A Method for Rapid Evaluation of Saturation Factors in *In Vivo* Surface Coil NMR Spectroscopy Using B_1 -Insensitive Pulse Cycles

Rolf Gruetter,*† Chris Boesch, Ernst Martin and Kurt Wüthrich†

University Children's Hospital, MR Unit, Steinwiesstr. 75, 8032 Zürich, Switzerland

For a rapid determination of saturation factors in surface coil NMR spectroscopy, the recently published (90° - τ - 180° - τ - 90° - τ -) SUFIR sequence was modified with adiabatic pulse cycles. Signals were acquired after each of the two 90° pulses and they were stored in different memory blocks. The deleterious offset-dependent saturation effects inherent in adiabatic 90° excitation pulses were minimized by suitable combinations of adiabatic pulses that sweep from either side of the spectrum onto resonance, and by setting the carrier frequency in the middle of the spectrum. The practical use of the proposed method is illustrated with the determination of ^{31}P NMR saturation factors in human calf muscle.

INTRODUCTION

In vivo surface coil NMR spectroscopy¹ has become a powerful tool² for non-invasive studies of metabolism in perfused organs, intact animals and human tissue.³⁻⁵ Experiments with surface coils are often performed under conditions where the repetition time is comparable to or shorter than T_1 of the observed nuclei.^{4,5} As a consequence, if the longitudinal relaxation rates differ for the various observed metabolites, as is normally the case under *in vivo* conditions, the relative signal intensities may not faithfully reflect relative concentrations, unless the integrals are scaled with the corresponding saturation factors.^{4,6} As an alternative, fully relaxed spectra acquired with repetition times, $TR \geq 5T_1$ of the most slowly relaxing resonance may be used, but such spectra have a low effective S/N per unit time. In any case, information on T_1 values in the systems studied is desirable. Standard methods, such as the full inversion recovery or saturation recovery experiments include the acquisition of fully relaxed spectra and are too time consuming for everyday use.^{7,8} For this purpose we therefore looked for an alternative method that allowed saturation factors to be estimated without the need for long repetition times.

This paper reports the adaptation of a two-point inversion recovery sequence⁹ to surface coil NMR spectroscopy. The inherent sensitive response of the sequence to pulse imperfections and, as a consequence, to the intrinsic strong B_1 -variations of the surface coil, could be overcome with the use of

adiabatic excitation (half passage) and inversion (full passage) pulses.^{10,11} The strong off-resonance effects inherent in adiabatic excitation pulses were minimized by a special compensation scheme, so that the effects of the pulses on the experiment were to a good approximation B_1 -insensitive over the whole spectral range used. With this method we obtained T_1 values of all major ^{31}P signals at 2.35 T in the calf muscle of six healthy volunteers with only a few minutes measuring time per T_1 determination.

EXPERIMENTAL

All experiments were carried out on a Bruker 2.35 T horizontal bore magnet with 35 cm free access (Bruker-Spectrospin AG, Fällanden, Switzerland).¹² ^{31}P spectra were acquired using a double-tuned surface coil with 5.5 cm diameter operating at 100 MHz for ^1H and at 40.5 MHz for ^{31}P . The unloaded 90° pulse width in the coil center was 25 μs at 95 W power. Six adults aged between 27 and 38 years volunteered to have the ^{31}P spectra of their calf muscle measured. The carrier frequency was at -3 ppm relative to PCr during all *in vivo* experiments. For the analysis of these data, the two FIDs S_1 and S_2 (stored in memory regions 1 and 2, respectively, as shown in Fig. 1) were sensitivity-enhanced with a 5 Hz exponential multiplication, zero-filled, Fourier transformed, and phase corrected with identical parameters. We then determined interactively the ratio $\lambda = S_2/S_1$ for each individual line by minimizing the difference spectrum $S_2 - \lambda S_1$ on a monitor, using standard Bruker software. This procedure proved to be insensitive to a non-zero baseline.

The performance of the different pulse shapes used was calculated by numerically solving the Bloch equations with a fourth-order Runge-Kutta algorithm.

* Author to whom correspondence should be addressed at Magnetic Resonance Center (MRC 126), Department of Molecular Biophysics and Biochemistry, Yale University, 333 Cedar Street, PO Box 3333, New Haven, CT 06510, USA.

† Institut für Molekularbiologie und Biophysik der Eidgenössischen Technischen Hochschule, ETH-Hönggerberg, 8093 Zürich, Switzerland.

