Predicting PSR Filters by Transverse Relaxation Enhancements

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ABSTRACT: The paramagnetic spin relaxation (PSR) filter allows the suppression of the NMR resonances of individual components in mixtures according to their Gd^{3+}-complexing ability. The difficulty in predicting this property hampers, however, the widespread application of this filter. Herein we describe that the PSR filter is dominated by the transverse relaxation enhancement (R_{2p}) experienced by nuclei in the presence of Gd^{3+} and so, that R_{2p} represents a reliable predictive tool of suppression in the 1D and 2D PSR filter of complex mixtures. The robustness of R_{2p} as a predictive tool in PSR filters has been demonstrated at different magnetic fields and for the ^{1}H, ^{13}C, COSY and HMQC filtering of commercial multicomponent compositions, including beverages and drugs.

INTRODUCTION

Recent advances in pulse sequences and NMR allow the analysis of individual components in complex mixtures by implementing filters that avoid the necessity of previous separations.[1] Widely used filters rely on differences in the relaxation times and diffusion coefficients of the components. As small molecules generally display larger relaxation times and shorter diffusion coefficients than high molecular weight species, these filters complement each other allowing the selective suppression of one of these two large groups of components in mixtures. The hurdle arises when selectivity within these groups is desired. It is therefore of much interest the development of filtering strategies that based on alternative properties could expand the limits of traditional filters.

Our research group has recently described the use of Gd^{3+} as a paramagnetic spin relaxation (PSR) agent for the selective suppression of the ^{1}H and ^{13}C resonances of Gd^{3+}-complexing components in mixtures (1D and 2D NMR).[2] The PSR filter relies on the faster relaxation of nuclei in a paramagnetic environment.[3] Gd^{3+} has seven unpaired electrons and a high electronic correlation time (τ_s *ca* 10^{-8} s), which being larger than the rotational correlation time (τ_r) of small molecules and most macromolecules, affords overall correlation times (τ_c) dominated by τ_r. In this scenario, the Solomon-Bloembergen-Morgan equations predict a dramatic reduction of the transverse relaxation time (T_2) for those species in fast chemical exchange with Gd^{3+}.[4] Because of the inverse proportionality between T_2 and the spectral line width in NMR spectra,[5] this ultimately results in their selective line-broadening/suppression without affecting the resolution and chemical shift of other components in a mixture.[2,6]

The PSR filter benefits from a rather low sensitivity to size and molecular weight that makes it compatible with classical relaxation and diffusion filters. A limitation that might hamper its wide adoption by the community is a reduced predictive character, mainly restricted to anionic species being generally easier to filter than neutral/cationic ones. In our effort to grant PSR with a predictive tool we have turned our attention to the transverse relaxation enhancements (R_{2p}) experienced by nuclei in the presence of Gd^{3+}. The transverse relaxation rate of nuclear spins in a paramagnetic environment ($R_2=1/T_2$) is given by

$$R_2=R_{2d}+c\cdot R_{2p} \qquad (1)$$

where R_{2d} is the transverse relaxation rate in the absence of paramagnetic effects ($R_{2d}=1/T_{2d}$) and c the concentration of the paramagnetic species.[7] Bearing in mind that T_{2d} values for common molecules and polymers usually lay in a short 0.1-3 s range, it was hypothesized that successful PSR suppressions must rely on large R_{2p} values more than on the original R_{2d}. With the aim of assessing R_{2p} as a predictive tool in PSR, the R_{2p} values of a series of more than forty small molecules and polymers of interest in the pharmaceutical and food industries have been determined in the presence of Gd^{3+}, and the outcome of the PSR filter in relevant compositions in these fields analysed in the light of the R_{2p} of the constituents. As a result, three large groups of molecules and polymers have been disclosed that according to their R_{2p} can be predictably filtered in complex mixtures by the simple addition of Gd^{3+}, alone or in combination with T_2 filters; confirming R_{2p} as a convenient predictive tool of selectivity in PSR filters.

EXPERIMENTAL SECTION

Materials. $Gd_2(SO_4)_3{\cdot}8H_2O$ was purchased from Aldrich. Chitosan·HCl was obtained from FMC BioPolymer (M_w 80000 by SEC-MALLS, degree of acetylation 14% by ^{1}H NMR). Dextran from *Leuconostoc mesenteroides* (M_n 33698, M_w 65794, by GPC), mannan from *Saccharomyces cerevisiae* (M_n 34000, M_w 36000, by SECMALLS), citric acid, glucuronic acid, lactose, poly-L-lysine hydrobromide (PLL) (M_n 12400, M_w 16100, by LALLS), poly(methacrylic acid) sodium salt (PMAA) (M_w 29600 by GPC), myo-inositol, D-galacturonic acid monohydrate, codeine, sodium cyclamate, and paracetamol were purchased from Fluka. Methyl α-D-glucopyranoside, sucrose, acetylsalicylic acid, sodium ascorbate, taurine, saccharin, lidocaine, warfarin, D-glucosamine hydrochloride, β-glycerol phosphate disodium salt pentahydrate, chondroitin sulfate sodium salt (M_n 66784, M_w 114098, by GPC), and polyacrylamide (PAM) (M_w 5200000) were obtained from Sigma. Isomannide, L-isoleucine, 3-methoxypropionic acid, and poly-

