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pCEST: Positive Contrast Using Chemical Exchange Saturation Transfer

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Abstract

Chemical Exchange Saturation Transfer (CEST) contrast utilizes selective pre-saturation of a small pool of exchanging protons and subsequent detection of the decrease in bulk water signal. The CEST contrast is negative and requires detection of small signal change in the presence of a strong background signal. Here we develop a Positive CEST (pCEST) detection scheme utilizing the analogous nature of the CEST and off-resonance $T_{1\rho}$ experiments and exploring increased apparent relaxation rates in the presence of the selective pre-saturation. pCEST leads to the positive contrast, i.e. increased signal intensity as the result of the presence of the agent and RF pre-saturation. Simultaneously substantial background suppression is achieved. The contrast can be switched “ON” and “OFF”, similar to the original CEST.

Keywords

CEST; PARACEST; positive CEST; off-resonance $T_{1\rho}$

1. Introduction

Chemical exchange saturation transfer (CEST) can be used to modify MR image contrast [1-4]. This approach is based on saturating a small pool of exchanging protons, endogenous or exogenous, and subsequent observation of the reduction of the water signal due to the exchange of the saturated spins with water. In a typical CEST experiment using small organic molecules, slowly exchanging –NH or –OH groups are pre-saturated via the application of selective RF irradiation, CW or pulsed. The CEST approach offers a variety of attractive features: it can be switched on and off at the operator's discretion; and, it can

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provide amplification so that some metabolites can be detected even when present at concentrations that are not usually observable in MRI [5]. Since the RF irradiation is applied at the characteristic frequency of an exchanging proton, multiple functional groups can be imaged simultaneously or sequentially [6]. Finally, the microenvironment of the functional group can affect CEST contrast. This has been exploited to image differences in pH or the presence of local metabolites [1,3-5,7-23].

There are two categories of CEST agents: (i) endogenous or exogenous diamagnetic molecules containing functional groups with exchanging protons such as –OH or –NH, in mobile proteins, peptides or small organic molecules – DIACEST [5,8,10-15,23]; and (ii) exogenous paramagnetic lanthanide (III) complexes containing exchanging –NH group or water molecules that exchange from the coordination sphere of the lanthanide – PARACEST [16,24-26]. PARACEST agents have a number of potential advantages. Because of larger frequency shifts between the exchanging resonance(s) and the water resonance, PARACEST agents can exhibit a wide range of exchange life times (from μsec to msec) and remain in the slow-to-intermediate exchange regime on the NMR time scale [25,27-31]. PARACEST agents with rapid exchange rates theoretically allow detection of much lower concentrations than typical for DIACEST agents. PARACEST agents have also been designed to report important biological indices, including: pH [16,17,32], temperature [18,33], metal ions [34,35], lactate [26] and glucose concentrations [19], and enzyme activity [20,21]. They have also been used for protein [36] and cell labeling [22]. The generation of the contrast effect may, however, require higher RF powers for PARACEST than DIACEST, potentially leading to SAR limitations in *in-vivo* studies.

CEST contrast is negative, i.e., the water proton signal *decreases* in tissue areas of interest. Typical changes in intensity that must be detected are small, on the order of 5%. When such small signal changes must be detected, background noise and background artifacts can strongly influence the observed effect. Background suppression techniques are widely used in MRI. In particular, Arterial Spin Labeling (ASL) employs background suppression to improve image quality [37-39]. ASL is of particular relevance to CEST since both techniques need to detect small signal changes and both employ pair-wise image subtraction: “on”/“off” images in the CEST case and “tagged”/“nontagged” in the ASL case. It was shown in the ASL case, that despite the background signal subtraction, instabilities in the background signal can still add substantial noise to the difference image [37]. These artifacts can be reduced by the suppression of the background signal. Such suppression in CEST studies will allow better utilization of the dynamic range to collect only a “useful” signal, i.e., the small signal originating from the contrast agent of interest. In ASL, background suppression is achieved by the application of multiple inversion pulses [37-39], a technique that is not directly applicable to CEST. Since CEST contrast is negative, it cannot be detected if the overall signal is suppressed. Positive CEST contrast is essential to employ simultaneous background suppression.

There are several examples where contrast agents or techniques originally developed to create negative contrast were re-designed to create positive contrast. One example is superparamagnetic iron oxide (SPIO) particles [40-44]. Originally, these particles were imaged using negative contrast generating sequences, but more recently, techniques generating positive contrast were created and are now used widely [40-44].

Here, we describe a general approach to create positive CEST signal (pCEST). The method utilizes a modification of the off-resonance spin-lock experiment. It uses saturation-transfer induced changes in relaxation rates to generate positive contrast. This concept was briefly mentioned by Ward *et al* [3], but, to our knowledge, has never been realized in practice. The method employs an inversion pulse followed by a saturation pulse and leads to a substantial

reduction of the background signal. In this paper, the method is presented in detail and demonstrated *in-vitro* with PARACEST agents.