Figure 1. The pulse schemes discussed in the text. (a), The SUFIR sequence consists of a 180° inversion pulse bracketed by two 90° excitation pulses and was originally introduced with hard rectangular pulses.⁹ The delay τ ($\tau \gg T_2^*$) is constant, and the FIDs acquired in the steady state are alternately stored in different memory blocks 1 and 2. (b) The pulse sequence of (a) with B_1 insensitive pulses and with compensation cycles for the limited off-resonance performance of the excitation pulses; α denotes the conventional adiabatic pulse, where the magnetization is spin-locked parallel to B_{eff} ; β denotes the corresponding *antipulse*, during which the spin-locking field B_{eff} is antiparallel to the magnetization; the inversion pulse is indicated by θ .

Definition of saturation factors

Throughout this paper we define the saturation factor, SF , when using 90° excitation pulses and a repetition time TR , as

$$\frac{S_0}{S(TR)} = SF = (1 - e^{-TR/T_1})^{-1} \quad (1)$$

where $S_0 = S(5T_1)$ represents the signal obtained under fully relaxed conditions.

Review of the original SUFIR method

We have used a scheme that relied on the recently introduced basic pulse sequence depicted in Fig. 1(a).⁹ It consists of a 180° pulse sandwiched by two 90° pulses, with fixed interpulse delays τ . The signals S_1 and S_2 acquired after the 90° pulses in different memory blocks are given by:⁹

$$S_1 = S_0(1 - e^{-\tau/T_1}) \quad (2)$$

$$S_2 = S_0(1 - e^{-\tau/T_1})^2 \quad (3)$$

These expressions are valid if transverse interference between pulses can be neglected ($T_2^* \ll \tau$), if only single exponentials are present, and if steady state conditions are established. From Eqns (2) and (3) we can derive the longitudinal relaxation rate T_1 and the relaxed signal S_0 :

$$T_1 = \frac{-\tau}{\ln(1 - S_2/S_1)} \quad (4)$$

$$S_0 = \frac{S_1^2}{S_2} \quad (5)$$

Eqns (4) and (5) are valid if the pulses are exactly 90° and 180° , respectively. This is clearly not the case for the RF field B_1 of a surface coil, which is strongly space dependent, especially when simple rectangular

pulses are used. A remedy for this problem can be found by using pulses which are insensitive to the spatial inhomogeneity of the transmitting RF field, as is shown below.

Adiabatic pulses

Adiabatic pulses have been shown to produce inversion and excitation over a large range of B_1 values.^{10,11,13–15} We focus on two pulse shapes that we have been using.

For *inversion*, the hyperbolic secant pulse has been introduced as an analytical solution of the Bloch–Ricatti equation.^{16,17} It acts as a selective inversion pulse that inverts the magnetization above a threshold in B_1 . By numerically solving the Bloch equations with a fourth order Runge–Kutta algorithm, we have calculated the inversion performance of a pulse of the hyperbolic secant type as a function of resonance offset and B_1 field strength. The z -magnetization after the pulse, M_z^+ , is shown as a contour plot in Fig. 2(a).

Figure 2. Performance of B_1 -insensitive (adiabatic) pulses. Contour plots of the z -magnetization after the pulse, M_z^+ , are drawn with the levels indicated on the right. The horizontal axis (offset) represents the chemical shift dimension $\Delta\nu = \gamma\Delta B/2\pi$. The vertical axis spans a ten-fold variation in the peak RF field strength $\nu_1 = \gamma B_1/2\pi$. (a), The hyperbolic secant (sech) pulse used for inversion. The pulse was 6 ms in duration, $\gamma B_{1,\text{sech}}(t) = 2\pi\nu_1/\cosh(\beta t)^{1+\mu}$ with $\mu = 8$ and $\beta = 1400 \text{ Hz}$.¹⁷ The grid that was interpolated consisted of 11 calculated points in the frequency direction $\Delta\nu$ (offset) and 10 points in the RF dimension (peak power). The shaded area indicates the region where $M_z^+ < -0.95$. The pulse profile is deteriorated at high RF field strengths due to pulse truncation effects. (b), The numerically optimized sin/cos pulse used for excitation. The modulation function of the 3 ms pulse was numerically optimized for a range of peak RF amplitudes ν_1 of 1–10 kHz, starting with the RF field 5 kHz off resonance (in the terminology used by Ugurbil *et al.*,¹⁵ the parameters were $\nu = 0.2$ – 2 , $A = 5200$). The optimized offset range was 2 kHz. Note that the pulse does not perform plane rotations, but is designed just to *excite* the magnetization.^{13,14} Contour lines were interpolated on a grid of 15×10 calculated points.

Note that the inversion bandwidth is constant over a large range in B_1 field strength. At high B_1 values it deteriorates because of pulse cutoff effects caused by the finite pulse length.

For excitation of the magnetization through rapid adiabatic half passage the sin/cos pulse¹⁰ was used. We have optimized this pulse numerically using a previously described procedure.¹⁵ In the contour plot of M_z^+ (Fig. 2(b)) two remarkable features emerge at high peak RF values ($\gamma B_1/2\pi > 2$ kHz): first, exact excitation ($M_z^+ = 0$) is achieved only on resonance, and second, M_z^+ varies linearly with offset over a wide range of B_1 values.

