Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Magnetic Resonance 170 (2004) 97–103
JMR Journal of Magnetic Resonance
www.elsevier.com/locate/jmr

In phase selective excitation of overlapping multiplets by gradient-enhanced chemical shift selective filters

Philip T. Robinson, Tran Nghia Pham, and Dušan Uhrín*

University of Edinburgh, School of Chemistry, Joseph Black Chemistry Building, West Mains Road, Edinburgh EH9 3JJ, UK

Received 27 April 2004; revised 8 June 2004
Available online 7 July 2004

Abstract

We have developed gradient-enhanced chemical shift selective filters (ge-CSSF) for inphase excitation of overlapping multiplets ^{1}H. This method relies on the constructive addition of on resonance signal while off resonance magnetization is eliminated by destructive interference due to variable chemical shift evolution. This is achieved by co-addition of several FIDs acquired with a gradually incremented chemical shift evolution period. Two variable-time and one constant-time ge-CSSFs are proposed that can be combined with TOCSY, NOESY, and ROESY mixing schemes yielding highly selective 1D experiments. Analytical and numerical expressions are derived to calculate the excitation profiles of the ge-CSSFs and to examine the effects of spin–spin relaxation, the length of the CSSF increment, and selective inversion pulses. We demonstrate, both theoretically and experimentally, that CSSFs yield fast signal separation for compounds with a range of spin–spin relaxation times and chemical shift differences as small as 1–2 Hz. The use of pulsed field gradients ensures that very clean spectra are obtained. The main application of these techniques lies in analysis of mixtures where severe spectral overlap prevents the use of simple 1D selective methods.

Keywords: NMR; Selective excitation; Chemical-shift selective filter; Analysis of mixtures

1. Introduction

Selective excitation of proton resonance is a well-established principle that has been exploited in numerous NMR experiments [1–6]. 1D selective experiments provide efficiently specific information that is either impractical or impossible to obtain by corresponding nonselective *n*D methods. Such experiments usually require at least some protons to have sufficiently unique chemical shifts to allow for their selective excitation. This may not be the case when dealing with a mixture of similar compounds differing only slightly in their chemical composition. The chemical shift differences between corresponding protons of related compounds are often smaller than the proton–proton coupling constants and their ^{1}H spectra frequently contain overlapping multiplets. As it may not be straightforward to separate such mixtures via chromatography, NMR techniques are needed that enable their characterization direct from the mixture.

At present, several selective 1D NMR experiments exist which can be considered for this challenging task. Double selective experiments [5,7–9], for example, such as 1D TOCSY–TOCSY, use semiselective excitation of overlapping proton resonances followed by an initial mixing step to generate magnetization of a limited number of protons. Providing some of these protons are sufficiently separated, they are then used for the second selective mixing step yielding signals of one spin system at a time. Of course there is no guarantee that the first transfer will yield a proton resonance that can be selectively excited and, clearly, the sensitivity of such experiments is not optimal.

Alternatively, in phase magnetization of one or two protons from the same spin system can be selectively excited by using a COSY transfer [10,11] that is then followed by an appropriate mixing period such as TOCSY or NOESY. These techniques require a single pair of coupled spins to resonate within the inversion

* Corresponding author. Fax: +44-131-650-7155.
E-mail address: dusan.uhrin@ed.ac.uk (D. Uhrín).

1090-7807/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.jmr.2004.06.004

bandwidths of two 180° pulses. Resonances present in only one of these areas are eliminated. This condition may be difficult to fulfill when trying to separate signals of similar compounds. Other potential drawbacks of this approach are that it requires a prior knowledge of the resonance assignment and for small coupling constants the efficiency of the initial COSY step could be low.

A third approach is to us line-selective inversion pulses [6]. Here, a zz-order state is created and converted to multiple quantum coherence by the action of a 90° nonselective pulse. The result is a 1D spectrum in which one multiplet is missing. Acquiring a regular 1D spectrum on alternate scans and subtracting the two thus yields a single multiplet with halved intensity. Of the three techniques discussed here, this is the most promising approach for resolving severely overlapping multiplets, providing the spectral lines are sufficiently separated to allow for their selective inversion.

In this contribution, we demonstrate that chemical shift selective filtration (CSSF) is a powerful method for separation of severely overlapping resonances and report on the development of gradient-enhanced chemical shift elective filters (ge-CSSF). These modified filters are more robust that the original CSSFs used by Hall and Norwood [12] in a 1D CSSF–COSY experiment. The new CSSFs selectively excite in phase magnetization and, when combined with appropriate mixing schemes, yield highly selective 1D ge-CSSF–TOCSY (NOESY, ROESY) experiments. The performance of these experiments is illustrated using a mixture of a trisaccharide, **I**, and its unknown degradation product.

