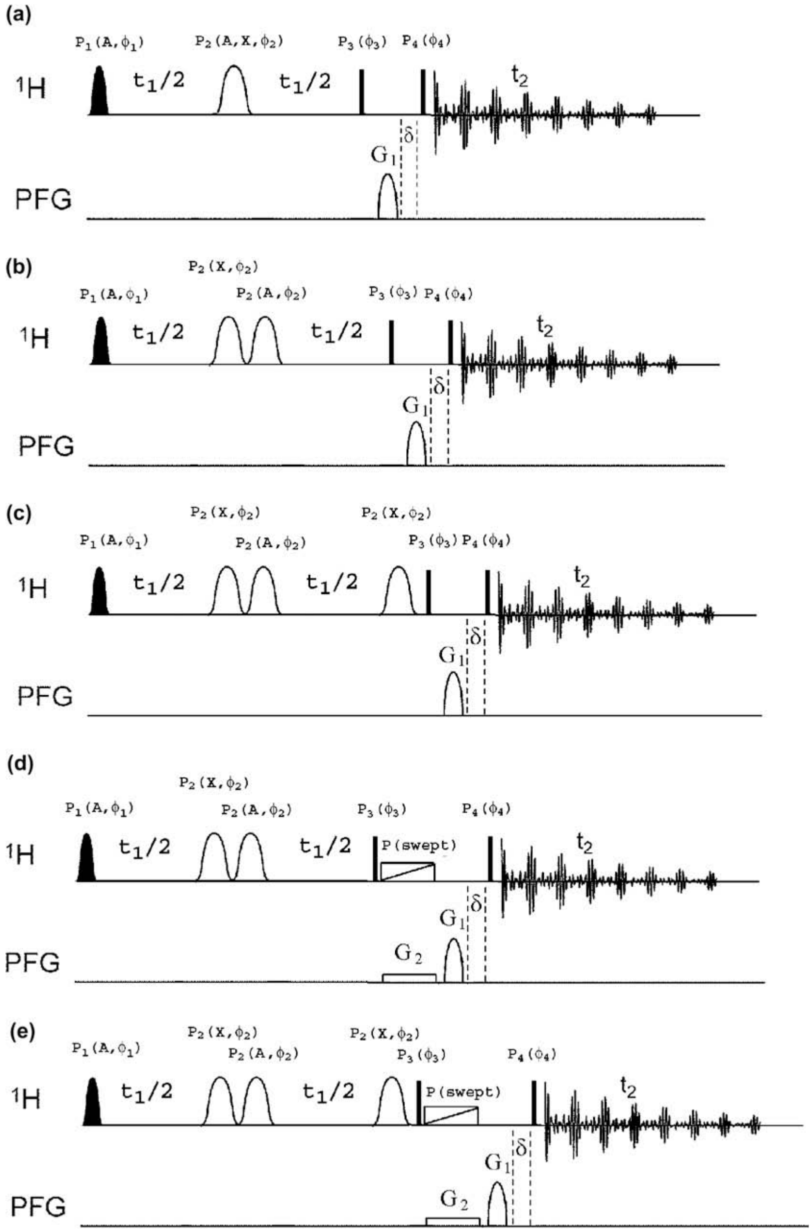

Fig. 1. Pulse sequences for the various SERFph experiments. Pulses $P_i(\mathrm{A},\phi_i)$ or $P_i(\mathrm{X},\phi_i)$ are applied semi-selectively to A or X with phase ϕ_i, $P_2(\mathrm{A,X},\phi_2)$ is applied to A and X simultaneously with phase ϕ_2, $P_i(\phi_i)$ are hard pulses with a phase ϕ_i, P(swept) is a frequency-swept π pulse applied simultaneously with a rectangular z field gradient G_2, G_1 is a *z* field gradient for the *z* filter. (a) The definition of the SERFph sequence which corresponds to the appending of one *z*-gradient filter at the end of a SERF sequence; (b) the sequence b-SERFph with two consecutive selective refocusing π-pulses in place of a single simultaneous π-pulse; (c) the b-SERFph sequence with the addition of a refocusing pulse on X at the end of the evolution time t_1. (d) The b-SERFph sequence with a zero-quantum filter include in the *z*-gradient filter and (e) the b-SERFph sequence with the both modifications present in the (c and d) SERFph sequences. The black gaussian shapes refer to semi-selective $\pi/2$ pulses; the empty gaussian shapes to semi-selective π pulses and the black sticks to hard $\pi/2$ pulses. The phase cycling is $\phi_1 = x$; $\phi_2 = x,-x$; $\phi_3 = -x,x$; $\phi_4 = x,y,-x,-y$; $\phi_{\mathrm{rec}} = x,y,-x,-y$.

resolution. The appending of the *z*-gradient filter at the end of the sequence generates the following equation for the FID, $S(t_1,t_2)$:

$$S(t_1,t_2) \propto \frac{1}{2}\cos(\pi T_{AX}t_1)\ \{\exp(-i[2\pi\nu_A + \pi T_{AX}]t_2) + \exp(-i[2\pi\nu_A - \pi T_{AX}]t_2)\}, \qquad (1)$$

where $T_{AX} = J_{AX} + 2D_{AX}$ is the total coupling between A and X proton spins (J_{AX} and D_{AX} are the scalar and dipolar coupling, respectively).

The signal in t_1 is modulated in amplitude by T_{AX} and, after a double Fourier transform and phase corrections, gives a 2D spectrum in pure absorption.

It has been demonstrated previously that this sequence can greatly simplify the extraction of proton–proton couplings, and allows the measurement of enantiomeric excess from the ^{1}H spectrum of a solution in a chiral liquid crystal solvent [11].

The simultaneous refocusing π pulse on A and X can be achieved with cosine-modulated shaped pulse, whose cosine modulation frequency is one half of the difference between the A and X resonance frequencies. Unfortunately, in this case, the two refocusing pulses correspond to the same *rf* field strength which can limit the applicability of the experiments. In this work we will demonstrate that this refocusing pulse can be applied as two separate, successive, pulses, under some specific experimental conditions, yielding better sensitivity than the initial pulse sequence [14,15].

The SERFph pulse sequence shown in Fig. 1b was tested on the H^3 and H^4 protons of propylene oxide dissolved in a chiral liquid