A correction of off-resonance effects can be based on general considerations concerning the performance of adiabatic excitation pulses. In our experiments, such a pulse was generated by sweeping a frequency-modulated pulse onto resonance through phase-modulation.¹⁰ It is mathematically equivalent to the use of a B_0 -sweep similar to continuous wave spectroscopy, together with an amplitude-modulated RF pulse of constant frequency. If longitudinal and transverse relaxation are neglected, the magnetization then precesses in the rotating frame around the effective field B_{eff} . Magnetization parallel or antiparallel to B_{eff} is spin-locked and does not precess. If the orientation of B_{eff} is changed slowly compared to its magnitude, which is a statement of the adiabatic condition,¹⁸

$$\frac{\gamma B_1}{2\pi} = \nu_1 \gg \frac{1}{\nu_1} * \frac{d\Delta\nu}{dt} \gg T_1^{-1}, T_2^{-1} \quad (6)$$

the spin-locked magnetization will follow the direction of the spin-locking field B_{eff} . The left inequality in Eqn (6) requires a large RF field compared to the change in RF frequency offset, or resonance frequency offset $\Delta\nu$, respectively. The right inequality in Eqn (6) restricts the duration of the sweep relative to the longitudinal relaxation time T_1 and the transverse relaxation time T_2 .

We now consider the original sin/cos pulse¹⁰ in more detail. During this pulse, the magnitude of B_{eff} has a constant value A at a particular RF field strength, and the orientation of B_{eff} is rotated about an axis in the transverse plane at a constant angular frequency, i.e., for a pulse of total duration T the components of B_{eff} are given by¹⁰

$$\gamma/2\pi * (B_{\text{eff}})_z(t) = A \cos(\pi t/2T) \quad (7)$$

and

$$\gamma/2\pi * (B_{\text{eff}})_{xy}(t) = A \sin(\pi t/2T). \quad (8)$$

The magnetization will follow B_{eff} , if the conditions (Eqn (6)) are fulfilled. A 90° excitation is achieved at $t = T$, when B_{eff} passes through the transverse plane. An exact 90° excitation will occur not only for $B_1 = (B_{\text{eff}})_{xy}(T)$, but also for different RF field strengths.

At a certain offset relative to the resonance condition, the z component of B_{eff} is altered according to the value of the offset frequency ΔB (Fig. 3(a)). When the pulse is turned off, the effective field, B_{eff} , and correspondingly the magnetization, is tilted at an angle of $(90^\circ - \alpha)$ from the transverse plane, resulting

Figure 3. Offset performance of rapid adiabatic excitation pulses. The pulse is applied along $+x$ in the rotating reference frame. The trajectories of the magnetization at a particular resonance offset are shown as solid arrows on the unit sphere. The time course of the spin-locking field B_{eff} during the pulse is indicated with dashed arrows. (a) The magnetization is spin-locked parallel to B_{eff} , given for the example of the original sin/cos pulse as

$$\gamma B_{\text{eff}}/2\pi = (\gamma B_1/2\pi * \sin(\pi t/2T), 0, A \cos(\pi t/2T) + \gamma \Delta B/2\pi).$$

(b) The antipulse: the magnetization is spin-locked antiparallel to B_{eff} , correspondingly given as

$$\gamma B_{\text{eff}}/2\pi = (-\gamma B_1/2\pi * \sin(\pi t/2T), 0, -A \cos(\pi t/2T) + \gamma \Delta B/2\pi).$$

in a non-vanishing residual z -magnetization (Fig. 3(a)). The angle α is given by

$$\tan \alpha = B_1/\Delta B = \nu_1/\Delta\nu, \quad (9)$$

where $\Delta\nu$ denotes the resonance offset relative to the carrier frequency in Hz. The residual z -component of the magnetization can also be seen in Fig. 2(b). Thus, distinct signals may exhibit different, offset-dependent saturation effects due to their dissimilar starting conditions of the z -magnetization after the pulse, M_z^+ . Consider now an adiabatic pulse where the initial B_{eff} is aligned along $-z$ (Fig. 3(b)), which corresponds to the classical adiabatic sweep from the other side of the spectrum onto resonance. The magnetization M (which is initially aligned along $+z$) is spin-locked antiparallel to B_{eff} . We term such an adiabatic pulse, during which M is antiparallel to B_{eff} , an *antipulse*. At the end of the antipulse, B_{eff} is the same as for the adiabatic pulse, but the magnetization at a particular offset corresponds to a different flip angle β and is associated with a 180° phase shift, since it is spin-locked antiparallel to B_{eff} during the pulse. The residual z magnetization of the adiabatic excitation pulse is equal to the negative z magnetization ($\cos \alpha = -\cos \beta$) of its antipulse (Fig. 3), while the transverse components are the same for the two pulses ($\sin \alpha = \sin \beta$). In the sections that follow this feature is exploited to improve the offset performance of adiabatic excitation pulses with respect to saturation effects in the pulse sequence of Fig. 1(a). Note that the offset performance is *qualitatively* independent of the particular pulse used, since it was derived using only that the adiabatic conditions (Eqn (6)) must be satisfied.