acrylic acid (PAA) samples: PAA 450000 (M_v 450000) and PAA 1800 (M_n 1022, M_w 1773) were purchased from Aldrich. Caffeine, D-mannitol, acetylcysteine, and sulfated β-cyclodextrin were purchased from Sigma-Aldrich. Polyvinylpyrrolidone (PVP) samples were obtained from Fluka (M_w 360000), Sigma (M_w 10000), and Aldrich (M_w 55000). PVP 10000 was purified by precipitation from MeOH-Et_2O before NMR experiments. Poly(ethylene glycol) (PEG) was purchased from Sigma (M_p 12703, M_w 10732, by GPC). Hyaluronic acid (M_w 160000) was obtained from Aldrich. Polyvinyl alcohol (PVA) (M_w 60000 by GPC) was obtained from Acros. Fructose was obtained from Azaconsa. Arixtra® (fondaparinux sodium) was kindly provided by GlaxoSmithKline. Flumil Forte® 600 mg was obtained from Pharmazam. Cafiaspirina® was obtained from Bayer. Acetylcysteine Mylan® 600 mg was obtained from Mylan Pharmaceuticals.

Each Flumil Forte® effervescent tablet contains 600 mg of acetylcysteine and citric acid as NMR visible excipient. Each Cafiaspirina® tablet contains 500 mg of acetylsalicylic acid and 50 mg of caffeine. Each Acetylcysteine Mylan® granules for oral solution contains 600 mg of acetylcysteine along with mannitol, citric acid, and sodium saccharin as NMR visible excipients.

Determination of molecular weight of polymers. Gel permeation chromatography (GPC) for chondroitin sulfate and PVA was performed on a Malvern Viscotek TDA 305 with refractometer, right angle light scattering and viscometer detectors. Samples were measured with a Suprema precolumn (5 μm, 8 x 50 mm), Suprema 30 Å (5 μm, 8 x 300 mm), Suprema 1000 Å (5 μm, 8 x 300 mm), and PLaquagel-OH-Mixed (8 μm, 7.5 x 300 mm) columns. The system was kept at 30 °C. 0.1 M NaN_3, 0.01 M NaH_2PO_4, pH 7.5 was used as eluent at a rate of 1 mL/min. Pullulan 200 kDa (PSS standard services) was used to calibrate the laser detector and a set of ten pullulan samples (180 Da to 355 kDa) was used to obtain the molecular weight based on retention time.

The molecular weight of chitosan and mannan was determined by SEC-MALLS. An Iso Pump G1310A (Hewlett-Packard) was connected to two PSS Novema GPC columns (10 μm, 30 Å, 8 × 300 mm; and 10 μm, 3000 Å, 8 × 300 mm). A PSS SLD7000 MALLS detector (Brookhaven Instruments Corporation) operating at 660 nm and a G1362A refractive index detector (Agilent) were connected on line. A 0.15 M NH_4OAc/0.2 M AcOH buffer (pH 4.5) was used as eluent. Polymer solutions were filtered through 0.2 μm pore size membranes before injection.

Determination of transverse relaxation enhancement (R_{2p}) values. ^{1}H R_{2p} values in Tables 1 and S1 were determined at 8 mg/mL in D_2O (500 MHz). To this end, transverse relaxation times were measured in the absence ($T_{2d}=1/R_{2d}$) and the presence ($T_2=1/R_2$) of 13 μM Gd^{3+}. In those species that at 13 μM Gd^{3+} revealed T_{2d}<150 ms and differences between T_{2d} and T_2 lower than 10% (in the range of the experimental error), reported R_{2p} values were obtained at 1 mM Gd^{3+} (dextran, PVA, PVP, chitosan, PLL). R_{2p} of warfarin was determined in 3% D_2O/DMSO-d_6. R_{2p} values in Tables 1 and S1 are weighted arithmetic mean values of all ^{1}H resonances in the sample. Maximum and minimum T_2 (T_2^{max}, T_2^{min}) and T_{2d} (T_{2d}^{max}, T_{2d}^{min}) values for all species are shown in Table S1.

Transverse relaxation (T_2) values were determined using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence using 16 values of spin-echo time (τ), with a minimum of 1.4 ms and a maximum of about 6 to 7 times the highest T_2. The delay between 180º pulses in the CPMG block was 1.4 ms and the number of scans was set at 32. The absolute signal integral or signal intensity (I) at each value of τ was fitted to the following equation:

