Accuracy of Exchange Rate Measurement using CUPS, a Novel Method for Kinetic Analysis.

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Introduction: A number of NMR techniques are available for determining spin-lattice relaxation times (T_1's) and kinetic rate constants in exchanging systems. Selective inversion and transient saturation transfer (TST) experiments require selective rf irradiation, leading to problems with rf spillover, off resonance effects, and incomplete saturation. A new method for measuring chemical exchange (CE) rate constants using progressive saturation, CUPS, (chemical exchange measurement using progressive saturation) is analyzed here. CUPS is based on the influence of chemical exchange on observed magnetizations during the one-pulse experiment [1], and also provides T_1 values which are not confounded by chemical exchange. We performed a detailed evaluation of CUPS in the presence of noise and flip angle errors, and compared our results with errors in the transient saturation transfer (TST) technique for analysis of exchanging systems

Methods and Materials: *Experimental Data:* CUPS was evaluated in three systems exhibiting exchange among phosphocreatine (PCr), ATP, and inorganic phosphate (Pi), mediated by creatine kinase and ATP synthase, using ^{31}P-MRS: 2-site exchange *in vitro* and in the rat leg, [2, 3] and 3-site exchange in the rat heart using physiologic parameters as previously described [4].

Simulated Data: Input values appropriate for the above 2- and 3-site exchange networks were used to generate M_{obs}(TR), with TR's equal to 0.2, 0.6, 1.0, 2.5, 3.5, and 15 s. To assess the effects of noise, normally distributed Gaussian noise was added to achieve SNR ratios for M_0(PCr) of 100000, 100, 50, 25, 20, and 10. Errors due to inaccurate flip angles were evaluated by generating data with flip angles of 80°, 85 °, 90 °, 95 °, and 100 °, but performing the CUPS parameter analysis assuming a flip angle of 90°.

Data Fitting: MATLAB, with the optimization toolbox (Mathworks, Natick, MA) was used for all analyses. Fits were performed in two ways. Either all of the system M_0's, T_1's, and k's were determined from the fit, or the M_0's were taken as fixed from separate experiments at long TR. The former case results in a 5-parameter fit for the *in vitro* CK reaction and the rat muscle CK reaction, and an 8-parameter fit for the heart. The latter results in 3-, and 5-parameter fits, respectively.

Results and Discussion: Figure 1 is a plot of observed magnetizations, M_{obs}, as a function of time for the rat heart experiments, with CUPS-generated 8-parameter fits superimposed. Numerical results are shown in Table 1; the results for the 5- and 8-parameter fits were similar. A comparable analysis for the *in vitro* and *in vivo* CK reactions showed that in these systems as well, 3- parameter and 5-parameter fits yielded substantially the same results. The effect of noise on the CUPS and TST analyses of heart data is illustrated in Table 2. We found that in general, errors resulting from CUPS were comparable to those from TST. The analysis of flip angle errors in the heart experiments, as well as in the other two systems, showed CUPS had a significant sensitivity to flip angle. The resulting errors were in fact on the same order as errors in T_1's derived in non-exchanging systems using progressive saturation.

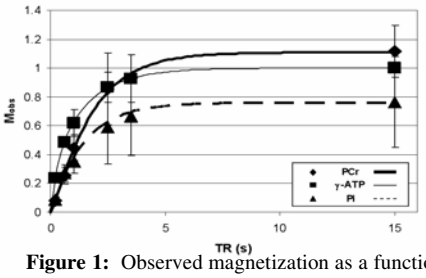

Figure 1: Observed magnetization as a function of TR and the corresponding model fit for metabolites in the isolated perfused rat **heart.**

		T_1(PCr) (s)	T_1(γ-ATP) (s)	T_1(Pi) (s)	$k_{PCr\rightarrow\gamma\text{-}ATP}$ (s^{-1})	$k_{Pi\rightarrow\gamma\text{-}ATP}$ (s^{-1})	M_0(PCr)	M_0(γ-ATP)	M_0(Pi)
Spencer et al., (4)		2.90	0.73	2.20	0.91	0.50			
CUPS	5	3.34	0.70	1.87	0.72	0.75	1.12	1.00	0.76
	8	3.35	0.69	1.83	0.75	1.04	1.11	1.00	0.73