Use of a cycle with antipulses

From the relationship $\cos \alpha = -\cos \beta$ between the conventional adiabatic pulse α and its antipulse β , a

pulse cycle can be constructed that minimizes off-resonance effects. We have concentrated on a scheme that relies on performing just one experiment with a minimal number of cycle elements. The basic experiment (Fig. 1(a)) was thus extended to six pulses as shown in Fig. 1(b). In terms of acquisition the sequence is still treated as a train of three pulses with the corresponding proper phase cycle.⁹

Using the relationships $\cos \alpha = -\cos \beta$ and $\sin \alpha = \sin \beta$ between the conventional adiabatic pulse and its antipulse, we can deduce the steady-state z -magnetizations prior to the excitation pulses and calculate the expected signal ratio S_2/S_1 . This calculation is given in Appendix A and yields for the scheme in Fig. 1(b), with $y = e^{-\tau/T_1}$,

$$\frac{S_2}{S_1} = (1 + y \cos \theta) \frac{1 + y^3 \cos^2 \alpha \cos \theta}{1 - y^3 \cos^2 \alpha \cos \theta}. \quad (10)$$

Assuming a perfect *inversion* pulse ($\theta = 180^\circ$), we can estimate the influence of the adiabatic *excitation* pulses, which is contained in the second factor of Eqn (10). For $\alpha = 90^\circ$ this factor is unity, otherwise it is always smaller than 1 and tends to yield rather overestimated T_1 values, which may help to reduce effects of slight 180° pulse imperfections. The y^3 term suggests that these effects will be important only at fast pulsing rates. For $\tau/T_1 > 0.5$ and $|\cos \alpha| < 0.5$, the error in S_2/S_1 introduced by the excitation pulses is smaller than 10%. Furthermore, the surface coil is most sensitive to those regions where the pulses perform best and S_2/S_1 deviates least from the desired value. Deeper lying tissue, for which $\cos \alpha$ deviates substantially from 0, contributes much less to the signal.

RESULTS

The filled squares in Fig. 4 (curve A) illustrate the extent to which the cycling scheme of Fig. 1(b) reduces the offset dependence of T_1 measurements. The open squares and open triangles in Fig. 4 (curves B and C) show that when using a single adiabatic pulse or a single antipulse, respectively, the T_1 measurements have a nearly linear dependence of T_1 on offset. The data points obtained with the adiabatic pulse resemble closely a mirror image, relative to $\Delta\nu = 0$, of the curve obtained only using the antipulse. These observations can be explained by Fig. 2(b) and 3, and are a typical feature of adiabatic excitation.

To test the practical performance of the sequence of Fig. 1(b) we applied it to various samples with different T_1 values and different coil loading effects. Based on the simulations presented in Fig. 2, we set the peak B_1 amplitude of the inversion pulse at 1.5 times the value of the excitation pulses. For large samples with strong loading effects, we obtained a constant T_1 value over a large range in pulse power. We have therefore used a fixed pulse power regardless of the sample being investigated. The peak RF power

Figure 4. Experimentally determined effects of the cycling scheme on the offset dependence of the T_1 measurements. (a) T_1 values were calculated from the two signals of Fig. 1(b) acquired with a delay $\tau = 335$ ms (filled squares). (b,c) The sequence was applied *without* cycling, using either one of the two adiabatic excitation pulses discussed in the text. The resulting T_1 values are strongly offset dependent (open squares and triangles). The sample was a bottle (3 cm in diameter and 5 cm in height) filled with a saturated solution of potassium phosphate in H_2O . Signal intensities were obtained from the peak heights of the magnitude spectra.

of the excitation pulses was 95 W. As a stringent test of the measurements performed with the scheme of Fig. 1(b) we compared fully relaxed spectra calculated with Eqn (5) with the corresponding experimentally measured fully relaxed spectra. The two spectra at the bottom of Fig. 5 show the two ^{31}P signals of a human calf muscle acquired in 4 min using the sequence in Fig. 1(b) with $\tau = 5.15$ s. The dashed line in the middle of Fig. 5 represents a spectrum calculated with Eqn (5) from the signals 1 and 2. Ideally, this spectrum would have no T_1 -dependent signal intensity distortions. The solid line, S_0 , displays a spectrum acquired under fully relaxed conditions with a simple Fourier transform experiment using the same adiabatic pulses for excitation as in the scheme of Fig. 1(b). Since the excitation profiles of both, S_0 and S'_0 , are influenced by the same off-resonance imperfections of the pulses ($\sin \alpha$ in Appendix A), these effects are cancelled in the difference spectrum shown at the top of Fig. 5, which contains only very small non-zero values.