2. Theoretical Background

In this section we will overview concepts and theoretical background relevant to the proposed detection scheme. The chemical exchange of magnetization between two pools, A and B, can be described by the Bloch-McConnell equations. In the presence of RF irradiation, the equations in the RF rotating frame are given by [45]:

$$\begin{aligned}
 \frac{d}{dt} M_x^A &= -\rho_{2A} M_x^A - \Delta_A M_y^A + k_B M_x^B \\
 \frac{d}{dt} M_y^A &= \Delta_A M_x^A - \rho_{2A} M_y^A + \omega_1 M_z^A + k_B M_y^B \\
 \frac{d}{dt} M_z^A &= -\omega_1 M_y^A - \rho_{1A} M_z^A + k_B M_z^B + R_{1A} M_0^A \\
 \frac{d}{dt} M_x^B &= -\rho_{2B} M_x^B - \Delta_B M_y^B + k_A M_x^A \\
 \frac{d}{dt} M_y^B &= \Delta_B M_x^B - \rho_{2B} M_y^B + \omega_1 M_z^B + k_A M_y^A \\
 \frac{d}{dt} M_z^B &= -\omega_1 M_y^B - \rho_{1B} M_z^B + k_A M_z^A + R_{1B} M_0^B
 \end{aligned} \tag{1}$$

where

$$\begin{aligned}
 \rho_{1A} &= R_{1A} + k_A, \rho_{2A} = R_{2A} + k_A \\
 \rho_{1B} &= R_{1B} + k_B, \rho_{2B} = R_{2B} + k_B \\
 \Delta_{A,B} &= \omega_{A,B} - \omega_{RF}
 \end{aligned}$$

In these equations, ω_{RF} and ω_I are the frequency, and amplitude of the RF irradiation (in rad units), respectively; Δ_A is the chemical shift offset from RF frequency for pool A, R_{1A} ($=1/T_{1A}$) is the spin lattice relaxation rate of pool A; R_{2A} ($=1/T_{2A}$) is the transverse relaxation rate of pool A; τ_A is the mean lifetime of a proton in A; and, $k_A = 1/\tau_A$ is the transition rate of a nuclei leaving pool A. Similar definitions apply to B. In the following we will designate the free water as pool A and the agent-bound water as pool B. The value of k_A is defined by the detailed-balance relationship:

$$\begin{aligned}
 k_A &= \left(\frac{M_0^B}{M_0^A} \right) k_B = p_B k_{ex} \\
 k_B &= \left(\frac{M_0^A}{M_0^B} \right) k_A = p_A k_{ex} \\
 k_{ex} &= k_A + k_B
 \end{aligned} \tag{2}$$

M_0^A and M_0^B are the equilibrium magnetizations and p_A and p_B are the fractional populations of pools A and B, respectively (with $p_A = M_0^A / (M_0^A + M_0^B)$ and $p_B = M_0^B / (M_0^A + M_0^B)$). The chemical shift difference between the two pools is $\Delta\omega = \omega_B - \omega_A$. Our representation is in the RF rotating frame; hence, when the RF frequency is applied on-resonance with pool B: $\omega_{RF} = \omega_B$ and $\Delta_B = 0$. We designate this case (Figure 1, panel a) by “RF ON”. “RF OFF” will designate the case when the RF is applied at the frequency $-\omega_B$ with respect to water (i.e., $\omega_{RF} = \omega_A - \Delta\omega$, $\Delta_A = \Delta\omega$ and $\Delta_B = 2\Delta\omega$).

The solution of Eq. 1 yields magnetization as a function of the experimental RF saturation parameters: off-resonance values (Δ_A , Δ_B), intensity (ω_I) and the RF pulse length (T_{pw}). In a typical CEST experiment, a detection RF pulse is applied after the saturating RF pulse and the Z-magnetization is measured (Figure 1, panel b). The intensity, I , measured in the experiment is dependent on the three above mentioned RF parameters and is normalized by the signal intensity at the absence of RF preparation, I_0 . The display of I/I_0 as a function of

RF frequency with respect to water ($\Delta_{RF} \equiv \omega_{RF} - \omega_A$), at fixed ω_I and T_{pw} , is called the Z-spectrum. These saturation transfer experiments can be transient, i.e. with $T_{pw} < T_{1a}, T_{1b}$, or steady-state, with $T_{pw} \gg T_{1a}, T_{1b}$. Before translation to imaging, these experiments were widely known and used extensively in dynamic studies of solutions of various small molecules and proteins (e.g. see Ref.[46-52]).

In an off-resonance spin-lock experiment, the RF is applied and the relaxation times are measured parallel ($T_{1\rho} = 1/R_{1\rho}$) or perpendicular ($T_{2\rho} = 1/R_{2\rho}$) to the effective field [53-55]. The dynamics of magnetization, which are described by Eq. 1, are analogous for CEST and off-resonance $T_{1\rho}$ (or $T_{2\rho}$) experiments. The experiments differ in the initial condition, observable operator and whether the observation is transient or in steady-state. In a CEST experiment, the initial magnetization is always parallel to the Z-axis while in the $T_{1\rho}(T_{2\rho})$ experiment it is parallel (perpendicular) to the effective field. For CEST, I/I_0 is typically measured at a single fixed T_{pw} , while in the $T_{1\rho}(T_{2\rho})$ experiment the measurement is performed as a function of T_{pw} in order to calculate $T_{1\rho}(T_{2\rho})$. Yet, the time constants governing the experimental dynamics are the same and can be derived by solving Eq.1. Thus, the analytical expressions derived and used in connection with $T_{1\rho}(T_{2\rho})$ experiments can be applied to CEST as well. Such solutions will be used to qualitatively understand the experimental results presented below. The similarities between the spin-lock and CEST experiments were recently explored in greater detail by Jin *et. al.* [56].

A full solution of Eq.1 would result in a set of six characteristic time constants, corresponding to the eigenvalues of the problem. Unfortunately, a full analytical solution does not exist. Many approaches have been used to derive approximate analytical solutions for relaxation in exchanging systems during RF irradiation. In the dilute species and slow exchange regime, the expression for relaxation rate constant parallel to the effective field can be written [53]:

$$R_{1\rho} = R_{1A} \cos^2 \theta + (R_{2A} + R_{ex}) \sin^2 \theta \quad (3)$$

where

$$R_{ex} = \frac{p_A p_B \Delta \omega^2 k_{ex}}{\Delta_B^2 + \omega_1^2 + k_{ex}^2}$$

and $\theta = \arctan(\omega_1 / \Delta_A)$. This expression assumes: (i) an asymmetric population limit, $p_A \gg p_B$, and (ii) exchange rates much faster than relaxation rates ($k_{ex} \gg R_1, R_2$). Both assumptions are met with PARACEST agents. The expression predicts that the maximum contribution from exchange to the $R_{1\rho}$ occurs when the RF is applied on-resonance with pool B, i.e., $\Delta_B = 0$.

