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UCEPR: Ultrafast Localized CEST-spectroscopy with PRESS in phantoms and in vivo

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Abstract

Purpose—Chemical Exchange Saturation Transfer (CEST) is a contrast mechanism enhancing low-concentration molecules through saturation transfer from their exchangeable protons to bulk water. Often many scans are acquired to form a *Z*-spectrum, making the CEST method time-consuming. Here, an ultrafast localized CEST-spectroscopy with PRESS (UCEPR) is proposed to obtain the entire *Z*-spectrum of a voxel using only two scans, significantly accelerating CEST.

Methods—The approach combines ultrafast non-localized CEST spectroscopy with localization using PRESS. A field gradient is applied concurrently with the saturation pulse producing simultaneous saturation of all *Z*-spectrum frequencies that are also spatially encoded. A readout gradient during data acquisition resolves the spatial dependence of the CEST responses into frequency. UCEPR was tested on a 3T scanner both in phantoms and in vivo.

Results—In phantoms, a fast *Z*-spectroscopy acquisition of multiple pH-variant iopamidol samples was achieved with 4–7-fold acceleration as compared to the conventional CEST methods. In vivo, amide proton transfer (APT) in white matter of healthy human brain was measured rapidly in 48 s and with high frequency resolution (0.2 ppm).

Conclusions—Compared to conventional CEST methods, UCEPR has the advantage of rapidly acquiring high-resolution *Z*-spectra. Potential in vivo applications include ultra-fast localized *Z*-spectroscopy, quantitative, or dynamic CEST studies.

Keywords

ultrafast localized CEST; PRESS; broadband saturation; iopamidol; APT

INTRODUCTION

Chemical exchange saturation transfer (CEST) MRI provides a new contrast mechanism that reflects the chemical exchange of protons between low-concentration solutes and bulk water. Many CEST applications are currently being explored, such as molecular imaging (1–3), metabolite detection (4–6), gene reporting (7), and pH monitoring (8,9). In order to observe CEST contrast, the exchanging solute protons need to be in the slow exchange regime with the water protons, thus possessing chemical shifts distinct from water. A selective RF irradiation is then used to saturate this distinct frequency offset of the solute protons. The resulting saturation gets transferred to bulk water via chemical exchange, leading to a decreased water signal and negative MR contrast. Effectively, the exchangeable solute protons are detected indirectly via the decreased water signal. This process of continuous exchange of saturated protons of the small solute pool with the much larger water pool enhances the MRI sensitivity of the indirect detection. Even for solute concentrations unobservable using conventional MRI, the cumulative CEST effect is detectable with sufficiently long RF saturation (10,11).

Though theoretically (and in some experimental circumstances) only two acquisitions are required (with and without the RF), in many CEST acquisitions a so-called *Z*-spectrum is acquired, in which a sequence of images or spectra is collected as a function of varying the saturation RF offset frequencies (12–14). Typically, the offset frequencies are acquired symmetrically with respect to the water signal, although other schemes were also introduced (as discussed below). The water frequency is used as a reference and is set to 0 ppm. In such a point-by-point acquisition, the entire *Z*-spectrum is typically covered; the number of points in the *Z*-spectrum determines the number of total acquisitions. Thus, for a high-spectral resolution and/or wide-band *Z*-spectrum acquisition, a large number of scans is required, which results in long scanning times. In addition, a long TR is often required to reduce the average SAR during the long irradiation times required for maximizing the CEST effect. All of these factors make CEST acquisitions time consuming, thus limiting its applications in vivo and in clinical settings.

Several approaches had been proposed to shorten the total scan time by reducing the total number of points in the *Z*-spectra. A faster CEST data acquisition scheme with multiple scans covering only the essential points in the *Z*-spectrum has been proposed and applied in diamagnetic (DIACEST) (15) as well as paramagnetic (PARACEST) agents (16,17). Optimization of the frequency points has also been studied (18). Despite this progress, the underlying, sequential nature of the experiment remains, and the total time in all of these experiments remains proportional to the number of off-resonance frequencies acquired. Moreover, to the best of our knowledge, no study has used less than five points in the *Z*-spectra.

Other approaches have been proposed to accelerate CEST through reductions of the acquisition or saturation times (19–24). For example, a flexible CEST preparation period followed by a single shot steady-state free precession acquisition (FISP) has been proposed to achieve a 50-fold reduction in acquisition time (19,20). Alternatively, a rapid multi-slice CEST imaging has been proposed (21), where the repeated long saturation pulses have been

segmented unevenly into a long primary RF pulse to build the steady state CEST contrast and a repetitive short secondary RF pulse to boost the CEST contrast, thereby resulting in a significant reduction of total scan time. In another approach, a fast 3D CEST imaging sequence of the entire human brain in less than 10 min has been presented (22). In this method, 3D gradient- and spin-echo (GRASE) imaging with 2D sensitivity encoding (SENSE) has been applied as the imaging module to reduce scan time. A key-hole approach acquiring reduced resolution CEST images was also introduced (23). However, in all of these fast CEST methods, the number of total data acquisitions required to characterize the entire Z -spectrum is not reduced.

The accuracy of CEST is dependent on the precise identification of the off-resonance position, making the method very sensitive to the B_0 inhomogeneity. Thus, pixel-by-pixel B_0 correction methods are often used together with CEST. Several methods have been proposed, and can broadly be divided into two categories: (i) using information already present in the Z -spectrum and (ii) acquiring an “external” map of B_0 distribution. The first category encompasses methods from finding the minimum of the collected Z -spectrum (25) to fitting using Bloch equations (26) and multi-peak fitting (16,17,27). The advantage of the first group of methods is that they do not require additional scan time. However, the results of fittings can be unreliable for broad Z -spectra and low CEST effects characteristic for endogenous CEST at moderate (3T) fields, common for human applications. The second category includes Water Saturation Shift Referencing (WASSR) (28) and GRE-based B_0 mapping (29). These can be more reliable in humans in vivo. However, the additional scans prolong the total CEST scan time even further.