2. Results and discussion

Chemical shift selective filtration relies on the constructive addition of on-resonance signal while off resonance magnetization is eliminated by destructive interference due to variable chemical shift evolution [12–15]. This is achieved by co-addition of several FIDs acquired with a gradually incremented chemical shift evolution period. The frequency characteristics of CSSFs will be discussed in detail later. For now it is sufficient to say that their selectivity is directly proportional to the maximum chemical shift evolution interval, t_{max}.

Obviously, it is essential that only chemical shift evolution varies during the incremented period. Evolution due to J coupling must be either eliminated or kept constant during all increments of the CSSF. The former approach is easily implemented in heterocorrelated experiments [14,15] but for homonuclear experiments only the latter has been utilized, as in the original homonuclear 1D CSSF–COSY [12]. In this 1D CSSF–COSY, chemical shift selective filtration was achieved by changing the position of a nonselective 180° pulse within a fixed evolution interval. This method can therefore be characterized as a constant-time chemical shift selective filter (CT-CSSF). The same constant time delay also served to generate the antiphase magnetization required for the COSY transfer. Consequently, the selectivity of this CT-CSSF could not be adjusted freely. This experiment also suffered from cancellation artifacts, as the off resonance signals were eliminated by the CSSF only, using the principles of the difference spectroscopy.

A more flexible implementation of CSSFs is therefore required for homonuclear experiments that (i) does not impose any restrictions on their selectivity, (ii) produces in phase magnetization, and (iii) is free from cancellation artifacts. Two 1D ge-CSSF experiments that fulfill these criteria are shown in Fig. 1. Both use a combination of nonselective and selective 180° pulses together with pulsed field gradients. The simpler pulse sequence of Fig. 1A has all the attributes of the new CSSFs and

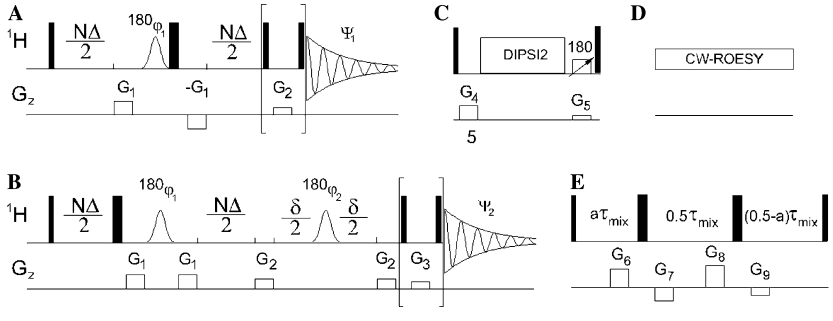

Fig. 1. Pulse sequences of 1D ge-CSSF experiments. Thick and thin rectangles represent 90° and 180° pulses, respectively, applied from the x axis unless specified otherwise. Selective 180° Gaussian pulses are represented by Gaussian envelope. (A) 1D ge-VT-CSSF; (B) 1D ge-DPFGSE-CSSF. For VT experiments $\delta = 4\,\mu s$, while for CT experiments $\delta = T - N\Delta$, where T is the constant time period; Δ is the increment of the CSSF, $N = 0, 1, 2, \ldots, n$, and $t_{max} = (N+1)\Delta$. When the parts of the pulse sequences (A) and (B) in square brackets are replaced by appropriate mixing schemes shown in (C)–(E), 1D-ge-CSSF–TOCSY, ROESY, and NOESY experiments, respectively, are obtained. The antiphase components of proton multiplets generated by the TOCSY transfer in (C) were suppressed by using an adiabatic inversion pulse applied during a low intensity (2 G/cm) pulse field gradient [21]. The timing of the inversion pulses inside the mixing interval of the NOESY experiment (E) depends on the value of the constant a that is typically set to 0.2–0.4 [22]. The following phase cycling was applied: $\varphi_1 = x, y$; $\varphi_2 = 2x, 2y$; $\psi_1 = x, -x$; $\psi_2 = x, 2(-x), x$. The following pulse field gradients were used $G_1 = 20$ G/cm, $G_2 = 13$ G/cm, $G_3 = 5$ G/cm, $G_4 = 17$ G/cm, $G_5 = 2$ G/cm, $G_6 = 15$ G/cm, $G_7 = -12$ G/cm, $G_8 = 25$ G/cm, and $G_9 = -7$ G/cm.