Table 1: Analysis of bioenergetic reactions in the isolated perfused rat heart. The top row shows T_1(PCr), T_1(-ATP), T_1(Pi), $k_{PCr\rightarrow\ -ATP}$, $k_{Pi\rightarrow\ -ATP}$, M_0(PCr), M_0(-ATP), and M_0(Pi) as determined in Ref. (4). The second and third rows contain the results from CUPS experiments performed in the present work under similar conditions to those used in Ref. (4). Fits using 5 and 8 parameters, as described in the text, are shown.

Conclusion: We have analyzed a novel method, CUPS, for determining spin-lattice relaxation times and rate constants in chemically exchanging systems which uses only the one-pulse experiment. The analysis has been performed for three systems demonstrating the typical exchange reactions of bioenergetics as studied by ^{31}P NMR. CUPS performs comparably to TST in the presence of realistic SNR, but is much easier to implement experimentally. In the case of CUPS, flip angle errors may be reduced by, for example, application of adiabatic or composite pulses. The errors in CUPS due to limited SNR and flip angle imperfections compare favorably to the errors in TST due to limited SNR, incomplete and spillover saturation, and off-resonance effects [5]. Due to the simplicity and rapidity of data collection as compared to other methods for monitoring exchange rates, CUPS may be especially useful for evaluating chemical parameters *in vivo* and in certain slowly-varying dynamic systems.

SNR(PCr)	T_1(PCr) (s)	T_1(γ-ATP) (s)	T1(Pi) (s)	$k_{PCr\rightarrow\gamma\text{-}ATP}$ (s^{-1})	$k_{Pi\rightarrow\gamma\text{-}ATP}$ (s^{-1})	M_0(PCr)	M_0(γ-ATP)	M_0(Pi)
	2.90	0.73	2.20	0.91	0.50	1.08	1.00	0.71
100000	2.90±0.0	0.73±0.0	2.20±0.0	0.91±0.0	0.50±0.0	1.12±0.0	1.00±0.0	0.76±0.0
100	2.95±0.03	0.73±0.0	2.22±0.02	0.92±0.02	0.50±0.02	1.12±0.0	1.00±0.0	0.77±0.0
50	2.97±0.04	0.72±0.0	2.24±0.02	0.94±0.02	0.52±0.02	1.12±0.0	1.00±0.0	0.77±0.0
25	3.21±0.16	0.73±0.01	2.38±0.05	0.93±0.03	0.60±0.03	1.12±0.0	1.00±0.0	0.76±0.0
10	3.86±0.31	0.72±0.01	3.07±0.22	0.97±0.08	0.74±0.09	1.13±0.0	1.00±0.0	0.77±0.0

SNR(PCr)	T_1(PCr) (s)	$k_{PCr\rightarrow\gamma\text{-}ATP}$ (s^{-1})	M_0(PCr)
	6.60	0.200	3.50
100000	2.90±0.0	0.910±0.0	1.12±0.0
100	2.89±0.01	0.911±0.0	1.12±0.0
50	2.92±0.01	0.908±0.0	1.12±0.0
25	2.91±0.03	0.911±0.0	1.12±0.0
10	3.09±0.06	0.906±0.01	1.12±0.0

Table 2: CUPS (left) and TST (right) analyses of bioenergetic reactions in the isolated perfused rat heart in the presence of noise. The mean ± SEM of the indicated parameter values resulted from fits using CUPS and TST with simulated M_{obs} data. Results were generated from Eq. [30] in Ref. [1] using parameter values from Ref. (4) as shown in the top row. One hundred realizations of simulated data with additive Gaussian random noise, using baseline parameters as shown in the top row of each table, were analyzed for each specified value of SNR(PCr). A fit that resulted in a parameter that was negative or an order of magnitude larger than the true value was excluded from the calculation.

References:

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