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Quantitative chemical exchange saturation transfer (qCEST) MRI – Omega plot analysis of RF spillover-corrected inverse CEST ratio asymmetry for simultaneous determination of labile proton ratio and exchange rate

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Abstract

Chemical exchange saturation transfer (CEST) MRI is sensitive to labile proton concentration and exchange rate, thus allowing measurement of dilute CEST agent and microenvironmental properties. However, CEST measurement depends not only on the CEST agent properties but also on the experimental conditions. Quantitative CEST (qCEST) analysis has been proposed to address the limitation of the commonly used simplistic CEST-weighted calculation. Recent research has shown that the concomitant direct RF saturation (spillover) effect can be corrected using an inverse CEST ratio calculation. We postulated that a simplified qCEST analysis is feasible with omega plot analysis of the inverse CEST asymmetry calculation. Specifically, simulations showed that the numerically derived labile proton ratio and exchange rate were in good agreement with input values. In addition, the qCEST analysis was confirmed experimentally in a phantom with concurrent variation in CEST agent concentration and pH. Also, we demonstrated that the derived labile proton ratio increased linearly with creatine concentration ($P < 0.01$) while the pH-dependent exchange rate followed a dominantly base-catalyzed exchange relationship ($P < 0.01$). In summary, our study verified that a simplified qCEST analysis can simultaneously determine labile proton ratio and exchange rate in a relatively complex *in vitro* CEST system.

Keywords

Chemical Exchange Saturation Transfer (CEST); Omega Plot; Quantitative CEST (qCEST) Analysis

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1. INTRODUCTION

Chemical exchange saturation transfer (CEST) MRI is a relatively new imaging technique that is sensitive to dilute CEST agents and microenvironmental properties such as pH and temperature (1–4). Recent studies have extended CEST imaging to investigate protein and pH changes in disorders such as acute stroke, tumor, and multiple sclerosis (5–11).

However, the experimentally obtainable CEST effect varies not only with labile proton concentration and exchange rate, but also with RF irradiation power, T_1 , and T_2 (12). As such, the commonly used CEST-weighted asymmetry analysis is oversimplified and there is a definitive need to develop quantitative CEST (qCEST) analysis for characterization of the underlying CEST properties (13,14).

Mathematical tools—including Bloch-McConnell equations, saturation time, and power dependence—have been established to determine labile proton ratio and exchange rate from conventional CEST-weighted information (15–22). Specifically, Dixon et al. demonstrated that the proton exchange rate can be determined independently of the paramagnetic CEST (PARACEST) agent concentration from the X-intercept of a plot of steady-state CEST intensity divided by the reduction in signal caused by CEST irradiation versus inverse RF power levels (i.e., $M_z/(M_0 - M_z)$ vs. $1/B_1^2$), and dubbed it the “omega plot” (23). However, because diamagnetic CEST (DIACEST) agents have relatively small chemical shifts, they are more susceptible to direct RF saturation (spillover) effects. We have shown that RF power-dependent CEST analysis may differentiate the labile proton ratio from the exchange rate (24,25). However, this approach relies on non-linear fitting of an empirical solution and is susceptible to multi-parametric non-linear fitting errors. Whereas the RF spillover effect could be estimated to correct for the simplistic asymmetry analysis, it requires accurate measurements of bulk water T_1 , T_2 , labile proton ratio, and exchange rate, which can be challenging without a priori knowledge (26,27).