will therefore be discussed first. The initial nonselective 90° pulse is followed by an incrementable period, $N\Delta$, with a train of two 180° pulses at its centre, the first of which is made selective. The magnetization within the inversion bandwidth of the 180° selective pulse therefore effectively experiences a 360° pulse, while the magnetization outside of this region is inverted by the nonselective 180° pulse. Providing that there are no mutually coupled spins within the inversion bandwidth of the 180° selective pulse, spins that are selectively inverted will evolve only under their chemical shifts during $N\Delta$. The pulse sequence of Fig. 1A thus constitutes a chemical shift selective filter and is referred to here as a 1D ge-VT-CSSF, where VT stands for variable-time. This adjective reflects the fact that the overall length of the CSSF increases with increasing chemical shift evolution. In addition, two pulsed field gradients with opposite polarity applied immediately before and after the 180° pulses dephase the magnetization outside of the selectively inverted region. Thus, the 180° selective pulse not only pre-selects the magnetization of interest, it also ensures that the J evolution is refocused and, in concert with PFGs, eliminates the magnetization outside of its inversion profile. This last point has two consequences. First, since the bulk of the off resonance magnetization is dephased by PFGs, difference spectroscopy is only used to eliminate signals from off resonance protons within the bandwidth of the selective pulse. This leads to acquisition of clean spectra with much reduced cancellation artifacts. Second, it allows fast incrementation of the CSSF. This is because CSSFs produce excitation sidebands at $\pm k/\Delta (k = 1, 2, \ldots, n)$ frequencies, where Δ is the increment of the CSSF [15]. Therefore the value of Δ would normally be small to move these sidebands beyond the edges of the spectra. When the magnetization outside of the inverted area is destroyed by PFGs, however, the excitation sidebands can be brought close to the zero frequency band. Long chemical shift evolution intervals required for separation of signals with very small chemical shift differences can thus be achieved very quickly. The 90°–PFG–90° pulse sequence element at the end of the filter selects only y magnetization while it removes the x magnetization. Without this purging element both absorptive and dispersive magnetization would be acquired. In real experiments this part of the pulse sequence is replaced by an appropriate mixing scheme (TOCSY, NOESY or ROESY) as shown in Figs. 1C, D and E. These mixing elements also utilize only the absorptive magnetization.

An alternative implementation of a CSSF shown in Fig. 1B is achieved by using the BSHD element originally proposed for the band selective homonuclear decoupled 2D experiments [16]. This pulse sequence utilizes a double-pulsed field gradient spin-echo (DPFGSE) and therefore compensates for phase anomalies within the inversion profiles of the selective pulses [17]. This may become an issue for longer selective pulses that need to be applied when closely resonating J-coupled protons are present. The addition of the second selective pulse increases the suppression of signals outside of the inversion bandwidths of selective pulses; otherwise both CSSFs select signals using the same principles. This alternative implementation of CSSFs in homonuclear experiments is referred to as 1D ge-DPFGSE–VT-CSSF, as the incrementation of the chemical shift evolution period is also achieved here in a variable-time manner. A distinct advantage of the DPFGSE scheme is that it can be easily modified to yield a constant-time CSSF, the merits of which will be discussed later.

Before proceeding with the illustrations of the proposed experiments let us first analyze the selectivity of CSSFs to choose the optimal parameters for the experiments. And, as we intend to use long chemical shift evolution intervals to achieve high chemical shift selectivity, it is also necessary to examine the effects of spin–spin relaxation on the frequency characteristics of CSSFs. Neglecting for now the effects of selective pulses, the absorptive frequency profile of a VT-CSSF in the presence of relaxation is given by the following integral:

$$I(\nu) = \int_{t=0}^{t=t_{\max}} \cos(2\pi\nu t)\exp(-t/T_2), \tag{1}$$

where ν is the frequency offset in Hz, t is the length of the chemical shift filter, and T_2 is the spin–spin relaxation time. This definition of the frequency characteristics of the CSSF presumes infinitely slow incrementation of the chemical shift evolution period, which is not the case in real experiments. We will show later, than in fact this is very good approximation of the real CSSFs considering the frequencies near the on resonance signal. The second reason for introducing this definition is that the integral given by Eq. (1) can be solved analytically:

$$I(\nu) = \frac{1}{t_{\max}[(1/T_2)^2 + (2\pi\nu)^2]}\left\{\left[\frac{-1}{T_2}\cos(2\pi\nu t_{\max}) + 2\pi\nu\sin(2\pi\nu t_{\max})\right]\exp(-t_{\max}/T_2) + 1/T_2\right\}, \tag{2}$$

which was normalized for convenience using the intensity of the on resonance signal in the absence of spin–spin relaxation ($\underset{T_2\to\infty}{I(0)} = t_{\max}$). In the absence of relaxation, Eq. (2) reduces to the sinc function: $I(\nu) = \sin(2\pi\nu t_{\max})/2\pi\nu t_{\max}$ with the first zero point at $\nu_{\text{zero}} = 0.5/t_{\max}$. Fig. 2 shows the frequency characteristics of a CSSF with $t_{\max} = 0.2$ s and T_2 values of 1, 0.2, and 0.1 s. On comparing these profiles, several useful observations can be made. First, as expected, the intensity of the zero frequency band is smaller for shorter spin–spin relaxation times. This decrease is modest, however, due to the fact that this is a VT-CSSF and so relaxation effects are only significant during the later