$$I(\tau)/I_0=\exp(-\tau/T_2) \quad (2)$$

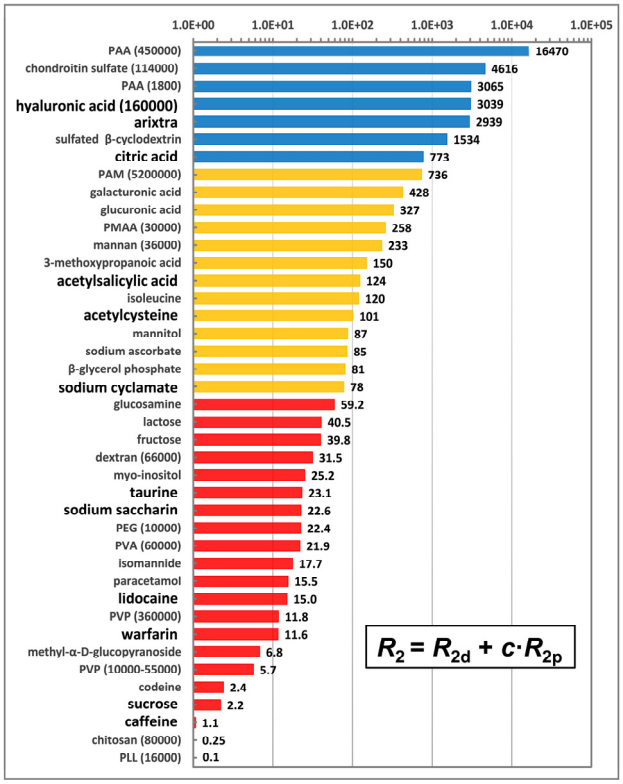
where $I(\tau)$ and I_0 are the observed signal integrals or intensities at a given value of τ and for τ equal to 0, respectively.

Sample preparation. A tablet of Cafiaspirina® was grinded and suspended in D_2O (1.5 mL). After stirring overnight it was filtered and lyophilized. The resulting white solid was dissolved in D_2O at the indicated concentration. Flumil Forte® and Acetylcysteine Mylan® were directly dissolved in D_2O. Arixtra® (fondaparinux sodium) provided as a powder was dissolved in D_2O at the indicated concentration. Mixtures were usually prepared in the range 1-20 mg/mL with relative concentrations between the components to ensure a comparable intensity of their signals in the NMR spectra.

RESULTS AND DISCUSSION

The transverse relaxation enhancement (R_{2p}) as a predictive tool for PSR filters. To evaluate the utility of R_{2p} as a predictive tool in PSR filters, various sugars, alkaloids, drugs, artificial sweeteners and food additives, aminoacids, vitamins, polyaminoacids, polysaccharides and synthetic polymers were selected and their R_{2p} determined (Table 1 and SI). Not unexpectedly, larger R_{2p} values arise in general for anionic species (polymeric and low molecular weight carboxylates, sulfates and phosphates) in agreement with their higher Gd^{3+}-complexing ability; followed by lower R_{2p} for neutral and cationic species. In outline, Table 1 justifies the successful PSR filters described in our previous report based on an empirical basis.[2] The large differences in R_{2p} between polyacrylic acid (PAA)/hyaluronic acid (HA)/citric acid on one side and lactose/dextran/polyvinylpyrrolidone (PVP)/methyl-α-D-glucoyranoside/caffeine on the other side might be used to explain the ^{1}H and ^{13}C PSR filtering of the former species in the NMR of their mixtures. In another example, the combined use of PSR and T_2-filters allowed the sequential suppression of the components in a ternary HA-mannan-PVP_{1000} mixture according to their R_{2p}. However, moving R_{2p} from *a posteriori* justification to a predictive tool of selectivity in PSR requires from a correct interpretation of Table 1. In other words, are successful PSR suppressions dictated by a minimum difference in R_{2p}? Or, is the relative position of the species in Table 1 the critical factor controlling efficiency? In addition, the low R_{2p} displayed by species like cyclamate, taurine or saccharin complicates the interpretation of PSR filters only on the basis of the anionic character of the species. With the objective of answering these questions, the efficiency of the PSR filter was analysed in mixtures of relevance in the pharmaceutical and food industry that comprise species covering the whole range of R_{2p} values in Table 1, namely: a dermal filler composed of HA and lidocaine as local anesthetic, a mixture of the commercial anticoagulants Arixtra® and warfarin, the beverage Red Bull® (taurine, sucrose, glucose, citric acid), the drugs Flumil Forte® (acetylcysteine and citric acid), Cafiaspirine® (acetylsalicylic acid and caffeine) and Acetylcysteine Mylan® (acetylcysteine, citric acid, sodium saccharin), and the artificial sweetener Natreen® (sodium cyclamate, sodium saccharin, citric acid).

TABLE 1. R_{2p} ($s^{-1}mM^{-1}$) values in D_2O (8 mg/mL, 13 μM Gd^{3+}, 500 MHz).



PAA [polyacrylic acid], HA [hyaluronic acid], PAM [polyacrylamide], PMAA [poly(methacrylic acid)], PEG [poly(ethylene glycol)], PVA [poly(vinyl alcohol)], PVP [polyvinylpyrrolidone], PLL [poly-L-lysine].

The PSR filter in mixtures with large difference in R_{2p}: a dermal filler, an anticoagulant composition and Red Bull®. We started analysing the selectivity of the PSR filter in two mixtures with components displaying quite different R_{2p} values such as, hyaluronic acid (HA)-lidocaine (R_{2p} 3039 *vs* 15) and Arixtra®-warfarin (R_{2p} 2939 *vs* 12). Dermal fillers based on HA are the most widely used injectables to augment facial volume. Commercial formulations including lidocaine as local anesthetic have been approved by the FDA.[8] Aristra® is the commercial trademark for the anticoagulant fondaparinux, a synthetic sulfated pentasaccharide derived from heparin that inhibits Factor Xa. The combined use of Aristra® with warfarin is indicated for the treatment of acute venous thromboembolism.[9] Based on the large difference in R_{2p} between the constituents in the mixtures, selective PSR suppressions were anticipated for fondaparinux and HA, the species with largest R_{2p}values. Figures 1 and S1 show that the simple addition of minute concentrations of Gd^{3+} to both mixtures resulted indeed in their selective filtering, confirming the predictive character of R_{2p} and the efficiency of the PSR filter in the analysis of complex mixtures.

