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Long repetition time experiments for measurement of concentrations in systems with chemical exchange and undergoing temporal variation–comparison of methods with and without correction for saturation

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Abstract

The purpose of this paper is to compare two methods for quantifying metabolite concentrations using the one-pulse experiment for a sample undergoing chemical exchange and subject to an intervention or other temporal variation. The methods, LATR-C (Long Acquisition TR (interpulse delay); Corrected for partial saturation) and LATR-NC (Long Acquisition TR; Not Corrected), are compared in terms of signal-to-noise ratio, SNR, per unit time and quantitation errors. Parameters relevant to the isolated perfused rat heart are used as a specific application, although the results are general. We assume throughout that spin–lattice relaxation times, T_1, do not change. For a given flip angle, θ, TR's are calculated which result in maximal SNR per unit time under 10%, 5%, and 1% constraints on quantitation errors. Additional simulations were performed to demonstrate explicitly the dependence of the quantitation errors on TR for a fixed θ. We find (i) if the allowed error is large, and when both metabolite concentrations and rate constants vary, LATR-C permits use of shorter TR, and hence yields greater SNR per unit time, than LATR-NC; (ii) for small allowed error, the two methods give similar TR's and SNR per unit time, so that the simpler LATR-NC experiment may be preferred; (iii) large values of θ result in similar constrained TR's and hence SNR per unit time for the two methods; (iv) the ratio of concentrations of metabolites with similar T_1 exhibit similar errors for the two methods.

1. Introduction

Measurement of concentrations is of importance in numerous NMR studies and is commonly performed using the one-pulse experiment analyzed by Ernst and Anderson (EA) in 1966 [1]. As shown by EA, the signal-to-noise ratio (SNR) per unit time of the experiment can be greatly improved using an interpulse delay, TR, which does not permit complete relaxation between pulses. The resulting partial saturation can be measured and used to correct subsequent concentration measurements.

Correction for partial saturation is typically performed in the following fashion. Consider a control period (Ctl), with metabolite magnetization M_0^{Ctl}, followed by an intervention period (Int), with metabolite magnetization M_0^{Int}. Throughout, concentrations are taken as directly proportional to magnetizations. Two spectra are acquired during Ctl, one with a short repetition time, TR^{S}, and flip angle $\theta \leqslant 90°$, selected for high SNR per unit time, and one with a long repetition time, TR^{L} permitting essentially complete relaxation, that is, $\mathrm{TR}^{\mathrm{L}} \cong 5 \times T_1$. The TR^{L} measurement is assumed to have a 90° flip angle in order to obtain optimal SNR per unit time and minimize measurement uncertainty, although this is not essential to the analysis. During Int, spectra with pulse parameters TR^{S} and θ are acquired. Letting $M_{\mathrm{obs}}(\theta, \mathrm{TR})$ denote the observed magnetization, the measured quantities are $M_{\mathrm{obs}}^{\mathrm{Ctl}}(\theta, \mathrm{TR}^{\mathrm{S}})$, $M_{\mathrm{obs}}^{\mathrm{Ctl}}(90°, \mathrm{TR}^{\mathrm{L}})$, and $M_{\mathrm{obs}}^{\mathrm{Int}}(\theta, \mathrm{TR}^{\mathrm{S}})$. Note that the following analysis requires a uniform flip angle throughout the sample in order that the measured resonance amplitudes for a given (TR, θ) may be unambiguously interpreted in

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terms of concentrations. This is typically achieved through use of a probe with a high degree of radio frequency field homogeneity, or, in the case of surface coils, through use of appropriate adiabatic pulses.

Regardless of whether or not chemical exchange (CE) is present, it has been shown [4] that

$$M_{\mathrm{obs}}^{\mathrm{Ctl}}(90^\circ, \mathrm{TR}^{\mathrm{L}}) = M_0^{\mathrm{Ctl}} \tag{1}$$

so that the saturation factor (SF) during Ctl is accurately given by

$$\mathrm{SF}^{\mathrm{Ctl}} = \frac{M_{\mathrm{obs}}^{\mathrm{Ctl}}(\theta, \mathrm{TR}^{\mathrm{S}})}{M_0^{\mathrm{Ctl}}}. \tag{2}$$