Our method, shown schematically in Figure 1, panel c, is a hybrid between the CEST and the off-resonance $T_{1\rho}$ experiment. Here, the inversion pulse was always used to prepare the magnetization so the initial magnetization was not always aligned with the effective field, as is required in $T_{1\rho}$ experiment. Also, the detection measures magnetization along the Z axis (like CEST) and not along the effective field. For reasons that will become apparent later, this method is named positive CEST (pCEST). In pCEST, the initial alignment changes with the off-resonance value and goes from anti-parallel (far from water resonance) to perpendicular (on-resonance with water) to the effective field. As discussed earlier, the rates governing these dynamics are the same for pCEST and for the spin-lock experiment. It should be noted that in the pCEST experiments shown here, the data fit well to a mono-exponential function. Since the magnetization was not prepared to detect pure $R_{1\rho}$ or $R_{2\rho}$,

the time constant measured here will be referred to as R_{Iapp} . As long as the initial magnetization is aligned with the saturating (spin-lock) field, i.e. for $\Delta_A > \omega_I$, R_{Iapp} is approximately equal to $R_{I\rho}$ and the Eq.3 is valid.

Based on Eq.3, the apparent relaxation rate with RF ON, $R_{Iapp}(RF\ ON)$, is greater than the relaxation rate with RF OFF, $R_{Iapp}(RF\ OFF)$ (I.e. the apparent relaxation time is shorter with RF ON). This difference will be utilized to generate positive contrast. The timing of the irradiation, T_{pw} , is adjusted to null the water signal when RF OFF: $T_{pw} = T_{null}(RF\ OFF)$. Changing the off-resonance irradiation to RF ON will result in a shorter apparent relaxation time, and, hence, a small positive signal at T_{null} . This is schematically illustrated in Figure 2. It should be emphasized that at the $T_{null}(RF\ OFF)$ (as well as at $T_{null}(NO\ RF)$) the system has not yet reached the steady-state. The method presented here relies on the transient state; this is one of its distinguishing features from standard CEST.

The steady state values of magnetization also change as a function of off-resonance frequency. The steady-state magnetization is smaller with RF ON than with RF OFF (Figure 2). The amount of the steady-state magnetization depends on a several parameters: p_B , τ_B , R_{IA} and R_{IB} and ω_I [28,57]; with ω_I the most relevant for the experiments described here. Hence, positive CEST signal requires adjustment of T_{null} and ω_I , as described in more detail in Section 5.2.

3. Quantitative Measures

Typically, the CEST magnitude is defined quantitatively as:

$$CEST = \frac{I(RF\ OFF) - I(RF\ ON)}{I_0} \times 100\%. \quad (4)$$

Note that this definition does not reflect a negative nature of the contrast. We will use it in order to remain in agreement with the accepted nomenclature.

Similarly, the change in the apparent relaxation rate will be described by:

$$\Delta R_{Iapp} = R_{Iapp}(RF\ ON) - R_{Iapp}(RF\ OFF). \quad (5)$$

Following Eq.(4), pCEST magnitude is defined as a positive number:

$$pCEST = \frac{I(RF\ ON) - I(RF\ OFF)}{I_0} \times 100\% \quad (6)$$

4. Methods

A diagram of the pulse sequence used in spectroscopy and imaging experiments is shown in Figure 1, panel c.

The sequence was tested using two PARACEST agents: a 10 mM solution of EuDOTA(glyOEt)₄³⁺ was used in spectroscopy; 0, 0.1, 1 and 10 mM solutions of EuDOTA(gly)₄⁻ in imaging. In addition, a 4% agar phantom containing a 10 mM EuDOTA(gly)₄⁻ agent was imaged. The spectroscopy experiments were performed on a Varian INOVA 400MHz vertical bore system using a high-resolution 5 mm probe. The imaging experiments were performed on a Bruker BioSpec 360MHz vertical bore system using a 20 mm diameter volume coil.

In the solution samples at 400 MHz, the R_{IA} in the absence of the irradiation was measured using inversion recovery sequence with the inversion times (TI) of 0, 1, 2, 6, 10 and 40 sec; the measured $R_{IA}=0.359\pm0.001$ sec. Apparent R_I was measured using sequence depicted in Figure 1, panel c, with detection block consisting of $\pi/2$ pulse followed by FID acquisition (inversion recovery sequence with irradiation during recovery time). The T_{pw} periods were equal to TIs listed previously. Irradiation offset (Δ_{RF}) varied from -35 KHz to +35 KHz in steps of 2 KHz. Same T_{pw} were used for all RF offsets. The Z-spectrum was acquired using the same offsets listed above, saturation time of 5s and B_1 of 1900 Hz (45 μ T).

In the agar sample at 360 MHz, the R_{IA} in the absence of the irradiation was measured using inversion recovery sequence with inversion times of 0, 0.2, 0.5, 0.8, 1.0, 1.2, 1.6, 2.0, 3.0, 5.0 and 10.0 sec; the measured $R_{IA}=0.432\pm0.004$ sec.

