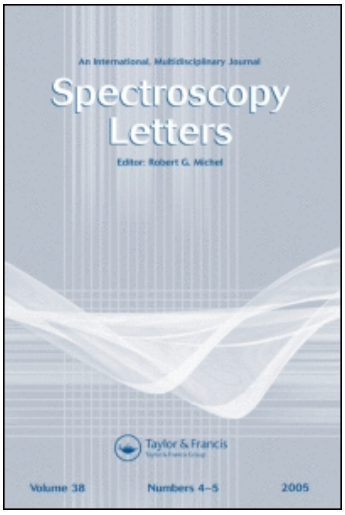

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Proton Decoupled ^{19}F NMR Spectroscopy of Drugs Used in Eye Treatment

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Proton Decoupled ^{19}F NMR Spectroscopy of Drugs Used in Eye Treatment

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Abstract: The potential for detecting fluorinated compounds in a 2.35 T nuclear magnetic resonance system was assessed to evaluate the possibility for *in vivo* monitoring of fluorinated drugs applied to the eye. Time-share proton decoupled ^{19}F NMR spectroscopy was implemented for signal enhancement in an experimental eye model. Signal-to-noise in ^{19}F NMR spectra of dexamethasone phosphate was enhanced by 56%. However, decoupling did not enhance S/N for ciprofloxacin. The obtained detection limit of 0.1–0.2 μmol did not enable detection of drugs at

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therapeutic concentrations in the eye. Higher magnetic field and improved coil and detection technology might enable future *in vivo* monitoring of drugs in the eye.

Keywords: Fluorine, time-share, decoupling, ciprofloxacin, dexamethasone

INTRODUCTION

Fluorine-19 nuclear magnetic resonance (NMR) spectroscopy is used for studying uptake of drugs and detection of metabolites of fluorine-containing compounds in living systems. Such a noninvasive method benefits from the absence of detectable endogenous fluorine and the advantageous NMR sensitivity of the ^{19}F nucleus (83% of the proton).[1] However, in a clinical setting or in animal experiments, an unsatisfactory detection limit may restrict the applicability. Maxwell[2] reported a detection limit of about 0.01 to 0.1 mmol for *in vivo* ^{19}F NMR spectroscopy. Macromolecular binding[3] and J-coupling to adjacent protons decrease ^{19}F signal intensities. Proton decoupling may then be used to collapse multiplet structures and thereby increase signal magnitudes.[4] In cases where significant interaction exists between the decoupler channel and the receiver, the specialized time-share decoupling method can be advantageous.[5]

Knowledge about intraocular penetration of drugs and their metabolism is very important for the treatment of eye diseases. Previously, NMR spectroscopy was applied in our laboratory to detect fluorinated drugs *in vitro* in tissue samples from the anterior segment of the eye.[6,7] The aim of the current study was to assess the detection limit for fluorinated drugs in a NMR spectroscopy/imaging system (field strength 2.35 T). As a first step, solutions of drugs were injected into a phantom eye for ^{19}F NMR spectroscopy. The purpose was to investigate the possibility for *in vivo* monitoring of fluorinated drugs in the eye in animal models. Numerous ophthalmic medications contain fluorine, including drugs used frequently as antibacterial and anti-inflammatory agents. In this study, two fluorinated drugs, the fluoroquinolone ciprofloxacin (CIP) and the corticosteroid dexamethasone phosphate (DXM-P), were investigated. The ^{19}F nucleus in these drugs exhibits J-couplings to neighboring protons, so decoupling of protons was tested as a means of enhancing ^{19}F signals.

EXPERIMENTAL

A phantom eye model (polypropylene sphere) with a volume of 2.75 mL was placed in the center of a 2-turn 25-mm ^{19}F transmit/receive surface coil, and this was positioned in a Bruker BioSpec spectroscopy/imaging system ($B_0 = 2.35$ T) (Bruker BioSpin GmbH, Rheinstetten, Germany). Decoupling pulses during acquisition were transmitted by a surrounding 70-mm

birdcage coil. A notch filter in the ^{19}F channel (loss $\leq$ 0.8 dB for ^{19}F, $\geq$80 dB for ^{1}H) and a noise filter in the ^{1}H channel did not prevent ^{1}H-pulse breakthrough into the receiver. Thus, time-share decoupling was utilized. This technique involves interleaving of decoupling pulses between the sampling points, with the receiver disabled during transmission of decoupling pulses. Two different methods for time-share decoupling were implemented, using 2,2,2-trifluoroethanol (triplet, $J_{FH} = 4.5$ Hz) as a test sample.