The goal of the present study was to develop a simplified yet accurate CEST quantification algorithm with which to solve the underlying CEST system. Specifically, recent studies have shown that the cross-term normalized (MTR_{Rex}) calculation corrects for the RF spillover effects (28,29). We applied an omega plot to analyze the RF spillover-corrected CEST effect for simultaneous determination of CEST agent concentration and exchange rate. To demonstrate this, we first simulated the classical 2-pool CEST effect using Bloch-McConnell equations and showed that omega plot analysis of CEST ratio (CESTR) calculated from conventional asymmetry analysis is prone to substantial error. Our study extended omega plot analysis to the RF spillover-corrected CEST effect, allowing accurate determination of labile proton ratio and exchange rate. We further validated the modified omega plot analysis in a relatively complex CEST phantom with concurrent variation in Creatine concentration and pH. Both the labile proton ratio and the exchange rate were determined from the modified omega plot analysis. Despite the differences in both Creatine concentration and pH, the proposed qCEST analysis yielded a labile proton ratio that was linearly proportional to Creatine concentration ($P < 0.01$) and dominantly base-catalyzed chemical exchange rate ($P < 0.01$). The simplified qCEST analysis resolves the underlying CEST system with full determination of labile proton ratio and exchange rate, augmenting the commonly used CEST-weighted analysis.

2. THEORY

Studies have shown (14,30) that the normalized signal intensity of CEST imaging can be described by

$$\frac{I}{I_0} = \left(1 - \frac{R_{1w} \cos^2 \theta}{R_{1\rho}}\right) \cdot e^{-R_{1\rho} \cdot TS} + \frac{R_{1w} \cos^2 \theta}{R_{1\rho}} \quad (1)$$

where TS is the saturation time, R_{1w} is the intrinsic longitudinal relaxation rate, and $\theta = \arctan(\omega_1 / \omega)$ with ω_1 and ω are the RF irradiation amplitude and offset, respectively. In addition, $R_{1\rho}$ is the longitudinal relaxation rate in the rotating frame,

$$R_{1\rho} = R_{1w} \cos^2 \theta + \left(R_{2w} + \frac{f_r \cdot k_{sw} \cdot \alpha}{(\omega_1 / \delta_s)^2 + (k_{sw} / \delta_s)^2} \right) \sin^2 \theta \quad (2)$$

where R_{2w} is the bulk water transverse relaxation rate, δ_s is the labile proton offset and α is

the labeling coefficient $\alpha = \frac{\omega_1^2}{p \cdot q \cdot (1 + (\Delta\omega - \delta_s) / p)^2 + \omega_1^2}$, in which $p = r_{2s} - \frac{k_{sw} k_{ws}}{r_{2w}}$, $q = r_{1s} - \frac{k_{sw} k_{ws}}{r_{1w}}$, $k_{ws} = f_r \cdot k_{sw}$, $r_{1w,s} = R_{1w,s} + k_{ws,sw}$ and $r_{2w,s} = R_{2w,s} + k_{ws,sw}$ (31). In addition, f_r and k_{sw} are the labile proton ratio and exchange rates, and R_{1s} and R_{2s} are the labile proton longitudinal and transverse relaxation rates, respectively.

We have $\alpha \approx \frac{\omega_1^2}{p \cdot q + \omega_1^2}$ for the label scan ($\omega = \delta_s$) and $\alpha = \frac{\omega_1^2}{p \cdot q \left(1 + 4 \left(\frac{\Delta\omega}{p}\right)^2\right) + \omega_1^2} \approx 0$ for the reference scan ($\omega = -\delta_s$). The steady state signal can be shown to be

$\frac{I^\pm}{I_0} = R_{1w} \cos^2 \theta / R_{1\rho}^\pm$, where $R_{1\rho}^+$ and $R_{1\rho}^-$ are $R_{1\rho}$ for the label scan and reference scans, respectively. We have

$$R_{1\rho}^+ = R_{1w} \cos^2 \theta + \left(R_{2w} + \frac{f_r \cdot k_{sw} \cdot \alpha}{(\omega_1 / \delta_s)^2 + (k_{sw} / \delta_s)^2} \right) \sin^2 \theta \quad (3.a)$$

$$R_{1\rho}^- = R_{1w} \cos^2 \theta + R_{2w} \sin^2 \theta \quad (3.b)$$

To overcome the confounding direct RF saturation, a cross term-normalized CEST ratio solution has been proposed (28,29). Whereas Zaiss et al. denoted this as MTR_{ReX} , we use inverse CEST difference ($CESTR_{\text{ind}}$) here specifically to point out the inverse difference calculation, in contrast to conventional asymmetry analysis, which takes their difference without inversion. Specifically, we have