We observed the same quality of agreement between calculated and measured relaxed spectra with $\tau = 2.65$ s, indicating a good performance of the excitation pulses in the experiment of Fig. 1(b) (data not shown).

As a further test of the reliability of the T_1 measurements, we measured longitudinal relaxation times of the major ^{31}P metabolites in the calf muscle of six volunteers with identical experimental parameters. The results of these measurements are listed in Table 1. The individual measurements were performed either in 2 min with interpulse delay $\tau = 2.65$ s, or in 4 min with $\tau = 5.15$ s. The experiments were alternated and repeated three times,

Figure 5. Prediction of fully relaxed spectrum. At the bottom the spectra of the signals S_1 and S_2 acquired in 4 min (16 averages with interpulse delay $\tau = 5.15$ s) are displayed. The spectrum S_0 acquired under fully relaxed conditions with a simple pulse-and-acquire experiment ($TR = 35$ s) is shown in the middle of the figure as a solid line. Using Eqn (5), S_0' (dashed line) was calculated for those points where both S_1 and S_2 were well above the noise level and gave positive T_1 values, otherwise S_0' was set to zero. For the points where S_0' was not zero the difference of the calculated S_0' to the measured S_0 is shown at the top. The same quality of agreement was observed at $\tau = 2.65$ s (data not shown). The spectra were baseline corrected by manually fitting a fourth order polynomial in the range of 30 ppm to -30 ppm relative to the PCr position. In the range from 10 ppm to -5 ppm, an additional broad signal hump was removed using the same procedure.

Table 1. T_1 measurements of ^{31}P metabolites at 2.35 T in resting calf muscles of six volunteers

τ^a		Longitudinal relaxation times (s)				
		P_i	PCr	γATP	αATP	βATP
2.65 s	T_1^b	7.6 ^c	6.6	3.7	3.0	3.5
	Standard deviation (SD)	0.8	0.4	0.3	0.2	0.4
	Mean SD ^d	3.5	0.43	0.62	0.44	0.94
5.15 s	T_1^b	6.5	6.5	4.9	3.3	4.1
	Standard deviation (SD)	0.5	0.3	0.4	0.2	0.2
	Mean SD ^d	1.5	0.15	0.83	0.36	0.69

Mean of the relative differences, %, of pairs of T_1 values (SD), $n = 18$

	$-10(45)$	$-2.3(6.0)$	$28(18)^e$	$10(17)^e$	$15(22)^e$
--	-----------	-------------	------------	------------	------------

^a τ is the delay in Fig. 1(b).

^b Average T_1 of six volunteers. T_1 values were obtained for each volunteer by averaging three measurements (see also text).

^c Poor signal-to-noise of $S_2(P_i)$ in the resting muscle resulted in an overestimation of T_1 .

^d The SD of the three measurements obtained with each volunteer has been averaged over all six subjects.

^e Considered as significant using a paired Student's t -test and supported by a sign test ($p < 0.05$) (see also text).

resulting in a total of 36 T_1 measurements. The three T_1 values obtained for each volunteer and each τ value were averaged, and the corresponding standard deviations were computed. The mean of the thus obtained six standard deviations is given in the third row of Table 1 for $\tau = 2.65$ s, and in the sixth row for $\tau = 5.15$ s, respectively. This procedure excludes interindividual differences. With the exception of γATP , we note an improvement of roughly 1.4 in accuracy when going from $\tau = 2.65$ s to $\tau = 5.15$ s.

When compared to the data obtained with $\tau = 2.65$ s, the longitudinal relaxation times T_1 of all three ATP signals are longer at $\tau = 5.15$ s (row 1 and row 4 of Table 1). To test whether these differences are significant, we calculated the mean of the paired differences. Using a paired Student's t -test on a 5% level, the values of the ATP signals obtained at $\tau = 5.15$ s were found to be significantly different from those obtained at $\tau = 2.65$ s (row 7 in Table 1). This finding was also supported by the sign test.

The T_1 values measured with $\tau = 5.15$ s are close to those reported previously at 1.5 T,⁶ except for the P_i peak, where the present measurements yielded a higher value.

DISCUSSION

The treatment of error propagation due to *stochastic errors* is given in Appendix B for Eqns (1), (4) and (5). As in the original publication on the experiment SUFIR,⁹ we find an acceptable variation in the relative accuracy of T_1 for τ in the range from 0.5 to 3 times T_1 . For most metabolites the accuracy of the measurement increased according to Eqn (B2) when the interpulse delay was doubled (Table 1).