Time-Share Decoupling: Method A

The Bruker pulse program designed for homodecoupling of a region (*zghc*, Bruker BioSpin)[8,9] was applied with the WALTZ-16 scheme for proton decoupling during ^{19}F acquisition. In order to switch on and off the decoupling between the individual sampling points, this program uses an oversampling technique; that is, the signal is digitally sampled at a rate ($(33\,\mu s)^{-1}$) not visible in the final spectrum. The dwell time was 132 μs (time between each visible point), with the first 20% of this period being open for decoupler pulses.[10] Using a 1.0-ms decoupler pulse, the software consequently controlled the segmentation of these pulses between the sampling points.

Time-Share Decoupling: Method B

A 90° decoupler pulse (100 μs) was divided into 8, with one segment interleaved between each sampling point. The receiver was disabled during pulse segments (12.5 μs, 2/12 of dwell time) and a following delay (6.25 μs, 1/12 of dwell time). This delay prevented pulse feedthrough into the receiver.[11] Nine-twelfths of the dwell time was left for sampling. The decoupler pulses were phase cycled according to WALTZ-16 and MLEV-16 decoupling sequences.

These two methods for time-share decoupling were compared at 2.35 T with regard to decoupled bandwidth and signal-to-noise ratio (S/N). Acquisition parameters for ^{19}F NMR spectroscopy on the drugs ciprofloxacin (CIP) (Alcon Laboratories Inc., Fort Worth, TX, USA) and dexamethasone phosphate (DXM-P) (Sigma Chemical Co., St. Louis, MO, USA) were optimized for the best S/N using the Ernst angle.[12,13] This requires knowledge of spin-lattice relaxation times (T_1). Due to the surface coil's inhomogeneous field distribution,[14] the ^{19}F T_1 values were obtained by inversion recovery experiments at $B_0 = 11.8$ T (Bruker Avance DRX 500, Bruker BioSpin). The measurements were performed at room temperature with proton decoupling during acquisition.

Coupled and decoupled spectra were obtained for a series of concentrations of CIP (0.05–1.5 mM) and DXM-P (0.03–1.0 mM) at 2.35 T. CIP was dissolved in acetate buffer (pH $\sim$ 5.15) for increased solubility, and

DXM-P was dissolved in distilled water. The solutions were injected at variable concentrations into the phantom eye. Acquisition parameters for CIP were pulse angle 62°, acquisition time 250 ms, pulse repetition period 300 ms, spectral width 3.77 kHz, and data zero-filled to 2 K data points. The number of scans was 6 K, which gave a total experiment time of 31 min. For DXM-P the same values were used, except for the pulse angle (47°). Method A (*zghc*) was chosen for decoupling. S/N was calculated by the *sino* function[15] in the XWIN-NMR software using matched filters. The *sino* function uses the peak intensity as signal and a value of "white" noise in a selected signal-free frequency interval. Chemical shifts were referenced to trifluoroacetic acid at −72.0 ppm for fluorine and TSP (sodium-3′-trimethylsilylpropionate-2,2,3,3-d_4) at 0 ppm for proton.

RESULTS

The pulse program for homodecoupling of a region combined with WALTZ-16 decoupling (method A) resulted in a decoupled bandwidth of 600 Hz (Fig. 1a). Using method B with WALTZ-16 and MLEV-16 schemes, the