In contrast, $\mathrm{SF}^{\mathrm{Int}}$ is not obtained; this would require the lengthy measurement of $M_{\mathrm{obs}}^{\mathrm{Int}}(90^\circ, \mathrm{TR}^{\mathrm{L}})$, which is precisely what one wishes to avoid. Therefore, while strictly,

$$M_0^{\mathrm{Int}} = \frac{M_{\mathrm{obs}}^{\mathrm{Int}}(\theta, \mathrm{TR}^{\mathrm{S}})}{\mathrm{SF}^{\mathrm{Int}}} \tag{3}$$

the fully relaxed resonance amplitude during Int is instead estimated to be

$$M_0^{\mathrm{Int,estimated}} = \frac{M_{\mathrm{obs}}^{\mathrm{Int}}(\theta, \mathrm{TR}^{\mathrm{S}})}{\mathrm{SF}^{\mathrm{Ctl}}}. \tag{4}$$

This is strictly valid provided

$$\mathrm{SF}^{\mathrm{Ctl}} = \mathrm{SF}^{\mathrm{Int}}. \tag{5}$$

We now discuss the validity of this assumption.

The SF for an isolated resonance is given by EA as [1]

$$\mathrm{SF}(T_1; \theta, \mathrm{TR}) = \frac{(1 - \mathrm{e}^{-\mathrm{TR}/T_1}) \sin\theta}{(1 - \mathrm{e}^{-\mathrm{TR}/T_1} \cos\theta)} \tag{6}$$

assuming $\mathrm{TR} \gg T_2$ or spoiling transverse magnetization [2,3]. In this formulation, the validity of Eq. (5) requires only that T_1 remain unchanged between Ctl and Int.

The EA analysis does not incorporate CE, which is present in virtually all in vivo samples. More recently, we have treated the more general problem of a system with N species undergoing mutual CE and subjected to a repetitive one-pulse sequence [4]. We found that in the presence of CE, the SF of a resonance depends upon the T_1's and M_0's for all species in the exchange network as well as on the exchange rate constants (Eq. 34 of [4]). Therefore, when CE is present, Eq. (5) is assured to be valid only if all of these system parameters remain the same between the Ctl and Int periods; this is generally not the case in intervention experiments, so that an error in metabolite quantitation as determined by Eq. (4) will generally occur.

Two simple and practical methods of minimizing the error in quantitation due to the effects of CE are addressed in this paper. First, it has been demonstrated [4–7] that the error in the correction procedure defined by Eq. (4) is decreased for larger TR^{S}, although this results in a decrease in SNR per unit time [1,4]. This procedure with a relatively Long Acquisition TR will be designated the LATR-C method, with C denoting Corrected. Alternatively, a TR^{S} may be selected which is sufficiently large that $\mathrm{SF}^{\mathrm{Int}} \sim 1$, so that the metabolite concentration can be determined directly from the resonance amplitude without correction [4,5]. Again, this incurs a SNR per unit time penalty [1,4,5]. This procedure will be designated the LATR-NC method, with NC denoting Non-Corrected.

We define errors in quantitation during Int by [6,7]

$$\%\ \text{Error in } M_0^{\mathrm{Int(C\ or\ NC)}} = \frac{\left(M_0^{\mathrm{Int(C\ or\ NC)}} - M_0^{\mathrm{Int}}\right)}{M_0^{\mathrm{Int}}} \cdot 100, \tag{7}$$

where $M_0^{\mathrm{Int,C}}$ denotes the magnetization determined from LATR-C, and similarly for $M_0^{\mathrm{Int,NC}}$.

Both LATR-C and LATR-NC can be rendered arbitrarily accurate by increasing TR^{S} at the expense of SNR per unit time. For practical use, what must be determined is which of these two methods will provide superior SNR per unit time for a given degree of accuracy.

2. Simulations

We consider ^{31}P NMR spectroscopy of the three-site system comprised of PCr, γ-ATP, and P_i, in which phosphate transfer is mediated by the creatine kinase (CK) and ATP synthesis and hydrolysis reactions:

$$\mathrm{PCr} \underset{k_{\gamma\text{-ATP}\to\mathrm{PCr}}}{\overset{k_{\mathrm{PCr}\to\gamma\text{-ATP}}}{\rightleftarrows}} \gamma\text{-ATP} \underset{k_{\mathrm{P_i}\to\gamma\text{-ATP}}}{\overset{k_{\gamma\text{-ATP}\to\mathrm{P_i}}}{\rightleftarrows}} \mathrm{P_i}. \tag{8}$$

The values used for all simulations, as discussed in [7], were obtained from published data on the isolated perfused rat heart and are given in Table 1. The γ-ATP resonance is incorporated into the model, as it is in exchange with PCr and P_i and therefore affects these amplitudes. The β-ATP peak does not undergo significant exchange, and hence is, with respect to the errors under present discussion, a more accurate reflection of [ATP].