In pCEST imaging, the positive contrast preparation was combined with a Spin Echo (SE) sequence, one preparation per phase encode. SE was also used with CEST preparation and for reference image acquisition (image with no preparation at all). Imaging parameters (for reference image, CEST and pCEST images) were: TR/TE=15sec/11.4 msec, FOV 2×2 cm², matrix 128x128, slice thickness 2 mm, single slice and NEX=1. Total acquisition time was 32 min per image, same total time for CEST and pCEST images. Localized first order shimming was used to improve B_0 inhomogeneity. In all the experiments CW irradiation was used for pre-saturation with $T_{pw}=T_{null}(\text{RF OFF})=1.55$ sec and $\omega_1/2\pi=1960$ Hz (46 μ T) in the solution experiments and $T_{pw}=T_{null}(\text{RF OFF})=0.85$ sec and $\omega_1/2\pi=600$ Hz (14 μ T) in agar phantom experiments. These parameters were optimized for pCEST imaging. CEST images were acquired using the same pre-saturation parameters.

Numerical simulations of the Bloch-McConnell equations (Eq.1) were written in Matlab (The MathWorks, Inc). R_{IA} used in simulations (0.359 sec^{-1}) was determined experimentally, as described above. A typical R_{2A} was used in simulations (1.333 sec^{-1}), based on values used in literature (e.g. [58]). The parameters of the bound pool, R_{IB} and R_{2B} , were chosen to be equal to each other and equal to 2.5 sec^{-1} . It was shown previously that if the bound pool relaxation lifetimes are longer than few tens of milliseconds their influence on the results is minor [58,59]. The exchange lifetime of the bound pool, τ_B was estimated based on the overall qualitative visual similarity between experimental and simulated results and is equal to 30 μ sec. This value is faster than the exchange lifetime measured for the compound at 25°C [57]. The chemical shift difference was assumed to be 19 KHz. In the following we will refer to this combination of $R_{IA}, R_{2A}, R_{IB}, R_{2B}, \tau_B$ and $\Delta\omega$ as the “model system”. They are the same for all the simulations shown in this paper. An additional set of parameters, “experimental parameters”, will include $T_{pw}, \Delta_{RF}, \omega_1/2\pi$. The purpose of all simulations was to provide overall qualitative support for the experimental observations, and not quantitative fittings of the data.

5. Results and Discussion

5.1 R_{1app} measurements

First, the off-resonance dependence of the apparent relaxation rate (i.e., R_{1app} vs. irradiation offset relative to the water frequency (Δ_{RF})) was measured in a 10 mM solution of EuDOTA(glyOEt)₄³⁺. In this experiment R_{1app} is equal to the off-resonance $R_{1\rho}$ when the $\Delta_{RF} \gg \omega_I$ (far off-resonance) and is equal to $R_{2\rho}$ when $\Delta_{RF} = 0$ (on-resonance with water).

Figure 3 displays a standard Z-spectrum (panel a) and the apparent relaxation rates vs. Δ_{RF} (panel b): the RF intensity was constant at $\omega_I \sim 1900$ Hz (45 μ T); and, the saturation length for a standard Z-spectrum (T_{pw}) was 5 s. In general, the R_{1app} measurement requires more total time than the Z-spectrum, since multiple saturation lengths are acquired for R_{1app} and,

typically, only one for Z-spectrum. Here, the acquisition of the R_{1app} spectrum (Figure 3, panel b) took six times longer than the Z-spectrum (Figure 3, panel a). As expected from Eq. 3, the apparent relaxation rate R_{1app} increases when the frequency of RF equals that of the exchanging pool (i.e., $\Delta_B = 0$, RF ON). The correlation between the R_{1app} off-resonance profile and Z-spectrum is excellent. For these RF intensities and timings the CEST effect in the 10 mM solution of EuDOTA(glyOEt)₄³⁺ measured 0.59 while $\Delta R_{1app} = 0.46 \pm 0.05 \text{ sec}^{-1}$.

To the best of our knowledge, analytical expressions do not exist for all of the experiments described in the following. To provide overall qualitative support of the experimental results, simulations were performed using a two-pool exchange model (Eq.1) with relaxation and exchange rates typical for a PARACEST agent, and given in the Methods section; the results are shown in Figure 3 (panels a-b) overlaid on experimental data. Overall, the experimental data are in good qualitative agreement with simulations. It should be noted that no actual fitting of the data was attempted, which would require at least three exchanging pools (-NH, Eu³⁺-bound water, bulk water) for these agents. Figure 3, panels c-d, display dependence of R_{1app} profile on the agent's concentration. This dependence will be discussed in the greater detail in the following.

To study the dependence of the apparent relaxation rate on the RF saturation intensity, R_{1app} was measured (Figure 4, panel a) as a function of RF saturation intensity, ω_1 , keeping the saturation frequency on-resonance with the exchanging pool ($\Delta_B = 0$, $R_{1app}(\text{RF ON})$). $R_{1app}(\text{RF ON})$ increases with the increasing ω_1 . To evaluate the spill-over effect (i.e., direct influence of the RF saturation on the relaxation time), the measurements were also performed with RF OFF ($\Delta_A = \Delta\omega$ and $\Delta_B = 2\Delta\omega$, $R_{1app}(\text{RF OFF})$). While $R_{1app}(\text{RF OFF})$ increases slightly with increasing RF intensity due to the spill-over effect (Figure 4, panel a), the exchange induced saturation transfer results in a substantially higher increase for $R_{1app}(\text{RF ON})$. Simulations confirm this experimental observation (Figure 4, panel a). The overall agreement between the experiment and the simulation is very good, thereby providing additional validation to the experimental results. To our knowledge, no analytical expression describing the dependence of $R_{1\rho}$ on ω_1 in the slow exchange regime exists.

5.2 Positive CEST (pCEST) imaging

A change in apparent relaxation is necessary but not sufficient to create positive CEST contrast. In contrast to T₁ shortening agents, the steady state magnetization that is reached under the application of RF is smaller than the equilibrium magnetization (as illustrated in Figure 2). A higher RF intensity leads to larger changes in R_{1app} (Figure 4) but application of higher RF power will ultimately result in complete saturation of the water signal. Hence, the RF intensity should be chosen carefully.