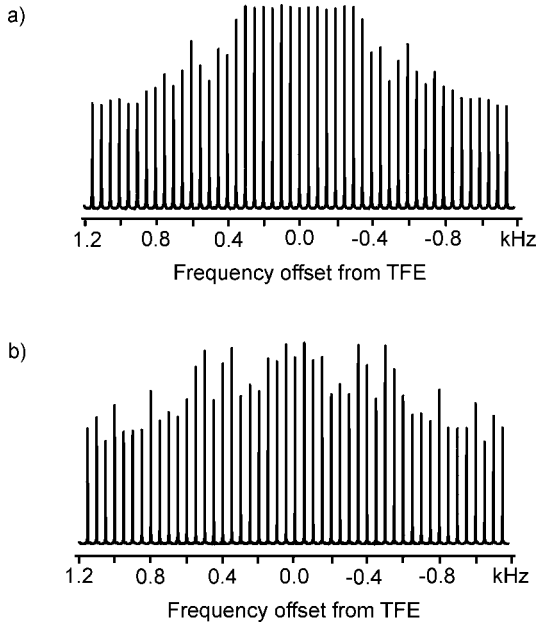

Figure 1. ^{19}F signal dependency on ^{1}H offset using time-share ^{1}H decoupling in a sample of trifluoroethanol (TFE, $J_{FH} = 4.5$ Hz). (a) Time-share method A (pulse program *zghc*, Bruker) with WALTZ-16 scheme, 1-ms ^{1}H pulses. (b) Time-share method B with MLEV-16 scheme, using 1/8 of a 90° decoupling pulse element during each dwell time.

peak amplitude of trifluoroethanol was increased by 30% and 59%, respectively. However, method B resulted in a decoupled bandwidth of only ~300 Hz (MLEV-16; Fig. 1b), and cycling sidebands and modulations in the peak height was introduced. Based on this, Bruker's method for homodecoupling in combination with WALTZ-16 scheme (method A) was chosen for detection limit assessment.

The ^{19}F signals from CIP and DXM-P were multiplets due to J-couplings to adjacent protons (Fig. 2a). Both drugs had two J_{FH}-couplings resolved ($J_{FH}/^{1}$H chemical shift: CIP 7 Hz/7.38 ppm, 13 Hz/7.48 ppm; DXM-P 10 Hz/4.41 ppm, 32 Hz/2.59 ppm), and DXM-P had additional long-range

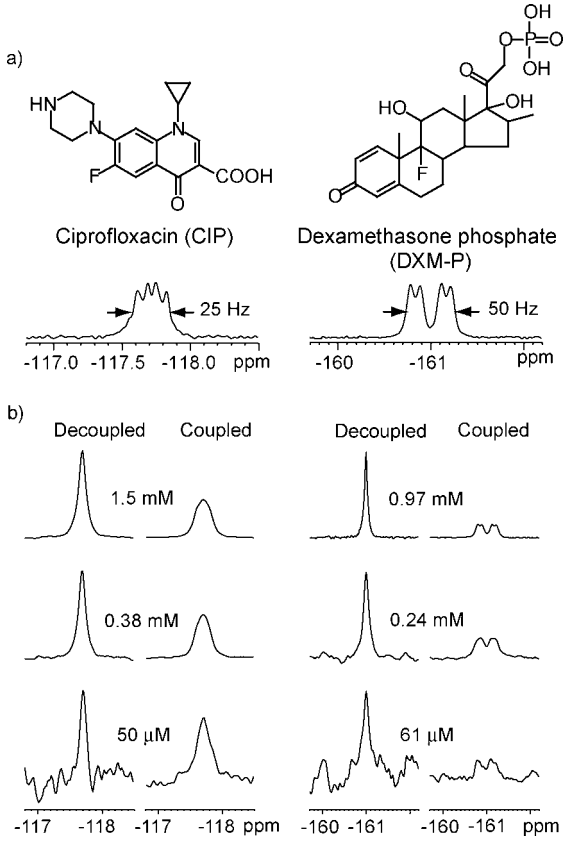

Figure 2. Chemical structures and ^{19}F NMR spectra of ciprofloxacin (left) and dexamethasone phosphate (right) ($B_0 = 2.35$ T). (a) High-resolution spectra showing the peak splitting (without filtering). (b) ^{1}H decoupled and coupled spectra of the drugs at different concentrations. The spectra are normalized with respect to the height of the decoupled peak at each concentration (applied exponential filters: CIP, 6 Hz; DXM-P, 7 Hz). Spectra were obtained during 31 min with 6 K transients.