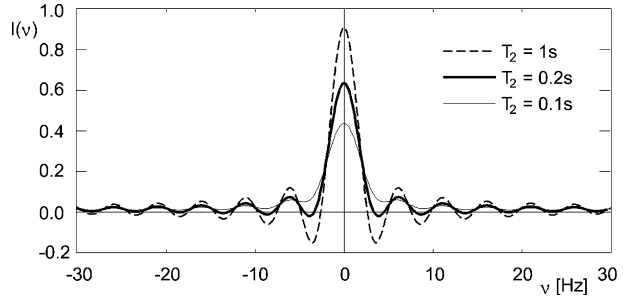

Fig. 2. The effects of the spin–spin relaxation on the frequency characteristics of VT-CSSFs. Individual profiles were calculated using Eq. (2) and $t_{max} = 200$ ms. T_2 was set to 1, 0.2, and 0.1 s, respectively.

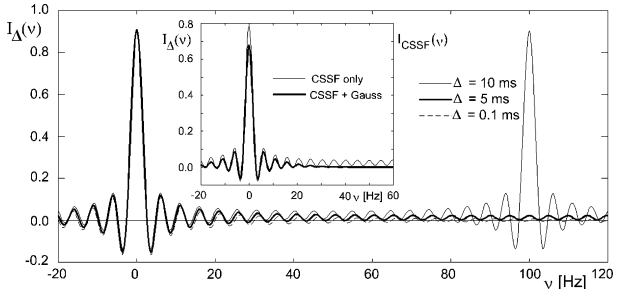

Fig. 3. The effect of the length of the CSSF increment, Δ, on the frequency characteristics of the VT-CSSFs according to Eq. (3). The following parameters were used to generate individual profiles: $t_{max} = 0.2$ s, $T_2 = 1$ s, and $\Delta = 0.0001$, 0.005, and 0.01 s. The inset shows comparison of CSSF profiles without (thin line, Eq. (3), $\Delta = 0.01$ s, $t_{max} = 0.2$ s, and $T_2 = 0.4$ s) and with two 30 ms Gaussian pulses as used in the 1D-ge-DPFGSE–VT-CSSF experiments (thick line, Eq. (4)).

increments of the filter. Such signal losses will usually be compensated for by acquiring the multiple scans needed to complete the filtration process. Second, the intensity of the side lobes relative to that of the central band decreases with increasing relaxation rates, so that, in fact, a modest relaxation improves the profile of VT-CSSFs. The third notable observation is that the frequency of the first point with zero intensity increases slightly for faster relaxing protons; e.g., from 2.5 Hz ($T_2 = 1$ s) to 3.35 Hz ($T_2 = 0.2$ s). As this point can be used to achieve a very clean separation of two overlapping signals, this observation suggests that in the presence of relaxation, t_{max} should be set longer than the value calculated from $0.5/\Delta\nu$, which is based solely on the chemical shift difference, $\Delta\nu$, between the overlapping protons. Finally, when spin–spin relaxation of 0.1 s, i.e., one half of the maximum duration of the CSSF, t_{max}, was used, the filter still showed relatively good separation, although it is no longer possible to find any point of zero intensity. Overall, then, the described effects of relaxation on the frequency characteristics of VT-CSSFs are reminiscent of the broadening of the excitation profiles of selective pulses in the presence of relaxation as described by others [18]. Since analogies can be drawn between CSSFs and rectangular DANTE pulses [19] this is not a surprising observation. The above analysis showed that the variable time CSSFs are efficient in separation of overlapping signals also in the presence of relaxation rates that are typical for small and medium sized molecules.

We now inspect the effect of finite increments, Δ, on the frequency characteristics of CSSFs. This can be done by replacing the integral in Eq. (1) by a sum and by setting $t = N\Delta$:

$$I_{\Delta}(\nu) = \frac{1}{N+1} \sum_{N=0}^{N=t_{max}/\Delta} \cos(2\pi\nu N\Delta) \exp(-N\Delta/T_2). \qquad (3)$$

Fig. 3 shows frequency characteristics of CSSFs calculated using Eq. (3) and the following parameters: $t_{max} = 0.2$ s, $T_2 = 1$ s, and $\Delta = 0.0001, 0.005$, and 0.01 s. It can be seen that increasing the length of the CSSF increment, Δ, increases the intensities of the side lobes and at the same time moves them in the direction of positive intensities. These effects are more pronounced at higher frequencies, while in the vicinity of the central band all three settings produced a similar picture. When the effects of shaped pulses are also considered, the frequency characteristic of CSSFs can be calculated using Eq. (4):

$$I_{CSSF}(\nu) = I_{\Delta}(\nu)[\exp(-1.2\tau_{180}^2\nu^2) \exp(-\tau_{180}/T_2)]^n, \qquad (4)$$

where τ_{180} is the length of a 180° Gaussian pulse in seconds and n is equal to 1 or 2 for the 1D ge-VT-CSSF or 1D ge-DPFGSE–VT-CSSF experiments, respectively. The inset of Fig. 3 shows the effect of two 30 ms Gaussian pulses used in a 1D ge-DPFGSE–VT-CSSF in which a 10 ms increment was employed. It can be seen that Gaussian pulses cause a rapid decrease in signal intensity at higher frequencies and fast incrementation of CSSFs does not have a detrimental effect on their frequency profiles. We typically use 5 and 10 ms increments in combination with 15 and 30 ms Gaussian pulses, respectively. This analysis also showed that close to the zero frequency the frequency characteristics of CSSFs is, to a good approximation, described by Eq. (2).