For the next set of experiments the RF saturation time, T_{pw} , was chosen to yield a small positive signal in the absence of RF (i.e., approximately null, $T_{null}(\text{NO RF})$). For the phantoms used here, $T_{null}(\text{NO RF})$ is approximately 2 sec. Figure 4, panel b, displays a signal intensity measured as a function of ω_1 for the RF of length $T_{null}(\text{NO RF})$ applied at RF OFF and RF ON. As can be seen from the figure, there is an optimal B₁ value at which the signal with RF ON is positive, while the signal with RF OFF is small. In our case the optimal B₁ is near 2000 Hz (47 μT) (Figure 4, panel b). The agreement with simulation (Figure 4, panel b) is excellent, with small differences as expected since the simulated model assumes only two pools and was executed for the overall qualitative support to the experiment. In addition, the experiment used $\Delta_{RF} = 17 \text{ kHz}$ for RF ON, the value also used in the simulation, which is slightly different from the assumed $\Delta\omega$ of 19 kHz.

This leads to a general scheme for the creation of positive CEST contrast by using an inversion recovery sequence with RF saturation between inversion and detection (Figure 1,

panel c). It should be noted, that saturation recovery cannot be used for this purpose since magnetization with RF ON is always smaller than with RF OFF.

To experimentally optimize pCEST, T_{pw} is initially set equal to $T_{null}(NO\ RF)$ and the RF intensity is varied, similar to the experiment shown in Figure 4, panel b. Once the optimal RF intensity is identified, T_{pw} is again made equal to $T_{null}(RF\ OFF)$, so that the RF OFF signal is null or slightly positive. We would like to emphasize that $T_{null}(RF\ OFF)$ is less than $T_{null}(NO\ RF)$ and depends on the RF intensity (see Figure 4,b). $T_{null}(RF\ OFF)$ and not $T_{null}(NO\ RF)$ is used in the experiment to compensate for the spill-over effects. We would like to emphasize that the $I(T_{pw})/I_0$ at $T_{null}(RF\ OFF)$ is null or small positive. For a particular type of tissue and contrast agent, this calibration should only have to be performed once. An RF intensity lower or higher than optimal, may be used but the intensity difference between the signals with RF ON and RF OFF will also be smaller (see Figure 4,b).

To compare pCEST and standard CEST contrast, the definition of the quantitative effect magnitude must be considered. CEST quantification (Eq. 4) employs normalization by I_0 . Alternately, normalization by $I(RF\ OFF)$ can be used [14], providing a better account of the spill-over effects. However, it is not applicable to the pCEST case since the RF OFF signal is close to zero. Using it for normalization will result in misleadingly large pCEST enhancement factors and experiment errors.

The images using the pCEST scheme are shown in Figure 5, panels a-c. The phantoms contained 0, 0.1 mM, 1 mM and 10 mM of EuDOTA(gly)₄⁻. For these experiments, the RF intensity was chosen to be approximately ~1960 Hz (46 μ T). At this ω_1 , the $T_{null}(RF\ OFF)$ was found to be 1.55 sec., which is shorter than the one used for Figure 4, panel b (2 sec). Several factors could contribute to the difference: (i) the imaging experiments were performed at a slightly lower field, 360 MHz vs 400 MHz used for spectroscopy; (ii) different agents are used, possibly leading to slightly shorter T_1 ; and, (iii) the influence of the direct saturation effects (as seen in Figure 5), resulting in a $T_{null}(RF\ OFF)$ that is slightly shorter than the 2 sec $T_{null}(NO\ RF)$ used in the spectroscopic measurement. From Figure 5, panel a it can be seen that a significant signal reduction was obtained in pCEST images with RF OFF. The background suppression is significant, though not absolute, due to small variations in T_1 and RF inhomogeneity. With RF ON, the signal increases, as expected (Figure 5, panel b). The signal intensities with RF ON increases with EuDOTA(gly)₄⁻ concentration.

Comparisons between pCEST and CEST images are also shown in Figure 5: panels (a-c) vs panels (d-f). A factor of three higher receiver gain was used for image acquisition with the pCEST scheme, than with CEST. The higher gain used reflects the fact that the pCEST images have much lower baseline intensity, due to background suppression. The positive nature of contrast in pCEST scheme versus negative in standard CEST is clearly illustrated in panel b vs panel e, images obtained with RF ON for both schemes. Note the very different intensity scale used for images acquired with pCEST and CEST schemes (as shown to the right of Figure 5, panels b and e). The pCEST magnitude (Eq.6) and CEST magnitude (Eq. 4) are listed on the panels c and f. The pCEST magnitude is $-0.2\pm0.5\%$, $-0.1\pm0.5\%$, $1.2\pm0.5\%$, $9.9\pm0.5\%$ for 0, 0.1 mM, 1 mM and 10 mM EuDOTA(gly)₄⁻, respectively. Thus, the pCEST magnitude for 0 and 0.1 mM is zero. The CEST magnitude is $0\pm2\%$, $0\pm2\%$, $9\pm1\%$, $53\pm2\%$ for 0, 0.1 mM, 1 mM and 10 mM of EuDOTA(gly)₄⁻, respectively. Similar to pCEST, the CEST magnitude for 0 and 0.1 mM is zero. Both pCEST and CEST effects increase with increasing agent concentration.