Table 1
Parameters used for the simulations

	T_1(PCr)	T_1(γ-ATP)	T_1(P_i)	M_0(PCr)	M_0(β-ATP)	M_0(P_i)	$k_{\mathrm{PCr}\to\gamma\text{-ATP}}$	$k_{\mathrm{Pi}\to\gamma\text{-ATP}}$
Heart								
Control	2.78	0.64	2.40	1.00	0.62	0.23	0.70	0.37
Ischemia	2.78	0.64	2.40	0.05	0.03	2.78	0.20	0.11

Therefore, it is the β-ATP resonance which we use for subsequent discussions of quantitation.

We first determined values of TR for flip angles of 30°, 60°, and 90° which give maximal root-mean-squared (RMS) SNR per unit time for the phosphorus-containing metabolites specified above, within the constraints of 10%, 5%, or 1% maximum quantitation error for each metabolite in the exchange network. For LATR-C, concentrations are accurately measured during Ctl, so that the error constraints apply only to concentrations during Int. For LATR-NC, on the other hand, the constraints apply to Ctl and Int individually. Note that the error due to CE for β-ATP is zero as it does not participate in the reaction scheme presented in Eq. (8). The SNR per unit time follows directly from the definition of SF's [4] and is

$$\text{SNR per unit time} \propto \text{SF}/\sqrt{\text{TR}}. \tag{9}$$

Because the duration of a control period is often dominated by set-up and preparation stabilization considerations, and because high time resolution is generally of more importance after an intervention than during a control period, our SNR analyses relate to the Int period.

We also investigated the sensitivity of quantitation errors to variation in TR. Errors were determined for $M_0^{\text{Int(C or NC)}}$(PCr), $M_0^{\text{Int(C or NC)}}$($\text{P}_\text{i}$), and the ratios $M_0^{\text{Int(C or NC)}}(\text{PCr})/M_0^{\text{Int(C or NC)}}(\text{P}_\text{i})$ and $M_0^{\text{Int(C or NC)}}(\text{PCr})/M_0^{\text{Int(C or NC)}}$(β-ATP). All simulations were performed using the Mathematica programming language (Wolfram Research, Champaign, IL).

3. Simulation results

3.1. Determination of TR with fixed error constraints

Table 2 shows the minimum values of TR required for magnetization determinations using LATR-C and LATR-NC for specified error constraints. Although the analysis was carried out with respect to the RMS SNR per unit time for the three resonances under consideration, results for SNR(PCr) per unit time are presented in Table 2 for concreteness. Because SNR per unit time is a decreasing function of TR, the minimum TR values calculated yield the maximal SNR per unit time for the given constraint. The literature values of TR = 2.1 s and $\theta = 60°$ [8] resulted in the greatest error, reaching 14% for PCr, but the largest SNR per unit time. In all cases, LATR-C permitted use of a smaller TR, and therefore resulted in larger SNR(PCr) per unit time, than LATR-NC. For each flip angle, the difference in the required TR, and hence in SNR(PCr) per unit time, between the two methods decreased with more stringent error constraints. Increasing θ also decreased the differences in the required TR between the two methods.

Table 2
Minimum values of TR required for specified error constraints using LATR-C and LATR-NC

θ	Percent error	TR (s)		SNR(PCr)/time	
		LATR-NC	LATR-C	LATR-NC	LATR-C
30°	10	2.08	0.89	0.66	0.89
	5	3.27	2.08	0.54	0.66
	1	6.83	6.24	0.38	0.40
60°	10	4.46	3.27	0.79	0.89
	5	5.92	5.25	0.70	0.74
	1	9.80	9.47	0.55	0.56
90°	10	5.84	5.05	0.81	0.85
	5	7.50	7.03	0.72	0.74
	1	11.6	11.2	0.59	0.60

The resulting SNR per unit time, SNR/time, was normalized to that obtained using the literature values of TR = 2.1 s, $\theta = 60°$.

3.2. Error magnitude with respect to variation in TR

Fig. 1 shows errors in $M_0^{\text{Int,C}}$(PCr) and $M_0^{\text{Int,NC}}$(PCr) with respect to TR. LATR-C yields significantly smaller errors as compared with LATR-NC at small values of TR. Values of TR greater than 6 s resulted in similar errors between the two methods. Increases in θ for fixed TR generally increased the systematic errors for both methods.