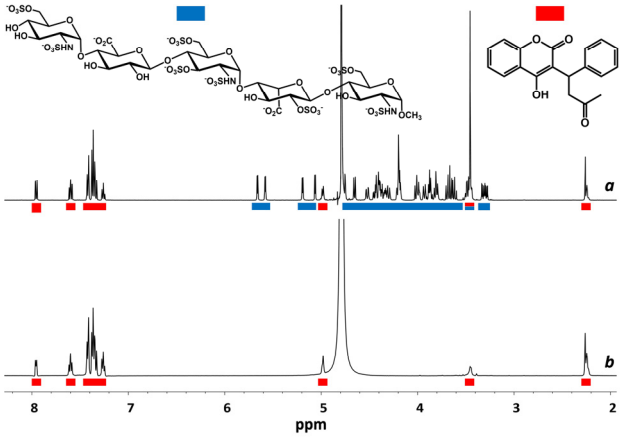


Figure 1. ^1H NMR spectra (D_2O, 500 MHz, 300 K) of a mixture of Arixtra® (4.8 mg/mL) and warfarin (1.1 mg/mL) before (a) and after (b) the addition of Gd^{3+} (30 μM).

Similar selectivity was achieved in the PSR filter of Red Bull®. Figure 2 shows NMR spectra of Red Bull® where the characteristic signals of the major constituents are easily identified: citrate (R_{2p} 773), taurine (R_{2p} 23), sucrose and glucose (R_{2p}<10). Because of the R_{2p} values of the constituents, the selective suppression of citrate was expected. Red Bull® represents, however, a more challenging mixture than the above examples. Citric acid displays a lower R_{2p} than HA and Arixtra®. This along with the more alike molecular weight of the constituents and the presence of a rather Gd^{3+}-complexing sulfate in taurine might result in extra hurdles for the suppression. Gratifyingly, the addition of Gd^{3+} to Red Bull® resulted in the very selective suppression of only the signals of citric acid in the ^1H NMR spectrum of the mixture. Identical selectivity was also revealed in COSY, HMQC, and even ^{13}C spectra (Figure 2), supporting the validity of ^1H R_{2p} data in the prediction of PSR filters in 2D and ^{13}C experiments.

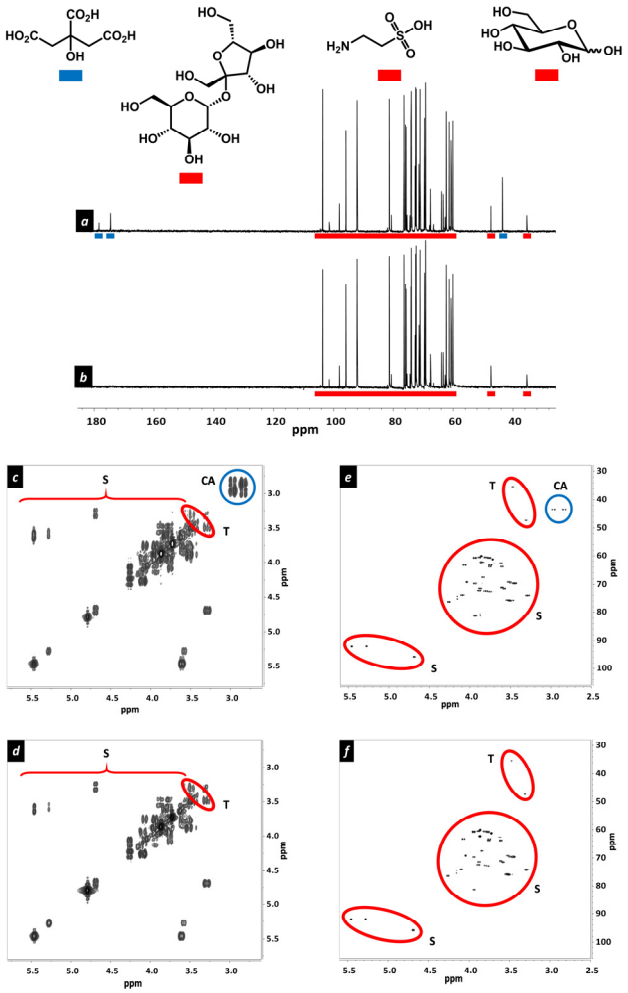


Figure 2. ^{13}C, ^{1}H-^{1}H COSY, and ^{1}H-^{13}C HMQC NMR spectra (D_2O, 500 MHz ^{1}H, 125 MHz ^{13}C, 300 K) of lyophilized Red Bull® (160 mg/mL) before (a, c, e) and after (b, d, f) the addition of Gd^{3+} (0.5 mM). CA=citric acid, T=taurine, S=sugars.