$$\begin{aligned}
 \text{CESTR}_{\text{ind}} &= \frac{I_0}{I_{\text{label}}} - \frac{I_0}{I_{\text{ref}}} = \frac{R_{1\rho}^+}{f_r \cdot k_{\text{sw}} \cdot \alpha} - \frac{R_{1\rho}^-}{R_{1w} \cos^2 \theta} \\
 &= \frac{R_{1w} \cos^2 \theta}{\frac{(\omega_1/\delta_s)^2 + (k_{\text{sw}}/\delta)^2 \sin^2 \theta}{f_r \cdot k_{\text{sw}} \cdot \alpha}}} \quad (4) \\
 &= \left(\frac{f_r \cdot k_{\text{sw}} \cdot \alpha}{R_{1w}} \right) \cdot \tan^2 \theta
 \end{aligned}$$

For slow exchange rate (i.e., $k_{\text{sw}} \ll \delta_s$) and RF amplitude substantially smaller than labile

proton chemical shift, we have $\tan^2 \theta \approx (\omega_1/\delta_s)^2$. We have $\text{CESTR}_{\text{ind}} = \frac{f_r \cdot k_{\text{sw}} \cdot \alpha}{R_{1w}}$, which eliminates the confounding RF spillover effect. Moreover, for dilute CEST agents undergoing slow and intermediate chemical exchange, the labeling coefficient can be

simplified as $\alpha \approx \frac{\omega_1^2}{\omega_1^2 + k_{\text{sw}} \cdot r_{2s}}$, and the relationship between $1/\text{CESTR}_{\text{ind}}$ and $1/\omega_1^2$ can be described by linear regression as

$$\frac{1}{\text{CESTR}_{\text{ind}}} \approx \frac{R_{1w}}{f_r \cdot k_{\text{sw}}} + \frac{k_{\text{sw}} \cdot (R_{2s} + k_{\text{sw}}) \cdot R_{1w}}{f_r \cdot k_{\text{sw}}} \cdot \frac{1}{\omega_1^2} \quad (5)$$

The intercept and slope of the linear regression from Eq. 5 can be shown to be

$$C_0 = \frac{R_{1w}}{f_r \cdot k_{\text{sw}}} \quad (6.a)$$

$$C_1 = k_{\text{sw}} \cdot (R_{2s} + k_{\text{sw}}) \cdot C_0 \quad (6.b)$$

If we assume that R_{2s} can be reasonably estimated, both the labile proton ratio and exchange rate can be determined.

$$k_{\text{sw}} = \frac{\sqrt{R_{2s}^2 + 4 \cdot C_1/C_0} - R_{2s}}{2} \quad (7.a)$$

$$f_r = \frac{R_{1w}}{k_{\text{sw}} \cdot C_0} \quad (7.b)$$

3. MATERIALS AND METHODS

Numerical Simulation

We simulated CEST MRI using a 2-pool exchange model in MATLAB (Mathworks, Natick MA) (16,32), assuming representative T_1 and T_2 of 2 s and 200 ms, respectively, for the bulk water, and 1 s and 20 ms for labile protons at 2 ppm at 4.7 T (25,33). We simulated a typical labile proton ratio and exchange rate of 1:1000 and 100 s^{-1} for a representative chemical shift of 2 ppm at 4.7 T. To evaluate the accuracy of the solution, we also simulated qCEST analysis for representative ranges of T_1 , T_2 , labile proton ratio, and exchange rate.

Phantom

We prepared gadolinium-doped (30 μM) phosphate-buffered saline (PBS, Sigma Aldrich, St Louis, MO), and varied both the creatine concentration and pH. Specifically, the creatine concentration started at 100 mM and was serially diluted to 80, 60, 40, and 20 mM, with the titrated pH starting at 6.4 and moving to 6.5, 6.7, 6.8, and 7.1, respectively. The solution was then transferred to NMR tubes and the tubes were sealed and inserted counterclockwise into a phantom holder. The phantom holder was filled with 1% agarose gel to minimize susceptibility mismatch.