From Eqns (B3) and (B4) we find that the relative error in the saturation factor SF is always smaller than the relative error in T_1 . For example, the saturation factors of αATP and βATP determined from the present T_1 measurements are twice as accurate as the T_1 (Table 1) for the example of a simple Fourier transform experiment with a repetition time TR of 6 s.

Since the presently described approach relies on evaluating two parameters T_1 and S_0 from two points along the relaxation curve, care must be exercised in its use because of *systematic errors* that may arise. A second measurement with a different interpulse delay τ may be helpful in assessing the sequence performance and in detecting deviations from the assumptions that form the basis of the method.

To detect relevant imperfections of the adiabatic pulses we examined T_1 values measured at $\tau = 2.65$ s and $\tau = 5.15$ s. T_1 of PCr is independent of τ (row 7 in Table 1), which indicates excellent performance of the sequence *in vivo*. In contrast, for the resonances of ATP we find significant differences ($p < 0.05$) between the relaxation times T_1 measured at $\tau = 2.65$ s and $\tau = 5.15$ s. These differences are less important for the corresponding saturation factors according to Eqn (B4), which may explain why excellent agreement was achieved at both τ values when the

fully relaxed spectra calculated from Eqn (5) were compared to the experimental, fully relaxed spectra (Fig. 5).

The difference was particularly large for the signal of γ ATP, although it was close to the carrier frequency where the experiment should perform best. Sequence performance may be impaired by imperfect inversion ($\theta < 180^\circ$) because of loss of magnetization during the inversion pulse due to T_2 . However, this could not account for all the observations, since in the aforementioned example of a human calf muscle the duration of the inversion pulse of 6 ms was short compared to T_2^* of ATP, as judged from the linewidths of the individual multiplett components (< 0.5 ppm) obtained without the 5 Hz exponential multiplication of the FID. Alternative explanations would be that the phosphorus nuclei of ATP have multiexponential relaxation characteristics, or that the T_1 measurements are influenced by chemical reactions, e.g., the creatine kinase reaction. The present use of the pulse sequence in Fig. 1 relies on the assumption of monoexponential longitudinal relaxation, and multiexponential relaxation can produce relaxation times with a complicated τ -dependence. It should be mentioned, however, that even in this situation the experiment of Fig. 1(b) is more efficient for determining saturation factors than inversion recovery (IR) or saturation recovery (SR) measurements, since the sensitivity obtained with a reasonable experimental time (typically below 1 h) is generally too low *in vivo* to allow the measurement of the necessary number of time-points required to fit a curve with more than one decay constant.

CONCLUSION

A method is presented that allows a rapid estimation of saturation effects. It is robust (coefficient of variation for PCr is of the order of 6%), fast (4 min measuring time) and compensates for off-resonance effects over the entire *in vivo* ^{31}P NMR spectrum at 2.35 T. Compared to earlier work,⁴ where the saturation factors were determined by recording fully relaxed spectra, the present approach of assessing the longitudinal relaxation time T_1 has the advantage that it gives results that can be applied with different TR without the need for recalibration, e.g., for comparison with published results. Although we demonstrated the method for ^{31}P , it is readily adaptable for surface coil spectroscopy with other nuclei, such as ^1H or ^{13}C , as long as adiabatic pulses are applicable and transverse interference is negligible, e.g., by quenching with gradient pulses. The presently used combination of two adiabatic pulses that sweep from either side of the spectrum onto resonance is of general importance. For example, in a simple Fourier transform experiment alternated application allows the carrier frequency to be set within the spectrum.

Acknowledgements

Financial support by the Swiss National Science Foundation (3.941–0.84 and 4.894–0.85.18), the Committee for the Promotion of Applied Scientific Research, the Kamillo-Eisner-Stiftung, Spectrospin, AG, Fällanden, and a special grant of the Eidgenössische Technische Hochschule are gratefully acknowledged.