The PSR filter in compositions with reduced R_{2p} difference between the components: Flumil Forte® and Cafiaspirina®. With the aim of further evaluating the potential of R_{2p} in the prediction of selective PSR filters, we proceeded to analyse two commercial drugs having components with close R_{2p} values: Flumil Forte® and Cafiaspirina®. Flumil Forte® is a mucolytic drug composed of the active ingredient acetylcysteine (R_{2p} 101) and citric acid (R_{2p} 773) as the only NMR-visible components. Cafiaspirina® is a widely used analgesic for the treatment of a variety of aches and pains. It combines acetylsalicylic acid (R_{2p} 124) with caffeine (R_{2p} 1). Our aim was to develop conditions for selectively filtering the species with largest R_{2p} (citric acid, acetylsalicylic acid) while leaving unaffected the other main components (acetylcysteine, caffeine).

In Flumil Forte®, the higher R_{2p} of acetylcysteine compared to PSR-inactive species seen in the previous section, like lidocaine/warfarin/taurine/sucrose, prevented the selective PSR filtering of citric acid in the ^{1}H NMR spectrum without affecting the resolution of acetylcysteine. Still, a clean suppression was possible at low Gd^{3+} concentrations when PSR was combined with a short T_2-filter (Figure 3). T_2-filters such as the Carr–Purcell–Meiboom–Gill (CPMG) and related sequences keep the magnetization (I) in the transverse plane before acquisition, allowing the magnetization of different nuclei to decay according to their T_2 values (the shorter the T_2, the faster the decay, eq 2).[10] Eventually, after a certain time (t), differences in T_2 can be exploited for the selective suppression of the signals of lower T_2.

$$I = I_0 \cdot \exp(-t/T_2) \qquad (2)$$

Figure 3 shows the normalized ^{1}H signal intensities (I/I_0) of citric acid and acetylcysteine in Flumil Forte® as a function of time before and after the addition of 20 μM Gd^{3+}; a concentration low enough to selectively induce a huge relaxation effect to citric acid while leaving practically unaffected the relaxation and resolution of acetylcysteine. These conditions do not account for the direct and complete suppression of citric acid, but this can be easily achieved by complementing the addition of Gd^{3+} with a very short 25 ms CPMG filter (Figure 3).

The relatively small dependence of T_2 on the magnetic field[11] encouraged us to test the robustness of this combined PSR-CPMG strategy at different fields. To this end, the PSR conditions developed at 500 MHz (20 μM Gd^{3+}/25 ms CPMG) were successfully implemented in spectrometers operating at 300 and 750 MHz, demonstrating the utility of the tool at fields different to that used in the determination of R_{2p} (Figure 3). The fidelity of PSR-CPMG was also demonstrated in 2D experiments where the CPMG sequence was used as an excitation block replacing the first excitation pulse.[12] This way, filtered COSY and HMQC spectra of Flumil Forte® were easily obtained by application of the same 20 μM Gd^{3+}/25 ms CPMG conditions to afford spectra showing only the signals of acetylcysteine. Alternatively, more straightforward conditions to filtered COSY, HMQC and even ^{13}C spectra were developed in the absence of the CPMG block by increasing the concentration of Gd^{3+} up to 0.2 mM, exploiting the lower signal resolution of 2D and ^{13}C experiments compared to ^{1}H (Figure S2).

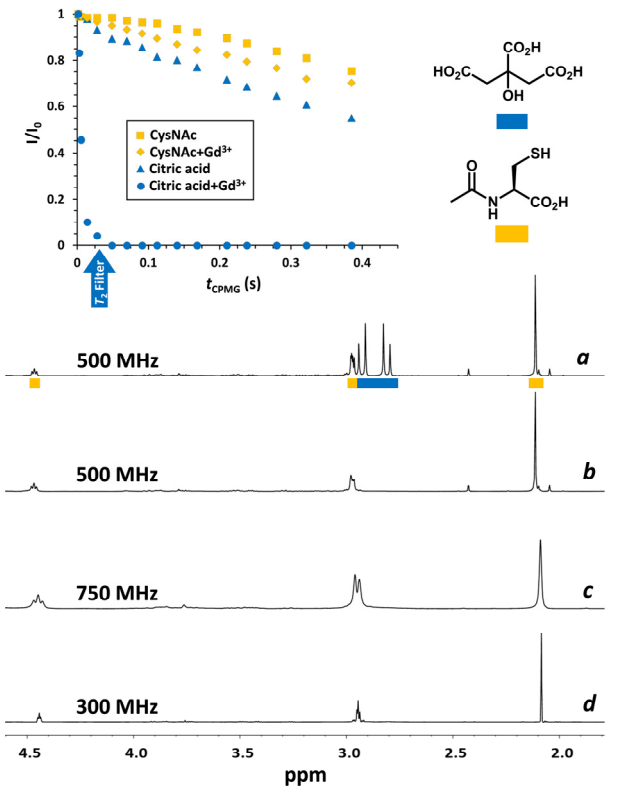


Figure 3. ^{1}H NMR spectra (D_2O, 300 K) of Flumil Forte® (20 mg/mL) before (a, 500 MHz) and after (b, 500 MHz; c, 750 MHz; d, 300 MHz) the addition of Gd^{3+} (20 μM) + T_2 filter (CPMG, 25 ms). Normalized ^{1}H intensities (I/I_0, 500 MHz) of citric acid (methylene protons) and acetylcysteine (acetyl group) in the mixture before and after the addition of Gd^{3+}.