Table 1. Longitudinal relaxation time T_1 in room temperature for ciprofloxacin (n = 4) and dexamethasone phosphate (n = 3) ($B_0 = 11.8$ T)

Ciprofloxacin		Dexamethasone phosphate	
Concentration (mM)	T_1 (ms)	Concentration (mM)	T_1 (ms)
0.3	372 ± 16	0.2	728 ± 50
1.5	360 ± 14	1.0	766 ± 8
3.0	351 ± 10[a]	2.0	770 ± 25

[a] n = 5.

couplings. Proton irradiation frequency was set specifically for each drug to minimize decoupler power (CIP + 260 Hz and DXM-P − 150 Hz relative to the water resonance), and acquisition parameter optimizations were based on the obtained T_1 values (Table 1).

The results from the concentration series experiments ($B_0 = 2.35$ T) in the phantom eye are shown in Fig. 3. CIP was detected at a concentration of 0.05 mM with a signal-to-noise ratio of 6.7 ± 0.6 (n = 3) without proton decoupling. Proton decoupling gave S/N = 4.7 ± 0.4 (n = 3), thus S/N decreased by 30% when decoupling was applied. The concentration of 0.05 mM in the phantom eye (2.75 mL) corresponds to an amount of 0.14 μmol of the drug inside the coil sensitive volume. DXM-P was detected at the concentration 0.06 mM with S/N = 3.4 ± 0.3 (n = 3) without decoupling and S/N = 5.3 ± 1.6 (n = 3) with decoupling as the lower limits, so decoupling increased S/N by 56%. Decoupled and coupled spectra of the two drugs, at different concentrations, are shown in Fig. 2b.

DISCUSSION

The possible detection limit for fluorinated compounds in the eye *in situ* was assessed. In the phantom eye, holding a volume of about 8–10 times a rabbit's aqueous humor, the lowest detected concentrations correspond to a detection limit of about 0.1–0.2 μmol. These results can be compared with previous results obtained after drug treatment of rabbit eyes. After topical application of CIP or DXM-P to the rabbit cornea, drugs have been detected in aqueous humor samples at concentrations of 0.1–0.15 mM.[6,16] As the volume of aqueous humor in the rabbit eye is approximately 0.3 mL, these reported drug concentrations correspond to 0.03–0.045 μmol CIP or DXM-P. In practice, the sensitivity requirement for monitoring of the drugs in the eye is estimated to be 10–50 μM. Thus, there is need for a 10- to 100-fold increase in sensitivity with the proposed technique. Higher magnetic fields