REFERENCES

- Ackerman, J. J. H., Grove, T. H., Wong, G. G., Gadian, D. G. and Radda, G. K. Mapping of metabolites in whole animals by P-31 NMR using surface coils. *Nature* **283**, 167–170 (1980).
- Williams, S. R. and Gadian, D. G. Tissue metabolism studied *in vivo* by nuclear magnetic resonance. *Quart. J. Exp. Physiol.* **71**, 335–360 (1986).
- Cady, E. B., Costello, A. M. de L., Dawson, M. J., Delpy, D., Hope, P., Reynolds, E. O. R., Tofts, P. S. and Wilkie, D. R. Non-invasive investigation of cerebral metabolism in newborn infants by phosphorous nuclear magnetic resonance spectroscopy. *Lancet* **i**, 1059–1062 (1983).
- Hope, P. L., Costello, A. M. de L., Cady, E. B., Delpy, D. T., Tofts, P. S., Chu, A., Hamilton, P. A., Reynolds, E. O. R. and Wilkie, D. R. Cerebral energy metabolism studied with phosphorous NMR spectroscopy in normal and birth asphyxiated infants. *The Lancet* **ii**, 366–370 (1984).
- Boesch, C., Gruetter, R., Martin, E., Duc, G. and Wüthrich, K. Variations in the *in vivo* P-31 MR spectra of the developing brain during postnatal life. *Radiology* **172**, 197–199 (1989).
- Roth, K., Hubesch, B., Meyerhoff, D. J., Naruse, S., Gober, J. R., Lawry, T. J., Boska, M. D., Matson, G. B. and Weiner, M. W. Noninvasive quantitation of phosphorous metabolites in human tissue by NMR spectroscopy. *J. Magn. Reson.* **81**, 299–311 (1989).
- Weiss, G. H. and Ferretti, J. A. Optimal design of relaxation time experiments. *Prog. NMR Spectroscopy* **20**, 317–335 (1988).
- Blackledge, M. J. and Styles, P. Measurement of localized P-31 spin-lattice relaxation rates *in vivo* using PMRFI-IR. *J. Magn. Reson.* **83**, 390–399 (1989).
- Canet, D., Brondeau, J. and Elbayed, K. Superfast T_1 determination by inversion-recovery. *J. Magn. Reson.* **77**, 483–490 (1988).
- Bendall, M. R. and Pegg, D. T. Uniform sample excitation with surface coils for *in vivo* spectroscopy by adiabatic rapid half passage. *J. Magn. Reson.* **67**, 376–381 (1986).
- Baum, J., Tycko, R. and Pines, A. Broadband and adiabatic inversion of a two-level system by phase-modulated pulses. *Phys. Rev. A* **32**, 3435–3447 (1985).
- Boesch, C. and Martin, E. Combined Application of MR imaging and spectroscopy in neonates and children: installation and operation of a 2.35 T system in a clinical setting. *Radiology* **168**, 481–488 (1988).
- Ugurbil, K., Garwood, M. and Bendall, M. R. Amplitude- and frequency-modulated pulses to achieve 90° plane rotations with inhomogeneous B_1 fields. *J. Magn. Reson.* **72**, 177–185 (1987).
- Ugurbil, K., Garwood, M., Rath, A. R. and Bendall, M. R. Amplitude- and frequency/phase-modulated refocusing pulses that induce plane rotations even in the presence of inhomogeneous B_1 fields. *J. Magn. Reson.* **78**, 472–497 (1988).
- Ugurbil, K., Garwood, M. and Rath, A. R. Optimization of modulation functions to improve insensitivity of adiabatic pulses to variations in B_1 magnitude. *J. Magn. Reson.* **80**, 448–469 (1988).
- Silver, M. S., Joseph, R. I. and Hoult, D. I. Highly selective $\pi/2$ and π pulse generation. *J. Magn. Reson.* **59**, 347–351 (1984).
- Silver, M. S., Joseph, R. I. and Hoult, D. I. Selective spin inversion in nuclear magnetic resonance and coherent optics through an exact solution of the Bloch-Ricatti equation. *Phys. Rev. A* **31**, 2753–2755 (1985).
- Freeman, R. *A Handbook of Nuclear Magnetic Resonance*, pp. 1–4. Longman Harlow, Essex (1988).

Received 18 April 1990; accepted (revised) 11 July 1990.

APPENDICES

Appendix A: calculation of Eqn (10)

To illustrate the calculation of Eqn (10), we follow the time course of the z -magnetization of the six-pulse sequence of Fig. 1(b) under steady state conditions. Neglecting transverse interference ($T_2^* \ll \tau$), the calculation of the effect of any combination of pulses and relaxation periods on the longitudinal magnetization requires an alternating series of two mathematical operations.

1. An operator P_γ describes the effect of a pulse with a *nominal* flip angle γ on the z -magnetization as

$$M_z^+ = M_z^- \cos \gamma \equiv P_\gamma(M_z^-), \quad (\text{A1})$$

where the z -magnetization prior to the pulse is given by M_z^- and the z -magnetization after the pulse is denoted by M_z^+ .