MRI

The experiments were conducted using a 4.7-T small-bore Bruker scanner (Bruker Biospec, Billerica, MA). We acquired multi-parametric MRI with single-shot echo planar imaging (EPI) readout (slice thickness = 5 mm, field of view (FOV) = 52×52 mm, image matrix = 96×96 , bandwidth=227 kHz) under room temperature. For CEST imaging, we varied the RF irradiation amplitude by increments of 0.5 μT from 0.5 to 2.5 μT , with label and reference offsets set to ± 1.9 ppm (375 Hz at 4.7 T). The repetition time (TR)/saturation time (TS)/echo time (TE) were 10 s/5 s/47 ms, respectively, and the number of signal average (NSA) was 2 (scan time ~ 1 min). We also acquired Z-spectra from -2.5 to 2.5 ppm with intervals of 0.05 ppm (TR/TS/TE=10 s/5 s/47 ms, NSA=1, scan time ~ 17 min) under representative RF irradiation power levels of 0.5, 1, and 2 μT . We used asymmetric spin echo (ASE) MRI for mapping B_0 inhomogeneity, with echo times shifted by 1, 3, 5, and 7 ms (TR/TE=10s/54 ms, NSA=2, scan time ~ 1 min 36 s). In addition, T_1 - and T_2 -weighted images were obtained using the inversion recovery sequence with seven inversion intervals (TI) from 0.1 to 7.5 s (recovery time (Tr)/TE=10 s/25 ms, NSA=2, scan time ~ 3 min), and spin-echo EPI (five TEs from 50 to 500 ms, TR=10 s, NSA=2, scan time ~ 2 min).

Data Processing

Images were processed in MATLAB (Mathworks, Natick, MA). CESTR was calculated as $\text{CESTR} = (I_{\text{ref}} - I_{\text{label}})/I_0$. $\text{CESTR}_{\text{ind}}$ was calculated as given in Eq. 4, where I_{ref} and I_{label} are the reference, label scans with RF irradiation applied on the reference and labile proton frequency shift, respectively. I_0 is the control scan without RF irradiation. The B_0 map was derived by fitting the phase map (ϕ) against the off-centered echo time (τ) using

$$\Delta B_0 = \frac{2\pi}{\gamma} \frac{\phi}{\Delta\tau}. \text{ The } T_1 \text{ map was obtained by least-squares fitting of the signal intensities (I)}$$

as a function of inversion time ($I = I_0 [1 - (1 + \eta) e^{-\text{TI}/T_1}]$), where η is the inversion efficiency and I_0 is the equilibrium signal. The T_2 map was derived by fitting the signal intensity as a function of the echo time, $I = I_0 e^{-\text{TE}/T_2}$. The results were reported as mean $\pm$ standard deviation and P-values less than 0.05 were considered statistically significant.

4. RESULTS

Fig. 1 shows simulated Z-spectra for three RF power levels -0.5 , 1 , and $2 \mu\text{T}$ – for a representative chemical shift of 2 ppm, with a labile proton ratio and exchange rate of 1:1000 and 100 s^{-1} , respectively. A CEST effect can be easily observed at 2 ppm for a B_1

level of 0.5 μ T. However, the Z-spectral intensity decreased at higher RF power levels due to greater saturation and the CEST effect at 2 ppm became less apparent (Fig. 1a). Indeed, the CEST effect, measured using the conventional CEST ratio (CESTR), initially increased with the RF power level, peaking and then decreasing for RF power levels greater than 1 μ T (Fig. 1b). It is important to note that because $\text{CESTR}_{\text{ind}}$ compensates for the RF spillover effect, it consistently increased with RF power level (Fig. 1b). Indeed, it overlapped with and could not be resolved from the spillover effect-corrected CEST effect (gray dashed line) estimated from the empirical solution, with a $0.01 \pm 0.015\%$ difference between the two (15). Furthermore, the omega plot analysis showed a poor linear relationship between the $1/\text{CESTR}$ and $1/B_1^2$ due to the RF spillover effect, with the normalized labile proton ratio and exchange rate with respect to the input values being 0.92 and 0.72, respectively. In contrast, the omega plot analysis of $\text{CESTR}_{\text{ind}}$ showed a normalized labile proton ratio and exchange rate of 0.99 and 1.01, respectively. Because RF irradiation amplitude is more often given in μ T, we used B_1 instead of ω_1 , as this can be easily converted (i.e. $\omega_1 = 2\pi\gamma B_1$, where γ is the gyromagnetic ratio).