Fig. 2 shows errors in $M_0^{\text{Int,C}}(\text{P}_\text{i})$ and $M_0^{\text{Int,NC}}(\text{P}_\text{i})$ with respect to TR. Again, errors in $M_0^{\text{Int,C}}(\text{P}_\text{i})$ were significantly smaller than errors in $M_0^{\text{Int,NC}}(\text{P}_\text{i})$ for short TR, with the difference between the two methods decreasing for increasing TR.

Fig. 3 shows errors in $M_0^{\text{Int,C}}(\text{PCr})/M_0^{\text{Int,C}}(\text{P}_\text{i})$ and $M_0^{\text{Int,NC}}(\text{PCr})/M_0^{\text{Int,NC}}(\text{P}_\text{i})$ with respect to TR. Quantitation errors for the two methods are similar in magnitude, with errors resulting from use of LATR-NC being somewhat lower than those from LATR-C. Because the general trends for the errors in PCr and P_i shown in Figs. 1 and 2 are similar, the error in their ratio is sig-

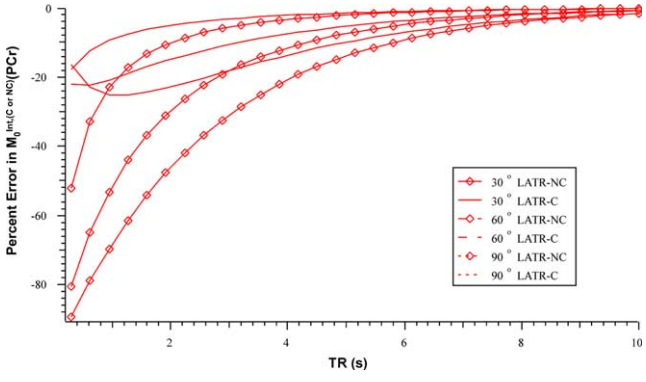

Fig. 1. Dependence on TR of quantitation errors in $M_0^{\text{Int,C}}$(PCr) and $M_0^{\text{Int,NC}}$(PCr).

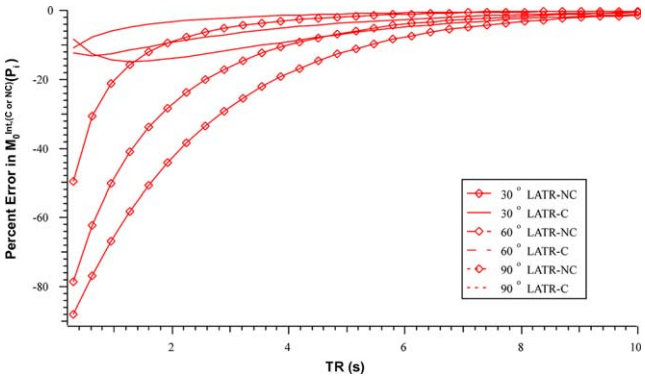

Fig. 2. Dependence on TR of quantitation errors in $M_0^{\mathrm{Int,C}}(\mathrm{P_i})$ and $M_0^{\mathrm{Int,NC}}(\mathrm{P_i})$.

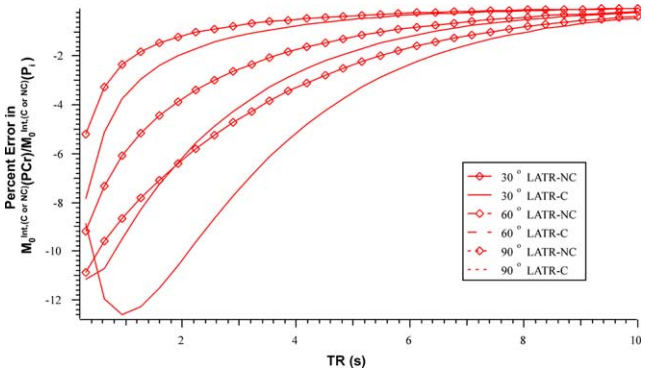

Fig. 3. Dependence on TR of quantitation errors in $M_0^{\mathrm{Int,C}}(\mathrm{PCr})/M_0^{\mathrm{Int,C}}(\mathrm{P_i})$ and $M_0^{\mathrm{Int,NC}}(\mathrm{PCr})/M_0^{\mathrm{Int,NC}}(\mathrm{P_i})$.

nificantly smaller. All ratio errors were below 15% for the range of TR and θ investigated.