As for Cafiaspirina®, the smaller difference in R_{2p} between acetylsalicylic acid and caffeine (*ca* 100 $s^{-1}mM^{-1}$) anticipated the suppression of the former as a difficult task. Indeed, the direct PSR filter turned unfeasible by the simple addition of Gd^{3+} (Figure 4). Only the acetyl group and H6 of acetylsalicylic acid, the nuclei in closer proximity to the complexing carboxylate, could be completely removed from the ^1H NMR spectrum of the mixture. The viability of a combined PSR-CPMG strategy was then evaluated. Figure 4 shows the normalized ^1H signal intensities (I/I_0) of acetylsalicylic acid and caffeine in Cafiaspirina® as a function of time before and after the addition of 1 mM Gd^{3+}. As in the previous example, the higher R_{2p} of acetylsalicylic acid compared to caffeine translates into a much faster relaxation in the presence of Gd^{3+}, which can be exploited in a selective suppression when combined with a 200 ms CPMG filter (Figure 4). Here again, the selectivity of the PSR-CPMG filter was further extended to COSY and HMQC, allowing the selective visualization of only the caffeine signals by using the same conditions as the ^1H spectrum. As for the magnetic field, the application of the filtering conditions developed at 500 MHz to spectrometers operating at 300 and 750 MHz also proceeded with complete selectivity (Figure S3).

Selectivity in PSR filters is dominated by R_{2p} rather than the original T_{2d} values. The experiments shown above confirm R_{2p} as a useful tool for the reliable prediction of selective PSR filters. An analysis of these suppressions in the light of the R_{2p} values in Table 1 suggests that the difference in R_{2p} among the components in a mixture might not be the key factor controlling the feasibility of a filter compared to their relative position in the table. Thus, species at the top of Table 1 can be selectively filtered in the presence of others at the bottom by the simply addition of Gd^{3+}, while the combined use of Gd^{3+} and T_2-filters of different length is required for selective suppressions among species at the top-medium and medium-bottom parts. These characteristic behaviours led us to propose three groups of compounds in Table 1 according to their ease of suppression by PSR, namely blue (B), yellow (Y), and red (R) at the upper, medium, and bottom parts, respectively. By studying the selectivity of PSR filters in two-component mixtures of species selected all along Table 1, not only the existence of the BYR groups was verified and their borders identified, but general suppression conditions among them unveiled (see below). Table 2 depicts successful and failed suppressions within these pairs, while NMR spectra and particular filtering conditions are included in Figures S4-S14.

- B-R: Easiest suppressions to accomplish. Concentrations of Gd^{3+}<0.4 mM for ^1H, COSY and HMQC, and larger concentrations for ^{13}C (smaller gyromagnetic ratio of ^{13}C than ^1H).
- B-Y: Same conditions as B-R complemented with CPMG filters up to 25 ms for ^1H, COSY and HMQC. ^{13}C filters are feasible without CPMG by increasing the concentration of Gd^{3+} (also of application for COSY and HMQC).
- Y-R: 0.4-2.0 mM Gd^{3+} complemented with 25-300 ms CPMG for ^1H, COSY and HMQC.

As an example to illustrate that selectivity in PSR is dominated by R_{2p} rather than the original T_{2d} values (T_{2d} in Table S1), an unfeasible filter in terms of T_{2d} was considered to involve acetylsalicylic acid, the Y species with largest T_{2d} (T_{2d}=3.4 s; R_{2p} 124), and dextran, a R component among those with lowest T_{2d} (T_{2d}<0.1 s; R_{2p} 31). For a successful PSR filter in such a mixture, a selective relaxation enhancement on acetylsalicylic acid is required in order to afford final T_2 values below those of dextran. Figure 5 shows the dependence of the T_2 of both species on the concentration of Gd^{3+}. Clearly, 0.2 mM Gd^{3+} represents a threshold from which T_2 values of acetylsalicylic acid become lower than dextran. Increasing the concentration of Gd^{3+} up to 1.8 mM resulted in even larger differences in T_2 that could be exploited for the selective filtering of acetylsalicylic by application of a short 20 ms CPMG.