Fig. 2 shows experimentally measured Z-spectra from two representative RF power levels: 0.5 and 2 μ T. At the weak RF power level (i.e., 0.5 μ T), the CEST effect increased with Creatine concentration and decreased with pH (Fig. 2a). At 2 μ T, the CEST effect showed very little difference between vials (Fig. 2b). This can be seen in both the CESTR (Fig. 2c) and $\text{CESTR}_{\text{ind}}$ (Fig. 2d) maps. While $\text{CESTR}_{\text{ind}}$ was approximately the same as CESTR at lower power levels, it increased consistently with RF power level and became substantially higher than CESTR at 2.5 μ T (Fig. 1b).

In Fig 3, we evaluated CESTR and $\text{CESTR}_{\text{ind}}$ as a function of B_1 level. Specifically, Fig. 3a shows that, for the compartment with high Creatine concentration and low pH (i.e. 100mM, pH=6.4), CESTR quickly increased with RF power level, peaked between 1 and 1.5 μ T, and then decreased at high RF power levels. In contrast, for the compartment with 20 mM Creatine at a pH of 7.1, CESTR did not peak until power levels higher than 2 μ T (Fig. 3a). This is consistent with our prior finding that the optimal RF power level increases with exchange rate and hence pH (25). In contrast, we found that $\text{CESTR}_{\text{ind}}$ consistently increased with RF level, suggesting that $\text{CESTR}_{\text{ind}}$ calculation effectively compensates for the RF spillover effect (Fig. 3b). Indeed, Fig. 3c shows that the modified omega plot analysis can accurately describe RF power-dependent $\text{CESTR}_{\text{ind}}$ ($R^2 = 0.997 \pm 0.003$).

The labile proton ratio and exchange rate maps were determined from a modified omega plot analysis of $\text{CESTR}_{\text{ind}}$, per pixel. Despite concurrent variation of Creatine concentration and pH in each vial, we can distinguish the labile proton ratio difference (Fig. 4a) from the pH-dependent exchange rate variation (Fig. 4b). Indeed, the labile proton ratio linearly scales with Creatine concentration (Fig. 4c), which is given by $f_{\text{r_fit}} = 0.02 \times 10^{-3} \cdot [\text{Cr}] + 0.15 \times 10^{-3}$ ($R^2 = 0.998$, $P < 0.001$), where $[\text{Cr}]$ is Creatine concentration in mM. Notably, the 95% confidence interval of the slope was 1.90×10^{-5} to 2.22×10^{-5} , in good agreement with the estimated labile proton ratio $f_{\text{r}} = 1.82 \times 10^{-5} \cdot [\text{Cr}]$ (25,27). In addition, Fig. 4d shows that the amine proton exchange rate can be described by a dominantly based-catalyzed chemical exchange formula (i.e., $k = k_0 + k_b \cdot 10^{\text{pH} - \text{pKw}}$), with $k_0 = 7.0$, $k_b = 1.4$ and $\text{pKw} = 4.8$ ($R^2 = 1.000$, $P < 0.001$), in good agreement with previous findings (25,34).

5. DISCUSSION

Our study demonstrated that the RF spillover-corrected CEST calculation can effectively compensate for direct RF saturation that confounds the commonly used asymmetry calculation (i.e., CESTR). Thus, an omega plot analysis of the RF spillover-corrected inverse CEST asymmetry calculation (CESTR_{ind}) allows simultaneous determination of the labile proton ratio and the exchange rate. To our knowledge, this is the first experimental demonstration of qCEST analysis in a complex CEST system with concurrent variation of CEST agent concentration and pH.