2. The effect of a relaxation period τ on the initial z -magnetization $M_z(0)$ can be calculated from the Bloch equations to be

$$M_z(\tau) = (1 - e^{-\tau/T_1})M_0 + e^{-\tau/T_1}M_z(0). \quad (\text{A2})$$

Using a normalized thermal equilibrium magnetization ($M_0 = 1$) and with the abbreviation $y = e^{-\tau/T_1}$ we define the operator R_τ , describing the relaxation effects in Eqn (A2) as

$$M_z(\tau) = (1 - y) + y^* M_z(0) \equiv R_\tau(M_z(0)). \quad (\text{A2}')$$

The pulse train in Fig. 1(b) can be calculated using (A1) and (A2'). Starting with the z -magnetization m_{1a} prior to the first pulse (the corresponding signal is stored in memory block 1) we subsequently calculate the magnetization m_{2a} prior to the third pulse (antipulse, memory block 2) as

$$m_{2a} = R_\tau P_\theta R_\tau P_\alpha(m_{1a}),$$

and the magnetization prior to the fourth pulse (antipulse, memory block 1) is given through

$$m_{1b} = R_\tau P_\beta(m_{2a}).$$

In the same way we calculate the magnetization m_{2b} prior to the sixth pulse (memory block 2) as a function of m_{1b}

$$m_{2b} = R_\tau P_\theta R_\tau P_\beta(m_{1b}),$$

and obtain the z -magnetization m'_{1a} prior to the first pulse of the *next* cycle as

$$m'_{1a} = R_\tau P_\alpha(m_{2b}).$$

Steady state conditions lead to the equation

$$m_{1a} = m'_{1a}$$

which allows the explicit calculation of m_{1a} , m_{2a} , m_{1b} and m_{2b} . From the expressions for m_{2a} thus obtained,

$$m_{2a} = (1 - y) * \{(1 + y \cos \theta) + y^2 \cos \beta \cos \theta + y^3 \cos^2 \beta \cos \theta + y^4 \cos^2 \theta \cos^2 \beta + y^5 \cos \alpha \cos^2 \theta \cos^2 \beta\} / (1 - y^6 \cos^2 \alpha \cos^2 \theta \cos^2 \beta),$$

and m_{1a} ,

$$m_{1a} = (1 - y) * \{1 + y \cos \alpha + y^2 \cos \theta \cos \alpha + y^3 \cos \beta \cos \theta \cos \alpha + y^4 \cos^2 \beta \cos \theta \cos \alpha + y^5 \cos^2 \theta \cos^2 \beta \cos \alpha\} / (1 - y^6 \cos^2 \alpha \cos^2 \theta \cos^2 \beta), \quad (\text{A4})$$

m_{1b} and m_{2b} are obtained by interchanging α and β , since the pulse in Fig. 1(b) might equally well start with an antipulse. The flip angles of the conventional adiabatic pulse and its antipulse are linked through the relations

$$\cos \alpha = -\cos \beta, \quad \sin \alpha = \sin \beta.$$

The signals acquired in the memory blocks 1 and 2 are given by

$$S_1 = \sin \alpha(m_{1a} + m_{1b})$$

and

$$S_2 = \sin \alpha(m_{2a} + m_{2b}).$$

which results in the ratio S_2/S_1 given by Eqn (10), since the y^2 , y^4 and y^5 terms in the numerator of Eqns (A3) and (A4) cancel.

Appendix B: calculation of error propagation factors

Assuming the noise to be uncorrelated and the rms noise to be the same for both S_1 and S_2 , the error in T_1 can be calculated from Eqns (2–4) in units of the signal-to-rms-noise ratio $(S/\sigma)_0$ of the fully relaxed spectrum obtained with an identical number of scans (but not in the same experimental time). Using Gaussian error propagation we obtain for the relative error in T_1 ,

$$\frac{\sigma\langle T_1 \rangle}{T_1} = k_{T_1}(x) * \frac{1}{(S/\sigma)_0}. \quad (\text{B1})$$

The dimensionless *error propagation factor*, $k_{T_1}(x)$, can be expressed as a function of $x = \tau/T_1$ by

$$k_{T_1}(x) = \frac{e^x[(1 - e^{-x})^2 + 1]^{1/2}}{x(1 - e^{-x})}. \quad (\text{B2})$$

For a given repetition time TR , the signal intensities are saturated according to their T_1 values, and their intensities have to be multiplied with the saturation factor defined in Eqn (1) to yield the thermal equilibrium signal. The error propagation factor $k_{SF}(y)$ of the saturation factor SF is defined as

$$\frac{\sigma\langle SF \rangle}{SF} = k_{SF}(y) * \frac{\sigma\langle T_1 \rangle}{T_1} \quad (\text{B3})$$

with

$$k_{SF}(y) = \frac{y}{(e^y - 1)}, \quad (\text{B4})$$

where $y = TR/T_1$. Since $k_{SF}(y) < 1$, the relative error in SF is always smaller than the relative error in T_1 . The uncertainty of S_0 calculated through Eqn (5) is given by the error propagation factor $k_{S_0}(x)$ through

$$\frac{\sigma\langle S_0 \rangle}{S_0} = k_{S_0}(x) * \frac{1}{(S/\sigma)_0}, \quad (\text{B5})$$

with

$$k_{S_0}(x) = \frac{[(1 - e^{-x})^{-2} + 2]^{1/2}}{x}. \quad (\text{B6})$$