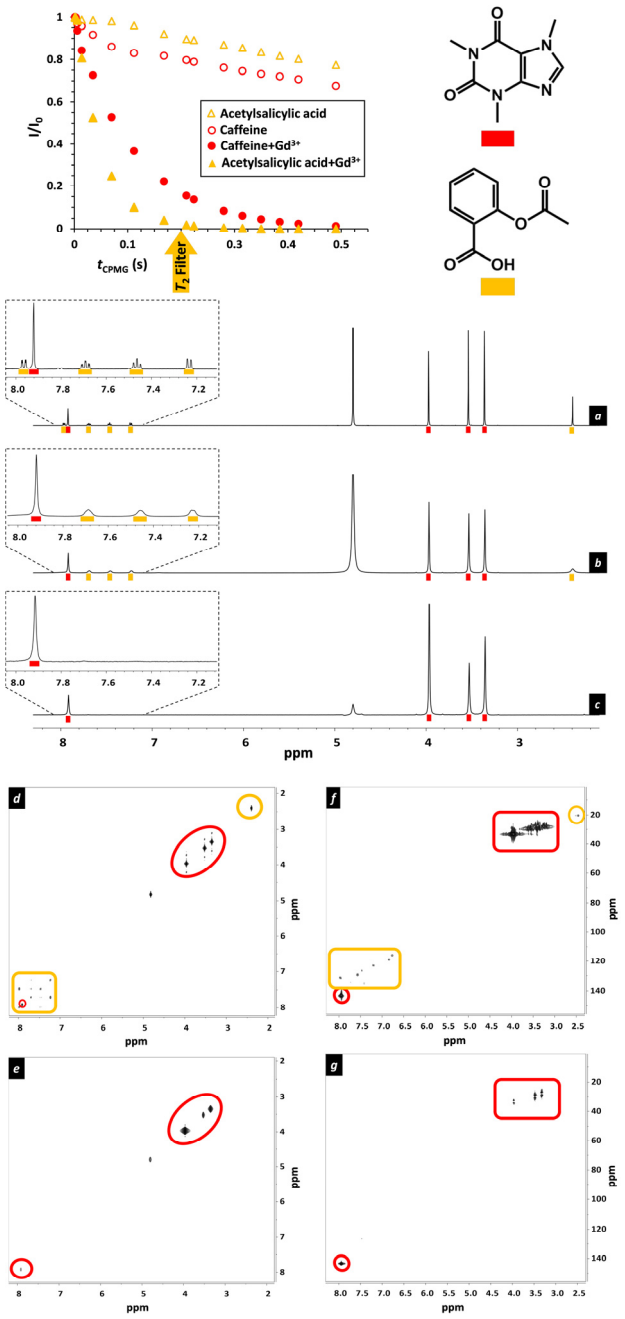
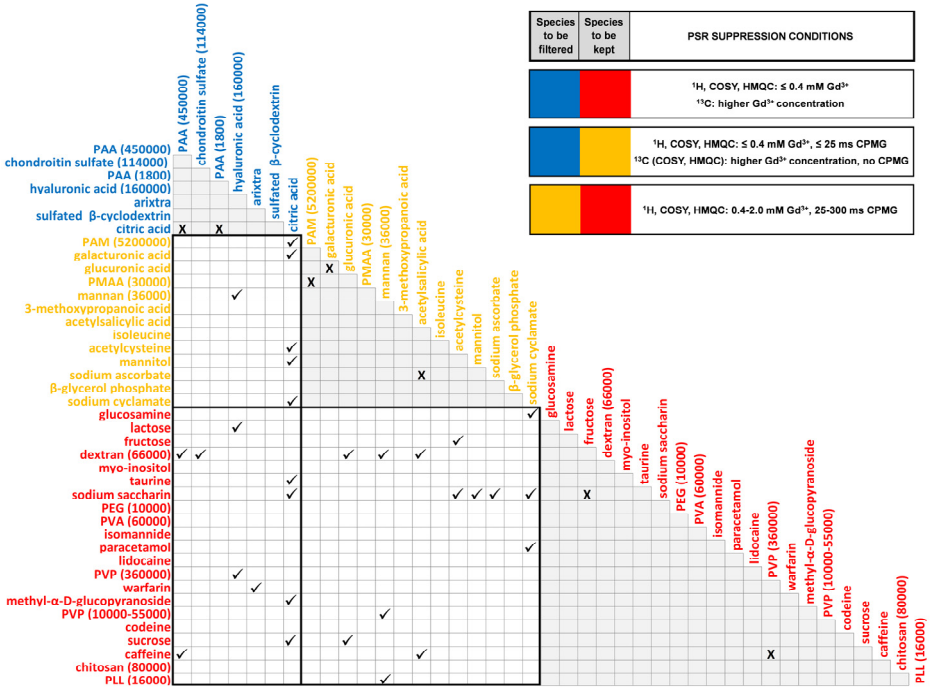


Figure 4. ^1H, ^1H-^1H COSY, and ^1H-^{13}C HMQC spectra (D_2O, 500 MHz, 300 K) of Cafiaspirina® (4 mg/mL) before (a, d, f) and after (b) the addition of Gd^{3+} (1 mM), and after the addition of Gd^{3+} (1 mM) + T_2 filter (CPMG, 200 ms) (c, e, g). Normalized ^1H intensities (I/I_0) of acetylsalicylic acid (H5) and caffeine (aromatic H) in the mixture before and after the addition of Gd^{3+}.

TABLE 2. Successful and failed PSR suppressions in two-component mixtures (D_2O, 500 MHz).



Blue/Red pairs PAA/caffeine, HA/lactose, HA/PVP(360000), PAA(450000)/dextran, and citric acid/methyl-α-D-glucopyranoside taken from ref. 2.

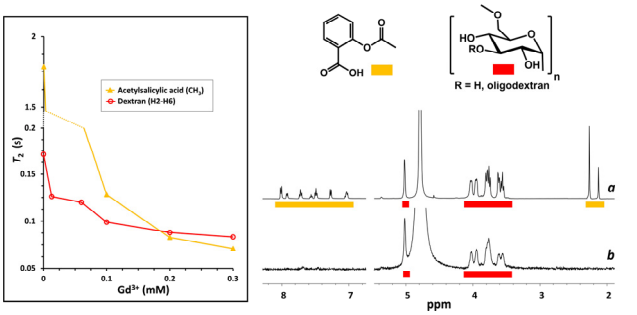


Figure 5. Right panel: ^{1}H NMR spectra (D_2O, 500 MHz, 300 K) of a mixture of dextran (1 mg/mL) and acetylsalicylic acid (1 mg/mL) before (a) and after (b) the addition of Gd^{3+} (1.8 mM) + T_2 filter (CPMG, 20 ms). Left panel: Dependence of ^{1}H T_2 of dextran and acetylsalicylic acid on the concentration of Gd^{3+}.