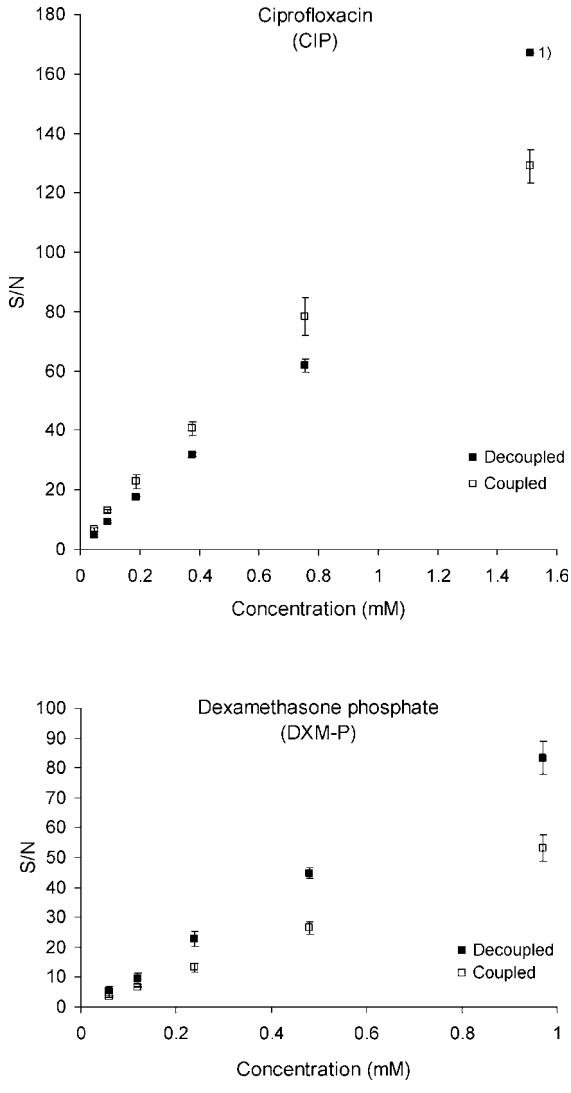

Figure 3. Signal-to-noise ratio (S/N) in ^{19}F NMR spectra for a series of concentrations of ciprofloxacin and dexamethasone phosphate, respectively, with and without ^{1}H decoupling (n = 3). Fourier transforms with exponential multiplication of 6 Hz (CIP) and 7 Hz (DXM-P) were applied (matched filters). $^{1)}$For the 1.5 mM measurement of CIP, only one measurement is displayed due to corrupted data.

and improved coil and detection technology might enable *in vivo* monitoring of therapeutic drugs in the eye.

Proton decoupling was expected to give a positive effect on the S/N. Due to high noise levels in the ^{19}F spectra under conventional WALTZ-16

decoupling, the time-share decoupling method was chosen. Li et al.[5] reported a threefold improvement in S/N by the time-share method compared with the conventional WALTZ-4 method for proton decoupled ^{19}F spectroscopy at 1.5 T, so time-share methods may be advantageous in *in vivo* proton decoupled ^{19}F NMR spectroscopy. In the current study, time-share proton decoupling demonstrated different effects on the signals from the two drugs investigated. The peaks in the coupled spectrum of CIP had a total width of 25 Hz (at half height) and showed no baseline separation (Fig. 2a). Collapse of the multiplet by decoupling led to a 2.5-times increase in peak height at the most. However, this signal enhancement during decoupling was exceeded by increased noise (Fig. 2b). DXM-P had a total width of 50 Hz in the coupled spectrum (Fig. 2a). The two halves of the signal were nearly baseline separated having the largest coupling of the two drugs (32 Hz), and decoupling gave a 4.3-times increase in peak height. The increase in signal intensity exceeded the introduced excess noise from the decoupler. Thus, the S/N was changed in opposite directions for the two drugs when decoupling was applied. In addition to the insufficient noise filtering, this is related to the width of the multiplet structure. Generally, the potential for signal improvement through decoupling is dependent on the field homogeneity. For DXM-P, experiments at 11.8 T (linewidth $\Delta\nu_{1/2} \sim 0.5$ Hz) revealed a large increase in signal intensity (>16 times) by effective decoupling of all proton–fluorine couplings in this drug. Mimicking the decoupled spectra to the spectra obtained at 2.35 T (by applying a 13 Hz line-broadening) gave an $\sim$6 times increase in signal intensity, which indicates that further improvements in decoupling efficiency are possible for the *in situ* setup.

Attempts were made on T_1 determinations at 2.35 T. By using a surface coil, the measurements were probably biased by a variation in flip angles over the sample volume.[14] Also, S/N was low in these experiments. The molecular weights imply short correlation times (CIP, M_W 331; DXM-P, M_W 516). Therefore, extreme narrowing conditions were assumed, implying that T_1 is independent of the magnetic field B_0.[17,18] Thus, data obtained at 11.8 T could be transferred to 2.35 T. A mistakenly estimated T_1 would diminish the value of S/N only to a small extent, as shown by Becker et al.[13]

CONCLUSIONS

In summary, the time-share decoupling method has been used for proton decoupled ^{19}F NMR spectroscopy on a Bruker BioSpec 2.35 T system. A surface coil was used for the ^{19}F signal and a birdcage coil for decoupling. The applicability of the time-share method was demonstrated when conventional decoupling failed to succeed. It was shown that the gain in S/N by this method is dependent upon the coupling pattern of the actual drug. At the lowest tested concentration, time-share ^{1}H decoupling gave a 56% increase in S/N for dexamethasone phosphate compared with the coupled

case. Thus, ^{1}H decoupling is advantageous for this drug. Decoupling did not enhance S/N for ciprofloxacin. With our setup, the lowest concentration for detection of fluorinated drugs was about 0.1–0.2 μmol ^{19}F. For monitoring drugs directly in the eye, however, refinement of the method is necessary to enable measurements of relevant drug concentrations. This includes optimization of the coil design, decoupling methods, and radiofrequency filters. Nevertheless, the method might advantageously be applied to studies at higher magnetic fields.

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