Having investigated various aspects of CSSFs, we now utilize our conclusions in the following demonstration of the efficacy of the modified CSSFs using the mixture of trisaccharide, **I**, and its unknown degradation product. Primed letters are used to label the resonances of the degradation product that is approximately 2.5 times less abundant than compound **I**. A 600 MHz ^{1}H NMR spectrum of the mixture of the two compounds (Fig. 4A) shows that even the anomeric protons are not sufficiently separated to allow for their straightforward selective excitation; a 1D TOCSY spectrum (Fig. 4B) acquired with a semiselective excitation of protons resonating at around 4.58 ppm shows signals of several spin systems. Subsequent 1D ge-VT-CSSF–TOCSY spectra used the minor (Fig. 4C) and the major (Fig. 4D) dou-

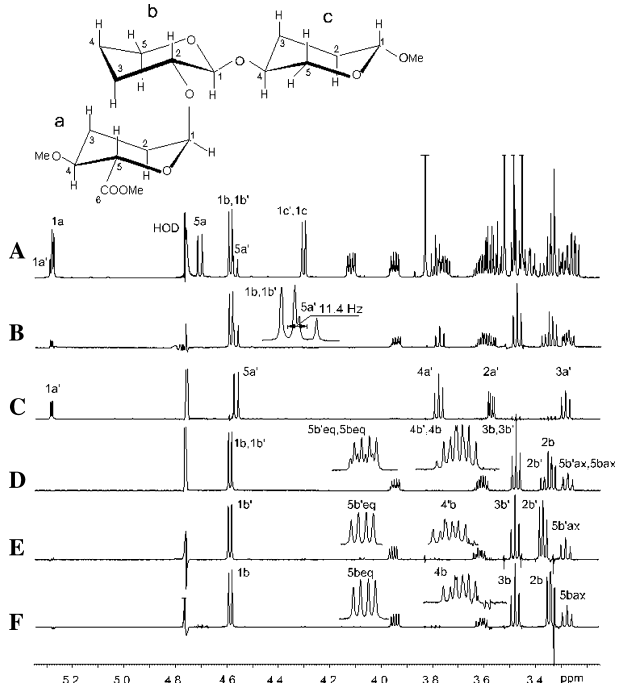

Fig. 4. Signal separation by 1D ge-CSSF–VT-TOCSY experiments. (A) 1D ^{1}H spectrum of a mixture of a trisaccharide **I** (major compound) shown in the inset, and its unknown degradation product (minor compound). Selected resonances are labeled using primed latter for the minor compound. (B) 1D TOCSY spectrum acquired using 16 scans and the semiselective excitation of protons 1b, 1b′, and 5a′. (C and D) 1D ge-VT-CSSF–TOCSY spectra (pulse sequence of Figs. 1A + 1C) with the filtration ($t_{max} = 45$ ms) through protons 1b, 1b′, and 5a′, respectively. A total of 20 scans were accumulated per spectrum. (E and F) 1D ge-DPFGSE–VT-CSSF–TOCSY spectra (pulse sequence of Figs. 1B + 1C) with the filtration through protons 2b and 2b′, respectively; $t_{max} = 200$ ms, $\Delta = 10$ ms, and $\tau_{180} = 30$ ms. A total of 42 scans were accumulated per spectrum. All other parameters are given in Section 3. Spectra (C and E) were plotted with a vertical scale twice as high as those used for spectra (D and F).

blet shown in the inset of Fig. 4B for the chemical shift selective filtration. The chemical shift difference of 11.4 Hz between the two signals was used to calculate the length the CSSF ($t_{max} = 0.5/11.4$) in order to utilize the first zero intensity point of the filter. While the spectrum in Fig. 4C contains protons from a single spin system (1a′–5a′), the spectrum in Fig. 4D shows signals from both b and b′ rings. Separation of these spin systems based on protons 1b and 1b′ was not possible because the two protons have identical chemical shifts. Nevertheless, inspection of this spectrum reveals two overlapping multiplets of protons 2b and 2b′ resonating at around 3.35 ppm that could be used for chemical shift selective filtration and two 1D ge-VT-DPFGSE-CSSF–TOCSY spectra were thus acquired. As protons 2b and 2b′ resonate in the crowded region of the spectrum a longer t_{max} (200 ms) was used than the one calculated on the basis of chemical shift difference between the two protons. These experiments yielded clean subspectra containing signals of only one spin system (Figs. 4E and F). While a 15 ms Gaussian pulse was used to acquire the spectra of Figs. 4B–D, a 30 ms pulse was used in the last two experiments because of the small chemical shift separation between the coupled protons 2b and 3b (or 2b′ and 3b′).