Overall, the pCEST has lower effect magnitudes compared to standard CEST. The total experimental time of the pCEST and CEST experiments was the same (32 min), allowing

direct comparison of the effect sizes shown above. Numerical simulations and experimental data indicate that the pCEST effect is about one-third to one-fifth that of standard CEST. At the same time, while the absolute pCEST effect magnitude decreased, so did the observed standard deviation. The contrast-to-noise ratio for CEST vs. pCEST is 9% vs. 2.4% for 1mM and 27% vs. 20% for 10mM, i.e. factors of about one-quarter to three-quarter, very strongly dependent on concentration. Hence, though the pCEST sequence leads to better dynamic range utilization and allows for use of higher receiver gains without receiver overflow, its absolute sensitivity is lower than that of the standard CEST scheme.

5.3 pCEST effect vs concentration

To further study the dependence of the pCEST effect on concentration, a series of simulations were performed. Figure 3, panel d, displays dependence of R_{Iapp} on concentration; corresponding Z spectra are shown in Figure 3, panel c. $R_{Iapp}(RF\ ON)$ increases linearly with concentration, as predicted by Eq.3 [53]. As becomes evident from the simulated spectra, at very high concentrations, exceeding tens of mM, exchange broadening becomes apparent, leading to increasing direct saturation effects for both, Z-spectra and R_{Iapp} spectra.

The experimental dependence of pCEST effect on concentration, as seen on Figure 5, panel c, is shown in Figure 6, panel a, overlaid over the numerical simulation. Similar to CEST, the effect increases with the increasing concentration, albeit always remaining smaller than the corresponding CEST effect.

As was shown above, pCEST effect is dependent on RF intensity (Figure 4, panel b). To explore the interplay between concentration and ω_1 influences, a series of simulations of pCEST and CEST effects was performed, and the results are shown in Figure 6, panels b and c. Overall, the dependence is similar to CEST, however, there is a slight deviation at higher concentrations and higher B_1 values.

To explore this differentiating feature further, additional simulations were performed, the detailed results of which are shown in the Figure 7. In these simulations $T_{pw}=T_{null}(NO\ RF)=\ln 2 T_{IA}$. Based on this preliminary numerical assessment, at very high concentrations of agents (on the order of several tens of mM) the exchange contribution leads to significant changes to R_1 and R_2 already at the absence of RF [48,53]. This, in turn, leads to changes of $T_{null}(NO\ RF)$ and, hence, $T_{null}(RF\ OFF)$. Using $T_{null}(RF\ OFF)$ unadjusted to exchange effects leads to smaller or negative pCEST effect (see Figure 7, left top and left middle panels). It should be noted that such high concentrations of PARACEST agent are unrealistic *in-vivo*. For endogenous DIACEST agents, that could be present in tens of millimolar concentration $T_{null}(RF\ OFF)$ is adjusted from the beginning to compensate for exchange contribution to intrinsic R_1 [60]. Moreover, at extremely high concentrations (hundreds of mM to M) and high RF intensity, exchange broadening leads to increased direct saturation, which may ultimately lead to negligible CEST effect (Figure 7, top right panel). At the same regime the pCEST effect passes through a minimum and increases again (Figure 7, top left panel). Overall, these data suggest that at higher concentrations the behavior of pCEST effect is rather complicated, strongly T_{pw} and ω_1 dependent, and deviates in the overall performance from CEST. At the same time pCEST effect dependence on concentration at the lower range is similar to CEST (Figure 7, bottom right and bottom left panels). We dare to speculate that in this regime quantification techniques, similar to the ones derived for CEST (such as QUEST, QUESP [61], Omega-plot [57], just to name a few) could be derived for pCEST.

5.4 pCEST in the presence of a Magnetization Transfer pool

Similarly to CEST experiments, the presence of magnetization transfer (MT) pool is expected to change overall dynamics in the system by generation of additional magnetization exchange pathways. To evaluate the influence of magnetization transfer effects present in tissue, the experiments were conducted using a 4% agar phantom containing 10mM of EuDOTA(gly)₄⁻. As expected, this required re-adjustment of the $T_{null}(RF\ OFF)$, with the new value equal to 0.85 sec. Moreover, monitoring the signal dependence vs RF intensity, analogous to the experiment presented earlier in Figure 4, indicated that the optimal RF intensity was 600 Hz (14 μ T). However, the ω_1 dependence demonstrated much shallower profile than the one shown in Figure 4. We speculate that the change of the optimal B_1 value can result from several factors including: (i) a potentially slower water exchange rate in the agar vs. solution leading to a different optimal B_1 value [59]; and, (ii) the influence of MT pool potentially “competing” with CEST [58]. Images (Figure 8) of the agar phantom were obtained using pCEST (panels a-b) and CEST (panels d-e). Using the pCEST sequence the signal with RF OFF was substantially suppressed, while the signal with RF ON increased. The pCEST magnitude was $1.4 \pm 0.9\%$. Using a CEST scheme the effect magnitude was $4 \pm 1\%$. The image intensities were lower for both CEST and pCEST compared to the same agent concentration observed in solution. The exact behavior of the system, leading to the decreased effects for either CEST and pCEST is rather complicated and beyond the scope of this paper. However, it was shown before that the presence of MT results in a decreased PARACEST effect [62]. We anticipate, but have not shown, that the origin of this difference is due to slower water/proton exchange in agar. The slower exchange rate leads to decreased optimal B_1 value compared to solution [59]. It is also known that decreased exchange lifetimes result in a decreased CEST effect. Based on the analogy between CEST and pCEST, one would anticipate a decreased pCEST effect as well. Moreover, the presence of an additional MT pool of protons results in more exchange pathways and generally more complicated dynamics. Despite the complicated dynamics, background suppression with pCEST RF OFF was achieved, and the intensity increase with RF ON is evident (Figure 8, panels a-b). The ratio of pCEST and CEST magnitudes is similar to that observed for samples in pure water. Although the pCEST effect is relatively small, the sequence offers the benefit of “nulling” the MT effect and image intensity, regardless of how small, originates with pCEST and is predominantly due to the presence of the exchanging agent. All other effects are nulled or significantly reduced. In contrast, in the standard CEST experiment, the signal decrease is largely governed by the MT signal and, to a lesser degree, by CEST.