The B_1 -dependent spillover factor can be estimated using an empirical solution. However, this requires accurate measurement of a number of parameters, including T_{1w} and T_{2w} . While T_{1w} is not sensitive to dilute labile protons undergoing slow and intermediate chemical exchange, T_{2w} is more susceptible to chemical exchange (35). To address this, prior qCEST studies were limited to CEST systems of serially varied CEST agent concentration so the bulk water T_2 could be extrapolated from the measurements (25). This could be cumbersome in systems where neither CEST agent concentration nor pH was known. Indeed, due to the concurrent variation of Creatine content and pH, phantom T_{1w} (1.97 ± 0.07 s) and T_{2w} (1.38 ± 0.14 s) showed no significant correlation with Creatine concentration. In addition, the coefficient of variation (COV) was calculated from multiple vials. COV for T_{2w} was substantially larger than for T_{1w} (10.4% vs. 3.8%), consistent with the observation that T_{2w} is more susceptible to the underlying CEST system (35). Because the inverse CESTR calculation corrects for the RF spillover effect without T_{2w} estimation, it substantially simplifies qCEST post-processing. It is important to note that determination of R_{2s} is necessary for solving the labile proton ratio and the exchange rate from the linear regression analysis (Eq. 7). Our simulation showed that as long as R_{2s} can be reasonably estimated, the underlying CEST system could be quantified using the proposed qCEST analysis. It is necessary to note that we used an RF irradiation time of 5 s in our study. For typical relaxation rates, labile proton ratio and exchange rate of the creatine phantom, the

transient term (i.e. $\left(1 - \frac{R_{1w} \cos^2 \theta}{R_{1\rho}}\right) \cdot e^{-R_{1\rho} \cdot TS}$) is attenuated over 90% for a typical B_1 of 1.5 μ T. In addition, Taylor series expansion shows that the inverse CESTR calculation (Eq. 4) approaches the steady state faster than the reference and label signals independently (Appendix). Therefore, an irradiation time of 5 s is sufficient to reach the steady state for the proposed qCEST analysis. It is important to point out that our study indicates the number of exchangeable protons per Creatine molecule is 2, in contrast to around 4 protons per molecular reported by another group (29,34). Although Creatine has four exchangeable protons, the carboxyl group exchange rate is likely too fast for CEST detection. In addition, because two predicted pKa (<http://www.chemaxon.com/>) for creatine are 3.5 (-COOH) and 12.4 (guanidino group), only two protons from the guanidino moiety could be measured with CEST MRI. This is because the two protons of -NH₂ group cannot undergo exchange simultaneously. In addition, our simulation confirmed that for CW-CEST MRI, the proposed qCEST analysis provides accurate labile proton ratio and exchange rate determination (Fig. 1). Therefore, the difference in the number of exchangeable protons per Creatine molecule is likely attributable to differences in phantom preparation, RF irradiation scheme (continuous

wave vs. pulse-train) and quantification algorithms, which is beyond the scope of our work and could be further investigated.

To assess the accuracy of qCEST analysis, we further simulated qCEST solutions as a function of labile proton ratio, exchange rate, and bulk water T_{1w} and T_{2w} (Fig. 5). We assumed typical B_1 from 0.25 to 2 μ T. The simulations showed that the normalized labile proton ratio (Fig. 5a) and exchange rate (Fig. 5b) were reasonably accurate for typical labile proton ratio and exchange rate, except for very slow exchange when the assumption that $k_{sw} \gg R_{1s}$ may not be valid (Eq. 5). In addition, Figs. 5c and 5d show good measurement for typical T_{1w} and T_{2w} , except at very short T_{2w} . This is likely because the inverse CEST asymmetry calculation only corrects for the spillover effect up to the first order and higher order correction terms may be needed for very short T_{2w} , which is prone to severe RF spillover effects.