***R_{2p}* in the prediction of selective PSR filters in multicomponent mixtures.** Having stablished the utility of R_{2p} and the BYR groups in the prediction of PSR filters in two-component mixtures, we decided to test their potential in more complex systems. With this aim the drug Acetilcisteina Mylan® and the artificial sweetener Natreen® were selected as multicomponent preparations. Acetilcisteina Mylan® is a commercial mucolytic drug related to Flumil Forte®, which in addition to acetylcysteine (R_{2p} 101) and citric acid (R_{2p} 773), incorporates D-mannitol (R_{2p} 87) and sodium saccharin (R_{2p} 23). According to the position of each component in Table 1, our objective was to selectively filter citric acid (B component) in a first PSR-CPMG, followed by acetylcysteine and mannitol (Y components) in a second step. As seen in Figure 6, by using concentrations of Gd^{3+} and CPMG lengths within the limits indicated in Table 2, the two sequential PSR-CPMG filters proceeded as expected with complete selectivity. Gratifyingly, this two-step filtering strategy also revealed successful when analysing a mixture of the three components of Natreen® [citric acid (R_{2p} 773, B component), sodium cyclamate (R_{2p} 78, Y component), sodium saccharin (R_{2p} 23, R component)], confirming the utility of R_{2p} as a valuable tool in the prediction of selective PSR filters in complex mixtures(Figure S15).

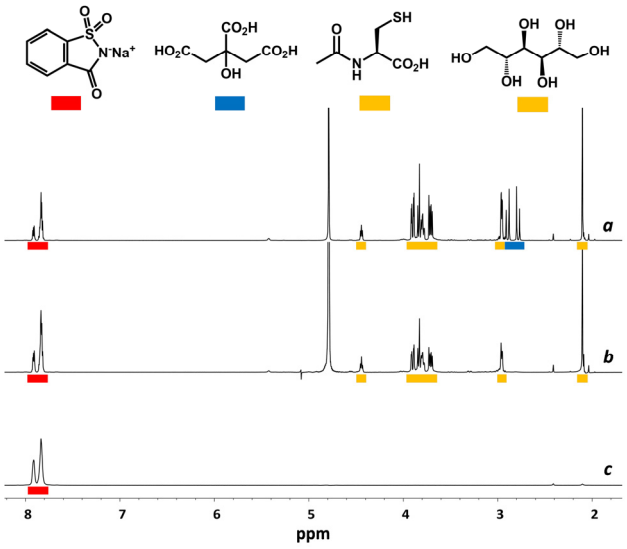


Figure 6. ^{1}H NMR spectra (D_2O, 500 MHz, 300 K) of Acetilcisteina Mylan® (25 mg/mL) supplemented with saccharin (12.5 mg/mL) before (a) and after the addition of (b) Gd^{3+} (20 μM) + 25 ms CPMG, and (c) Gd^{3+} (1.6 mM) + 200 ms CPMG.

CONCLUSIONS

The paramagnetic spin relaxation (PSR) filter has been recently described by our laboratory as a tool for the NMR suppression of the ^{1}H and ^{13}C resonances of individual components in mixtures according to their Gd^{3+}-complexing ability. It is based on the faster relaxation of nuclei in a paramagnetic environment and so, complements traditional filters like relaxation and diffusion filters. Herein we describe that the PSR filter is dominated by the transverse relaxation enhancement (R_{2p}) of the nuclei rather than the original T_{2d} values and so, that R_{2p} represents a reliable predictive tool of selectivity in the PSR filter of complex mixtures. The analysis of the R_{2p} of a series of small molecules and polymers of interest in the pharmaceutical and food industry (Table 1), along with the conditions of the PSR filter in various of their commercial mixtures, has led us to identify three large groups of compounds in Table 1 according to their ease of suppression by PSR (R_{2p} value) in ^{1}H, ^{13}C, COSY and HMQC experiments (Table 2). The robustness and fidelity of this filtering strategy has been demonstrated at magnetic fields different to that used in the determination of R_{2p} and for the sequential suppression of species in complex multicomponent compositions. The easy determination of R_{2p} will facilitate the incorporation of new species in Table 1, paving the way for the application of the PSR filter in the analysis of complex mixtures in quality control, natural product extracts, or the metabolic profiling of biological samples like urine and blood plasma.

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ASSOCIATED CONTENT

Supporting Information Available. Materials, determination of R_{2p} values, sample preparation, NMR spectra and conditions. This information is available free of charge via the Internet at http://pubs.acs.org.

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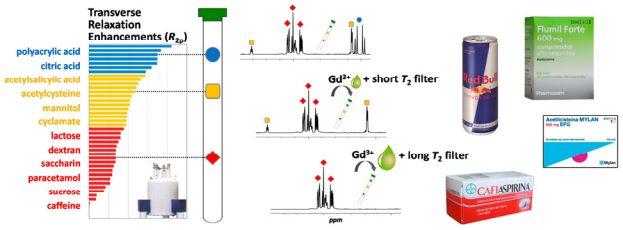

Predictive PSR Filters in the NMR of Mixtures