A severe test of the proposed CSSF filtration method is provided by the protons 1c and 1c′ whose chemical shifts differ by only 1.4 Hz. A 1D TOCSY spectrum with selective excitation of both protons shows resonances from both compounds (Fig. 5A). These were separated in two 1D ge-VT-CSSF–TOCSY spectra shown in Figs. 5B and C. The t_{max} was set to 400 ms. This value is slightly larger than the 357 ms calculated as 0.5/1.4 to account for the effects of relaxation as discussed above. This experiment illustrates that chemical shift selective filtration is a powerful method for separation of severely overlapping multiples and also works when the application of line selective pulses [6] would be problematic.

In the previous example, we have used a gradual incrementation of the CSSFs to achieve separation of two overlapping signals. It is possible, however, to achieve this much more quickly using only two increments of a CSSF, providing all other protons are outside of the inversion bandwidths of the selective pulses. In the first increment, no chemical shift evolution is allowed ($N = 0$) and in the second, chemical shift evolution of $\Delta = 0.5/\Delta\nu$ is used, where $\Delta\nu$ is the separation of two signals in Hz. This leads to a rotation of the off resonance signal by 180°. Addition of the two increments eliminates the off-resonance signal, leaving only the on resonance signal in the spectrum [20]. This procedure works with variable-time CSSFs only when the relaxation effects are so small that they can be neglected. However, when constant-time CSSFs are used, this method will always work, since equal attenuation of signals occurs in both spectra. A 1D ge-DPFGSE–CT-CSSF can easily be obtained by inserting decrementable

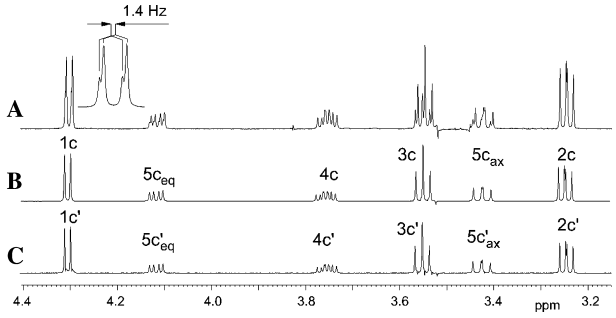

Fig. 5. Signal separation based on the chemical shift difference of 1.4 Hz between protons 1c and 1c′. (A) A 16 scan 1D TOCSY spectrum with semiselective excitation of protons 1c and 1c′. (B and C) 1D ge-VT-CSSF–TOCSY spectra with filtration via protons 1c and 1c′ and $t_{max} = 400$ ms. Two scans were acquired for each of 81 increments resulting in 162 scans in total. All other parameters are given in Section 3. The spectrum (C) was plotted with a vertical scale twice as high as that used for the spectrum (B).

intervals, $(T - N\Delta)/2$), around the second Gaussian pulse of the 1D ge-DPFGSE–VT-CSSF pulse sequence. Thus, the overall length, T, of the filter is constant during both increments. This procedure is illustrated on the signal separation of spin systems of ring a and a′ using filtration via protons 1a and 1a′. The constant time period of 89 ms calculated based on a 5.6 Hz separation between the two protons yielded clean separation of signals (Fig. 6) by acquiring only two increments of the filter. Gradual incrementation of the Δ period could, in principle, also be used in CT-CSSF experiments. The frequency characteristics of such constant-time filters is given by the $\mathrm{sinc}(2\pi\nu t_{\max})/2\pi\nu t_{\max}$ function attenuated by relaxation during the constant time, T, and the Gaussian pulses. Relaxation therefore does not alter the frequency characteristics of constant-time filters but does lead to a constant loss of signal that could be significant when longer evolution intervals are required. Our analysis of VT-CSSFs showed that their frequency characteristic improves in the presence of modest relaxation. We therefore recommend using the VT-CSSF methods for regular filtration and leave the CT method for the fast separation of two relatively isolated but overlapping signals.