Because CEST measurement is susceptible to field inhomogeneity, we collected a B_0 field map and found B_0 inhomogeneity to be -2 ± 1 Hz. In addition, we used a volume coil with an inner diameter of 72 mm to image samples within a relatively small phantom, with a diameter of 25 mm. Therefore, B_1 inhomogeneity was within 5% for the region of interest and no field correction was necessary during post-processing (36). For cases where field inhomogeneity is non-negligible, post-processing can be applied to minimize such artifacts. Briefly, B_0 inhomogeneity can be corrected for using interpolation or empirical solution-based approaches, and B_1 inhomogeneity can be corrected for per pixel based on a B_1 field map (36–38). Also, it would be interesting to test how emerging pulse sequences, sampling schemes, and processing routines can further enhance the sensitivity and improve the accuracy of qCEST analysis (39–42).

Our study demonstrated qCEST *in vitro*. Further investigation is needed to validate this work *in vivo*. Specifically, asymmetry analysis is commonly used during *in vivo* CEST/APT imaging and is susceptible to concomitant changes in T_1 , T_2 , magnetization transfer (MT), and the nuclear overhauser effect (NOE), providing CEST-weighted information (43,44). Therefore, *in vivo* CEST measurement is complex and subject to exchange processes from multiple pools, including amide, amine, and hydroxyl groups (45,46). It should be noted that substantial progress has been made in delineating the CEST effect from concomitant MT and NOE effects (47–50). Whereas pulsed-RF irradiation has been commonly implemented on clinical scanners, the relationship between continuous wave (CW) – and pulsed-RF irradiation has been elucidated (51–53). Moreover, recent advances have enabled pseudo-CW irradiation on clinical scanners (54). In summary, our work verified qCEST analysis in a relatively complex CEST system with simultaneous CEST agent concentration and pH variation. Further technical advancement and improved understandings of *in vivo* CEST imaging are needed to translate and establish qCEST MRI *in vivo*.

6. CONCLUSIONS

Our study demonstrated qCEST analysis in a relatively complex CEST system with concurrent modulation of CEST agent concentration and pH, determining both the labile proton ratio and the exchange rate using a modified omega plot analysis. The simplified

qCEST analysis augments conventional CEST-weighted calculation and thus promises to advance CEST MRI as a quantitative molecular imaging modality.

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7. APPENDIX

We derived the transient solution of $\text{CESTR}_{\text{ind}}$. Briefly, we have

$$\frac{I^{\pm}}{I_0} = e^{-R_{1\rho}^{\pm} \cdot \text{TS}} + \frac{R_{1w} \cos^2 \theta}{R_{1\rho}^{\pm}} \left(1 - e^{-R_{1\rho}^{\pm} \cdot \text{TS}} \right) \quad (\text{A.1})$$

The saturation time-dependent $\text{CESTR}_{\text{ind}}$ can be shown to be

$$\begin{aligned} \text{CESTR}_{\text{ind}} &= \frac{1}{e^{-R_{1\rho}^{+} \cdot \text{TS}} + \frac{R_{1w} \cos^2 \theta}{R_{1\rho}^{+}} \left(1 - e^{-R_{1\rho}^{+} \cdot \text{TS}} \right)} - \frac{1}{e^{-R_{1\rho}^{-} \cdot \text{TS}} + \frac{R_{1w} \cos^2 \theta}{R_{1\rho}^{-}} \left(1 - e^{-R_{1\rho}^{-} \cdot \text{TS}} \right)} \\ &= \frac{R_{1\rho}^{+}}{R_{1w} \cos^2 \theta} \cdot \frac{1}{1 - \left(1 - \frac{R_{1\rho}^{+}}{R_{1w} \cos^2 \theta} \right) e^{-R_{1\rho}^{+} \cdot \text{TS}}} - \frac{R_{1\rho}^{-}}{R_{1w} \cos^2 \theta} \cdot \frac{1}{1 - \left(1 - \frac{R_{1\rho}^{-}}{R_{1w} \cos^2 \theta} \right) e^{-R_{1\rho}^{-} \cdot \text{TS}}} \quad (\text{A.2}) \\ &\approx \frac{R_{1\rho}^{+}}{R_{1w} \cos^2 \theta} \cdot \left(1 + \left(1 - \frac{R_{1\rho}^{+}}{R_{1w} \cos^2 \theta} \right) e^{-R_{1\rho}^{+} \cdot \text{TS}} \right) - \frac{R_{1\rho}^{-}}{R_{1w} \cos^2 \theta} \cdot \left(1 + \left(1 - \frac{R_{1\rho}^{-}}{R_{1w} \cos^2 \theta} \right) e^{-R_{1\rho}^{-} \cdot \text{TS}} \right) \\ &\approx \frac{R_{1\rho}^{+} - R_{1\rho}^{-}}{R_{1w} \cos^2 \theta} + \left[\frac{R_{1\rho}^{+}}{R_{1w} \cos^2 \theta} \left(1 - \frac{R_{1\rho}^{+}}{R_{1w} \cos^2 \theta} \right) e^{-R_{1\rho}^{+} \cdot \text{TS}} - \frac{R_{1\rho}^{-}}{R_{1w} \cos^2 \theta} \left(1 - \frac{R_{1\rho}^{-}}{R_{1w} \cos^2 \theta} \right) e^{-R_{1\rho}^{-} \cdot \text{TS}} \right] \end{aligned}$$