There is a small intensity gradient observed in pCEST (panels a-b) and CEST (panels d-e) that is corrected in the pCEST magnitude image (Figure 8, panel c). This intensity gradient may stem from either B_0 or B_1 inhomogeneity, or both. The sensitivity of the CEST effect to both types of field inhomogeneities is well-documented [63]. Generally it is established that for reasonably homogeneous B_1 fields RF inhomogeneity plays a relatively minor role [63]. B_0 influence is more substantial: the shift in frequency results in erroneous RF offsets leading to less efficient saturation and smaller CEST effects. The B_0 homogeneity problem is especially pronounced for CEST agents, but less for PARACEST due to much larger chemical shifts and higher RF intensities used with PARACEST agents. In our studies we estimate the dispersion in B_0 to be ± 60 Hz. The RF intensities used (at least 600Hz) result in the suppression profile much higher than the B_0 dispersion [64]. Hence, we expect the influence of B_0 inhomogeneity to be negligible. Moreover, the intensity gradient observed in panels a-b and d-e cancels for pCEST (panel c) and (to a large degree) for CEST (f). This likely indicates that the intensity gradient originating in B_1 inhomogeneity and its influence on the excitation, refocusing and detection involved in Spin Echo sequence used for image acquisition.

There is some residual heterogeneity in the CEST image, but not in pCEST. Overall, the standard deviations observed in CEST were slightly higher than in pCEST (Figures 6f and 7f vs Figures 6c and 7c). These observations might indicate slightly decreased sensitivity of pCEST to field inhomogeneities. This will be investigated further in future studies.

It should be noted that the pCEST scheme, being of the same nature as CEST experiments, shares the same safety concerns for *in-vivo* application of PARACEST agents. The optimal RF saturation intensity and/or overall power might be too high for safe clinical usage. Despite these limitations, successful *in-vivo* PARACEST studies were demonstrated [65,66]. As figure 4 illustrates, pCEST is feasible with B_1 values less than optimal even though the effect size will be smaller. This is in complete analogy with CEST experiments, where B_1 values lower than optimal can be used, but the achieved effect size is also lower [59].

Next, an influence of multiple T_1 's should be discussed. The presence of several T_1 's reduces the effectiveness of the background suppression used here. If ideal background suppression and null signal with RF OFF could be achieved, it would eliminate the necessity for the control experiment. Only one experiment with RF ON would be needed, with the remaining signal only from the areas containing the agent. Currently, since background suppression is not ideal and not homogeneous (due to potential presence of multiple T_1 's in a tissue) a control experiment with the pCEST method is needed. Moreover, as was discussed earlier, B_0 inhomogeneities may contribute to the image and control experiment can be used to compensate for them, at least partially. Several routes can be investigated to improve the pCEST method with respect to T_1 sensitivity. Multiple inversion pulses can be added to extend the suppression over a larger range of T_1 's. For instance, fat has a shorter T_1 time and, hence, will not be nulled by T_{pw} used for a muscle. Fat suppression pulses can be used in combination with a pCEST scheme to suppress the fat signal. Moreover, the sequence can be combined with spatial suppression pulses if only part of an anatomy within an imaging slice is of interest. At the same time, based on the analogy with standard CEST, application of additional spatial selective or spectral selective pulses may lead to undesired saturation effects [67].

While we have demonstrated the application of pCEST to detect paramagnetic exchanging agents (PARApCEST), the method can easily be extended to DIACEST agents. Such extension would require some additional adjustments. First of all, since exchange rates are slower, the optimal B_1 is lower. In addition, if MT is present, the $T_{pw}(RF\ OFF)$ should be adjusted accordingly, as seen in the data presented here. Moreover, for DIACEST agents, due to a smaller chemical shift difference, direct saturation plays a very important role and $T_{pw}(RF\ OFF)$ should be adjusted for a particular RF saturation intensity. Despite these perceived difficulties, we have recently demonstrated preliminary DIApCEST results using glycosaminoglycans in cartilage *exvivo* [60]. This study will be discussed in a separate publication.

Conclusions

We have implemented a new scheme called pCEST that results in a true positive contrast based on CEST, with concurrent background suppression. The sequence is based on the off-resonance spin-lock experiment and employs influence of the saturation exchange on the apparent relaxation rate. The absolute effect sizes are smaller in pCEST than in CEST, but the pCEST sequence provides background suppression and therefore has better dynamic range. Potentially, in non-quantitative studies, if robust background suppression can be achieved, only one image of the agent presence could be acquired, with no need for a control image.

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Highlights

> Conventional Chemical Exchange Saturation Transfer generates negative contrast. > We introduce a method to generate true positive CEST contrast (pCEST). > In addition, PCEST achieves simultaneous background suppression. > The method utilizes analogous nature of CEST and off-resonance $T_{1\rho}$ experiments.

Figure 1.

Panel a: A schematic of an NMR spectrum of two pool system consisting of a larger population A (*e.g.*, bulk or ‘free’ water) in exchange with a smaller population B (*e.g.*, water bound to the PARACEST agent). The bold arrows mark application of RF on resonance with the exchanging pool B (RF ON) or the control experiment, with RF moved an equal frequency to the opposite side of population A (RF OFF). Panel b: A schematic of a pulse sequence utilized for saturation transfer experiments (T_{pw} is the RF pulse length). Panel c: A schematic of a pulse sequence utilized for apparent relaxation time measurements and for the creation of the positive CEST effect (pCEST); π is an inversion pulse.

Figure 2.