The 1D ge-CSSF–NOESY and ROESY pulse sequences are easily constructed by replacing the purging element of the ge-CSSF pulse sequences by appropriate pulse sequence elements (Figs. 1D and E). For the trisaccharide **I** the ROESY mixing at 600 MHz is more efficient than the NOESY due to the intermediate tumbling rate of this molecule. Two 1D ge-VT-CSSF–ROESY spectra shown in Figs. 7B and C were acquired using the filtration via protons 1a and 1a′. Both spectra show two major signals belonging to protons 2a, 2b and 2a′, 2b′, respectively, and a large number of small, long-range ROEs. This spectrum illustrates that the CSSFs can also be used when a large range of signal intensities

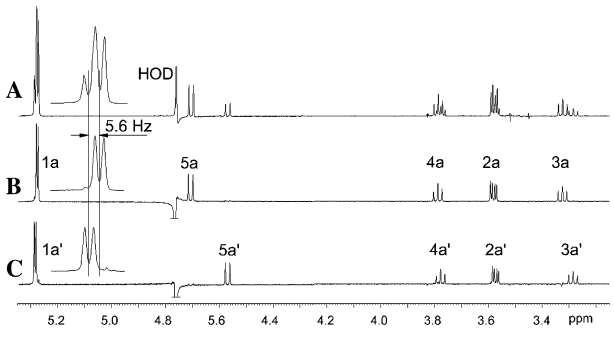

Fig. 6. Fast chemical shift selective filtration. (A) 1D TOCSY spectrum with semiselective excitation of protons 1a and 1a′. (B and C) 1D ge-DPFGSE–CT-CSSF–TOCSY spectra with filtration via protons 1a and 1a′ (pulse sequence of Figs. 1B + 1C), respectively. Two increments ($N = 0, 1$) were acquired using $T = 89.004$ ms and $\Delta = 89.0$ ms. Sixteen scans in total were acquired for each spectrum. All other parameters are given in Section 3. The spectrum (C) was plotted with a vertical scale twice as high as that used for the spectrum (B).

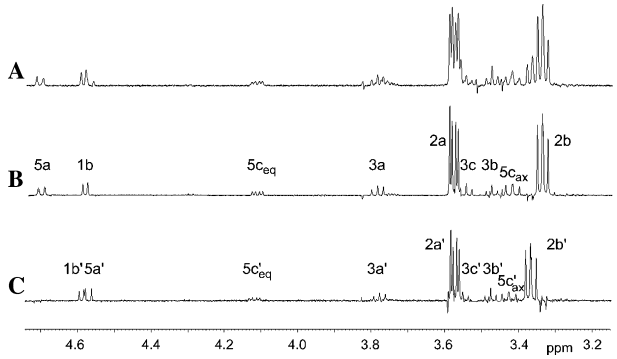

Fig. 7. 1D ge-VT-CSSF–ROESY spectra. (A) A 160 scan 1D ROESY spectrum with semiselective excitation of protons 1a and 1a′. (B and C) 1D ge-VT-CSSF–ROESY spectra (pulse sequence of Figs. 1A + 1D) with filtration ($t_{\max} = 100$ ms) via protons 1a and 1a′, respectively. Sixteen scans were accumulated in each of the 21 CSSF increments resulting in accumulation of 336 scans per spectrum. All other parameters are given in Section 3. The spectrum (C) was plotted with a vertical scale twice as high as that used for the spectrum (B).

can occur such as in the ROESY and NOESY spectra. Inspection of the frequency characteristics of CSSFs suggests that one has to proceed carefully here: the first few side lobes could have 10–20% of the intensity of the main band, the exact figure depending on the interplay between the length of the filter and spin–spin relaxation. In the case of only two overlapping resonances, it is therefore advantageous to make use of the first point of zero intensity in either VT- or fast CT-CSSFs. This may not be possible for a combination of very small chemical shift differences and fast spin–spin relaxation, though this is unlikely to happen for small molecules. When separation of several overlapping signals is attempted, cross talks are reduced significantly when using longer chemical shift evolution periods.

In conclusion, the results presented here illustrate that the gradient-enhanced chemical shift selective filtration is a powerful method for the separation of overlapping proton resonances. Two variable-time and one constant-time ge-CSSFs were designed and combined with mixing schemes that act on in phase magnetization. Analytical and numerical expressions were derived for the calculation of excitation profiles of the ge-VT and CT-CSSFs taking into account spin–spin relaxation. CSSFs yield fast signal separation for compounds with a range of spin–spin relaxation times and chemical shift differences as small as 1–2 Hz. The main application of these techniques lies in analysis of mixtures where sever spectral overlap prevents the use of simple 1D selective methods.

3. Methods and materials

All experiments were performed on a Bruker Avance 600 MHz NMR spectrometer equipped with a 5 mm

triple-resonance probe. The simple selective 1D spectra (e.g., Fig. 1B) were acquired using a single pulsed field gradient spin-echo and a 15 ms Gaussian pulse. Two scans were typically acquired for each CSSF increment. To improve the signal-to-noise ratio, this number can be increased by repeating a complete passage through all increments of the filter several times. In this way the effects of any long-term instabilities are minimized. The length of the CSSF increment, Δ, was 5 ms and a 15 ms 180° Gaussian pulse was used unless stated otherwise in figure captions. Mixing time of 140 ms was used for the TOCSY spectra of Figs. 1E and F and 6A–C and 100 ms for the spectra shown in Figs. 1B–D and 5A–C. A 300 ms CW spin lock ($\gamma B_1/2\pi = 2500$ Hz) was used in the ROESY experiments.