For dilute CEST agents undergoing slow and intermediate exchange, we have

$R_{1\rho}^{+} \approx R_{1\rho}^{-} \approx R_{1\rho}$, and $\text{CESTR}_{\text{ind}}$ can be approximated by

$$\text{CESTR}_{\text{ind}} \approx \frac{R_{1\rho}^{+} - R_{1\rho}^{-}}{R_{1w} \cos^2 \theta} \cdot \left(1 + \left(1 - \frac{R_{1\rho}}{R_{1w} \cos^2 \theta} \right) e^{-R_{1\rho} \cdot \text{TS}} \right) \quad (\text{A.3})$$

Specifically, the transient term is further modulated by $\left(1 - \frac{R_{1\rho}}{R_{1w} \cos^2 \theta} \right)$ from that of the label and reference scans independently. For typical experimental conditions, this term is reasonably close to 0.

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Fig. 1.

RF irradiation level-dependent CEST measurement. a) Simulated Z-spectra and asymmetry plots for three representative RF irradiation levels of 0.5, 1 and 2 μT . b) The CEST ratio (CESTR) calculated from asymmetry analysis and the inverse CEST ratio difference (CESTR_{ind}) as a function of B_1 level. c) Omega plot analysis of $1/\text{CESTR}$ vs. $1/B_1^2$ (Squares) and $1/\text{CESTR}_{\text{ind}}$ vs. $1/B_1^2$ (Circles).

Fig. 2.

CEST measurement from a creatine phantom. a) CEST Z-spectra and asymmetry plots under B_1 of 0.5 μT . b) CEST Z-spectra and asymmetry plots under B_1 of 2 μT . c) CESTR maps as a function of B_1 level. d) $\text{CESTR}_{\text{ind}}$ maps as a function of B_1 level.

Fig. 3. Experimental qCEST analysis. a) CESTR as a function of B_1 level. b) $\text{CESTR}_{\text{ind}}$ as a function of B_1 level. c) Omega plot analysis of the cross term normalized CEST ratio (i.e. $1/\text{CESTR}_{\text{ind}}$ vs. $1/B_1^2$).

Fig. 4. Experimental validation of qCEST analysis. a) Pixel-wise mapping of labile proton ratio. b) Pixel-wise mapping of labile proton chemical exchange rate. c) The labile proton ratio as a function of Creatine concentration. d) The numerically solved chemical exchange rate as a function of pH.

Fig. 5.

Evaluation of the accuracy of qCEST analysis. a) The normalized labile proton ratio as a function of labile proton ratio and exchange rate. b) The normalized exchange rate as a function of labile proton ratio and exchange rate. c) The normalized labile proton ratio as a function of T_{1w} and T_{2w} . d) The normalized exchange rate as a function of T_{1w} and T_{2w} .