A schematic of positive contrast generation using chemical exchange saturation transfer. Following inversion, the magnetization returns to steady-state with rates that are different for RF OFF and RF ON. At $T_{null}(RF\ OFF)$ the signal with RF OFF is null, but the signal with RF ON is small positive resulting in true positive contrast with CEST. For longer saturation time the system reaches steady-state, i.e. returns to standard CEST negative contrast.

Figure 3.

Panel a: experimental (dots) and simulated (solid line) Z-spectrum for a 10mM EuDOTA(glyOEt)₄³⁺. Panel b: The experimental (dots) and simulated (solid line) Δ_{RF} dependence of R_{1app} obtained from the same solution. The horizontal line in panel b is the R_{1A} value in the absence of irradiation. Model system parameters, as described in Methods section were used with 10mM PARACEST agent, $\omega_I/2\pi=1600$ Hz, and $T_{pw}=5$ sec for Z spectrum. Panels c and d: Simulated dependence of Z-spectra (panel c) and R_{1app} spectra (panel d) on the agent concentration. Same model system, ω_I , and T_{pw} for Z spectra was used as in panels a-b. Numbers next to the lines indicate concentration in mM that was used in the simulation.

Figure 4.

Panel a: Apparent relaxation rate (R_{1app}) dependence on RF intensity (ω_1) for 10mM EuDOTA(glyOEt)₄³⁺. Symbols correspond to experimental data: circles (RF ON) and squares (RF OFF). The lines correspond to simulated data: black (RF ON) and gray (RF OFF). The model system as described in Methods section was used in the simulations. Additional parameters: for RF ON $\Delta\omega_{RF}=17$ kHz, for RF OFF $\Delta\omega_{RF}=-17$ kHz, $T_{pw}=2.1$ sec (using experimental RF saturation parameters, same for CEST and pCEST) The simulation, included averaging over a ω_1 nominal value $\pm 5\%$ to mimic experimental RF inhomogenities. Panel b: Normalized signal intensity, $I(\omega_1)/I_0$, obtained in the 10mM EuDOTA(glyOEt)₄³⁺ solution using the pulse sequence shown in Figure 1, panel c, with $T_{pw} = T_{null}(NO RF)$ and varying RF intensity (ω_1). Symbols correspond to experimental data: circles (RF ON) and squares (RF OFF). The lines correspond to simulated data: black (RF ON) and gray (RF OFF). The same simulation parameters as described for panel a were used.

Figure 5.

Images of phantoms with 0, 0.1 mM, 1 mM and 10 mM of EuDOTA(gly)₄⁻ acquired with pCEST (panels a-c) and CEST (panels d-f) experimental schemes. Numbers on panel (d) correspond to phantom agent concentration and location. Panels (a) and (d) show images acquired with RF OFF; panels (b) and (e) show images acquired with RF ON. The images are normalized by the reference image intensity acquired with no preparation. The same intensity scale (left of panel b) was used for the pCEST images (panels a-b). Likewise, the same intensity scale (left of panel e) was used for the CEST images (panels d-e). Note the different intensity scale used for pCEST and CEST images. Panels (c) and (f) display quantitative pCEST and CEST magnitude maps, respectively. Numbers on panels (c) and (f) correspond to mean ROI values, of pCEST and CEST maps, respectively. A factor of three higher receiver gain was used for image acquisition with the pCEST sequence relative to CEST.

Figure 6.

Panel A: Experimentally observed pCEST (squares) and CEST (circles) effects vs concentration in EuDOTA(gly)₄⁻ solution. Black and gray lines correspond to simulated CEST and pCEST, respectively. The model system as described in Methods section was used in the simulations. Additional parameters: for RF ON $\Delta\omega_{RF}=17$ kHz, FOR RF OFF $\Delta\omega_{RF}=-17$ kHz (using experimental value). Panels b and c: Simulated CEST (panel b) and pCEST (panel a) effects vs. concentration for a series of RF intensity values: 100 Hz, 700 Hz, 1300 Hz, 1900 Hz, 2500 Hz and 3100 Hz. The RF intensity values are marked next to the lines, on the panels b and c. Model system as described in Methods section was used in the simulations. Additional parameters: for RF ON $\Delta\omega_{RF}=19$ kHz, for RF OFF $\Delta\omega_{RF}=-19$ kHz, $T_{pw}=T_{null}(NO\ RF)=1.927$ s (ideal conditions, same RF saturation parameters used for CEST and pCEST)

Figure 7.

Numerical simulations of pCEST and CEST effect as a function of concentration and RF intensity ($\omega_1/2\pi$). Three concentration ranges are shown: 0 to 1 M (top row), 0 to 100 mM (middle row) and 0 to 10 mM (bottom row). White dotted horizontal lines show the area of the plot expanded in the row below. The model system parameters are given in the Methods section. In all the simulations shown $T_{pw} = T_{null}(NO\ RF) = \ln 2 T_{1A} = 1.927$ sec was used, same RF saturation parameters for pCEST and CEST.

Figure 8.

Images of phantom containing 4% Agar and 10 mM of $\text{EuDOTA}(\text{gly})_4^-$ acquired with pCEST (panels a-c) and CEST (panels d-f). Panels (a) and (d) show images acquired with RF OFF; panels (b) and (e) show images acquired with RF ON. The images are normalized by the reference image intensity acquired with no preparation. The same intensity scale (left of panel b) was used for the pCEST images (panels a-b). Likewise, the same intensity scale (left of panel e) was used for the CEST images (panels d-e). Note the different intensity scale used for pCEST and CEST images. Panels (c) and (f) display quantitative pCEST and CEST magnitude maps, respectively. Numbers on panels (c) and (f) correspond to mean ROI values, of pCEST and CEST maps, respectively.