References

[1] H. Kessler, H. Oschkinat, C. Griesinger, Transformation of homonuclear two dimensional NMR techniques into one-dimensional techniques using gaussian pulses, J. Magn. Reson. 70 (1986) 106–133.

[2] H. Kessler, U. Anders, G. Gemmecker, S. Steuernagel, Improvement of NMR experiments by employing semiselective half-gaussian-shaped pulses, J. Magn. Reson. 85 (1989) 1–14.

[3] K. Stott, J. Stonehouse, J. Keeler, T.L. Hwang, A.J. Shaka, Excitation sculpting in high-resolution nuclear-magnetic-resonance spectroscopy—application to selective NOE experiments, J. Am. Chem. Soc. 117 (1995) 4199–4200.

[4] T. Parella, High-quality 1D spectra by implementing pulsed-field gradients as the coherence pathway selection procedure, Magn. Reson. Chem. 34 (1996) 329–347.

[5] D. Uhrin, P.N. Barlow, Gradient-enhanced one-dimensional proton chemical-shift correlation with full sensitivity, J. Magn. Reson. 126 (1997) 248–255.

[6] C. Roumestand, C. Delay, J.A. Gavin, D. Canet, A practical approach to the implementation of selectivity in homonuclear multidimensional NMR with frequency selective-filtering techniques. Application to the chemical structure elucidation of complex oligosaccharides, Magn. Reson. Chem. 37 (1999) 451–478.

[7] L. Poppe, H. van Halbeek, NOE measurements on carbohydrates in aqueous-solution by double-selective pseudo-3D TOCSY–ROESY and TOCSY–NOESY—application to gentiobiose, J. Magn. Reson. 96 (1992) 185–190.

[8] D. Boudot, C. Roumestand, F. Toma, D. Canet, Pseudo-3D NMR of proteins with selective excitation by DANTE-z—one-dimensional TOCSY–NOESY experiment, J. Magn. Reson. 90 (1990) 221–227.

[9] M.J. Gradwell, H. Kogelberg, T.A. Frenkiel, Applying excitation sculpting to construct singly and doubly selective 1D NMR experiments, J. Magn. Reson. 124 (1997) 267–270.

[10] S. Bourg, J.M. Nuzillard, In-phase double selective excitation of coupled spin systems using excitation sculpting, J. Magn. Reson. 133 (1998) 173–176.

[11] G.J. Harris, S.R. Lowe, T.J. Norwood, In-phase selective excitation of overlapping multiplets, J. Magn. Reson. 142 (2000) 389–392.

[12] L.D. Hall, T.J. Norwood, A chemical-shift-selective filter, J. Magn. Reson. 76 (1988) 548–554.

[13] L.D. Hall, T.J. Norwood, An improved chemical-shift-selective filter, J. Magn. Reson. 78 (1988) 582–587.

[14] D. Uhrín, G. Batta, K.E. Kövér, One-dimensional heteronuclear chemical-shift correlation using chemical-shift-selective filters, J. Magn. Reson. 97 (1992) 54–64.

[15] K.E. Kövér, D. Uhrín, G. Szalontai, G. Batta, Easy implementation of selective inverse experiments with chemical-shift-selective filters, J. Magn. Reson. Ser. B. 101 (1993) 1–7.

[16] V.V. Krishnamurthy, Application of semi-selective excitation sculpting for homonuclear decoupling during evolution in multidimensional NMR, Magn. Reson. Chem. 35 (1997) 9–12.

[17] T.L. Hwang, A.J. Shaka, Water suppression that works—excitation sculpting using arbitrary wave-forms and pulsed-field gradients, J. Magn. Reson. Ser. A 112 (1995) 275–279.

[18] P.J. Hajduk, D.A. Horita, L.E. Lerner, Theoretical-analysis of relaxation during shaped pulses. I. The effects of short T_1 and T_2, J. Magn. Reson. Ser. A 103 (1993) 40–52.

[19] G.A. Morris, R. Freeman, Selective excitation in Fourier-transform nuclear magnetic-resonance, J. Magn. Reson. 29 (1978) 433–462.

[20] D. Uhrín, J.-R. Brisson, D.R. Bundle, Pseudo-3D NMR spectroscopy—application to oligosaccharides and polysaccharides, J. Biomol. NMR. 3 (1993) 367–373.

[21] M.J. Thrippleton, J. Keeler, Elimination of zero-quantum interference in two-dimensional NMR spectra, Angew. Chem. Int. Ed. 42 (2003) 3938–3941.

[22] K. Stott, J. Keeler, Q.N. Van, A.J. Shaka, One-dimensional NOE experiments using pulsed field gradients, J. Magn. Reson. 125 (1997) 302–324.