Errors in $M_0^{\mathrm{Int,C}}(\mathrm{PCr})/M_0^{\mathrm{Int,C}}(\beta\text{-ATP})$ and $M_0^{\mathrm{Int,NC}}(\mathrm{PCr})/M_0^{\mathrm{Int,NC}}(\beta\text{-ATP})$ with respect to TR were also calculated (data not shown). As β-ATP does not participate in the reaction scheme Eq. (8), and complete relaxation is assumed to occur in the measurement of $M_0^{\mathrm{Ctl}}(\beta\text{-ATP})$, results for $M_0^{\mathrm{Int,C}}(\mathrm{PCr})/M_0^{\mathrm{Int,C}}(\beta\text{-ATP})$ are identical to those for $M_0^{\mathrm{Int,C}}(\mathrm{PCr})$. For LATR-NC, errors in β-ATP, while small, are nonzero, as full relaxation is not assumed to occur and partial saturation is not corrected. Thus, errors in $M_0^{\mathrm{Int,NC}}(\mathrm{PCr})/M_0^{\mathrm{Int,NC}}(\beta\text{-ATP})$ were similar to those in $M_0^{\mathrm{Int,NC}}(\mathrm{PCr})$.

4. Discussion

The one-pulse sequence is frequently performed under partially saturated conditions. As previously shown [6,7] correction for partial saturation in biological samples undergoing an intervention leading to changes in any of the system M_0's, T_1's or exchange rates can lead to systematic errors which can be substantial. In previous work, the magnitude of these errors was described for heart, skeletal muscle, and brain [7,9].

Although the use of long TR experiments both with and without correction for partial saturation has previously been proposed [4,6] as a way to decrease quantitation errors, previous work did not include a comparison of these two distinctly different approaches. Therefore, in this work we sought to determine the preferred experimental approach to accurate metabolite quantitation using the one-pulse experiment in the presence of CE.

Table 2 demonstrates that for more generous error constraints and smaller flip angles, correction for saturation, that is, use of LATR-C, permits the use of a substantially smaller TR than does LATR-NC, resulting in greater SNR per unit time. As the error constraint becomes more stringent or flip angle increases, LATR-C and LATR-NC require comparably large TR and hence result in comparable SNR per unit time. Therefore, the simpler LATR-NC experiment, in which near-complete relaxation permits direct measurement of accurate resonance amplitudes, may be preferred for precise measurements.

It has been previously shown [4,7] that the error in certain metabolite concentration ratios may be smaller than that for a single metabolite concentration. Accordingly, in the context of clinical spectroscopy, which often requires short TR in the interest of brevity, metabolite ratios may be more precise indices of pathology than measurements of individual metabolite concentrations. For the LATR-NC method in particular, the error in the ratio [PCr]/[$\mathrm{P_i}$] is significantly smaller than that of either metabolite individually. This is attributable to the fact that, for the example chosen, $T_1(\mathrm{PCr}) \approx T_1(\mathrm{P_i})$ so that the degree of saturation of the two is comparable. In the case of LATR-C, this is largely accounted for by the correction procedure so that the errors in the ratio [PCr]/[$\mathrm{P_i}$] and in the individual metabolites are much more similar. Likewise, in the case of the ratio [PCr]/[β-ATP], because $T_1(\mathrm{PCr}) \gg T_1(\beta\text{-ATP})$, LATR-NC results in much larger errors than does LATR-C.

Aside from the SNR and accuracy tradeoffs delineated above, LATR-C has the disadvantage of requiring the measurement of a metabolite ratio, leading to an effective increase in noise of approximately $\sqrt{2}$ (although this consideration does not apply to quantitation with respect to an external standard with high SNR). This, combined with the overall simplicity of the LATR-NC experiment and the fact that the SNR benefits of LATR-C are fairly modest, indicates that the LATR-NC experiment may be preferred for accurate quantitation in the presence of CE. In addition, T_1 changes are more accurately accounted for by LATR-NC than by LATR-C. Nevertheless, as shown in Figs. 1 and 2, when using a small TR in order to improve SNR per unit time,

LATR-NC results in much larger errors in individual metabolite measurements, so that LATR-C may then be preferred in this case.

While the qualitative trends presented here apply generally, the numerical results, based upon specific parameter choices, are clearly only representative.

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