For most in vivo applications, the whole CEST spectrum is collected for the entire anatomy. At the same time, there are emerging CEST applications, such as the assessment the tumor stage (3) or the differentiation from necrosis (2), where only spatially-localized CEST information is required. In these cases, the exact location of the suspicious area is previously identified via conventional imaging methods, and CEST is acquired with the goal of further lesion characterization. As such, Z -spectroscopy localized only over the lesion area would be required and it can be done at a fraction of the time, as proposed below.

Recently, an ultrafast Z -spectroscopy (UFZ) has been proposed to acquire the whole Z -spectrum of a phantom with homogeneous CEST agent distribution using only two scans, independent of the bandwidth and the resolution of the Z -spectrum (30,31). This work is based on the rapid acquisition of magnetization transfer (MT) spectra published previously (32) and later used in quantitative analysis of pulsed MT (33). In this approach, a constant magnetic field gradient is applied during the RF saturation (“CEST” or “saturation” gradient), spatially encoding all saturation frequencies at once. A readout gradient along the same direction as the saturation gradient is applied during signal acquisition, thus resolving the spatial encoding of saturation frequencies back to frequency information in the form of a “chemical exchange” 1D object projection. Effectively, the CEST response over a continuous frequency range is acquired by a single case of RF saturation. Only one additional reference scan is required, without the RF saturation, to be used for signal normalization in samples with non-uniform shape. The continuous Z -spectrum is obtained by division of the two acquired 1D profiles, with and without the RF saturation. This method

is useful for the rapid characterization of CEST agents with a homogeneous distribution of the agent, where resolving spatial CEST information is not necessary. However, this method has limited in vivo utility where spatially localized information is required. Moreover, it is not straightforward to expand this method to 2D imaging, since the dimension used for encoding the Z -spectrum frequencies already occupies the dimension required in imaging for the second (frequency) spatial encoding.

Here, we propose an ultrafast spatially-localized CEST-spectroscopy utilizing Point Resolved Spectroscopy (PRESS) sequence for localization (UCEPR). UCEPR combines the time efficiency of UFZ with the localization encoding of PRESS (34), thus making it extendable to in vivo applications. In the following sections, the technique is described and several features of the UCEPR method are discussed. Examples of UCEPR are presented both in phantoms and in vivo. First, fast Z -spectroscopy of multiple iopamidol samples with different pH is used to illustrate the ability of spatially localizing continuous CEST profiles. Secondly, continuous Z -spectrum of amide proton transfer (APT) in the human brain is used to demonstrate the speed advantages of UCEPR in vivo. Both phantom and human UCEPR results are compared with conventional CEST imaging and spectroscopy techniques.

THEORY

In conventional CEST, the pulse sequence is composed of a long saturation RF pulse with a specific (with respect to water) off-resonance frequency ω_s , followed by an MR signal acquisition to record the magnetization of bulk water $M_z(\omega_s)$ (12,35). During the saturation process, only the spectroscopic features of the sample dictate the frequency offset required to saturate a particular group of protons (solute or solvent). All of the protons with the same chemical shift experience the same RF offset saturation.

In the non-localized UFZ, due to the application of an extra magnetic gradient $\mathbf{G}_C$ (the “CEST” or “saturation” gradient) concurrently with the saturation RF pulse, protons in different positions along the gradient experience saturation at different RF offset. In the simplest case when the center of the object coincides with the gradient isocenter, and assuming (without loss of generality) a saturation gradient along the x -axis, the saturation frequency offset ω at location x is $\omega(x) = \gamma G_C x$, where γ is the gyromagnetic ratio and G_C is the strength of $\mathbf{G}_C$. This offset frequency is the same for the whole y - z slice at location x , and it is caused only by the extra gradient during saturation. Thus, if the frequency of the saturation pulse is $\omega_s = 0$, only protons at location $x = 0$ experience on-resonance saturation. At all other locations, where $x \neq 0$, protons experience off-resonance saturation with a position-dependent RF frequency offset $\omega_C(x) = -\omega(x)$. Thus, the Z -spectrum bandwidth is determined by the gradient strength G_C and the dimension of the object along $\mathbf{G}_C$. Moreover, to a good approximation, there is a one-to-one correspondence between the off-resonance saturation frequency and the position along the x -axis. Note that this encoding is continuous, as it is performed along a continuous variable – the spatial x -axis. A readout gradient $\mathbf{G}_r$ is applied along the same x -axis during data acquisition, resolving this spatial encoding. A Fourier transform of the complex data generates a continuous Z -spectrum: the saturation response of the sample to a range of RF frequencies (32).

In order to remove the effects of signal variation due to inhomogeneous distribution of bulk protons along the x -axis (e.g. due to object's shape), a reference signal is acquired by switching off the RF saturation pulse, effectively acquiring the 1D profile of the object along the x -axis. Thus, only two scans (with and without RF saturation) are required to obtain the entire Z -spectrum in UFZ, leading to a significant reduction of CEST scan time. UFZ requires that: (1) the phantom has homogeneous CEST response along G_C (but can have inhomogeneous total proton distribution, to be corrected by the reference scan), and (2) proton diffusion is negligible during the acquisition period (30,31). The second requirement is easy to satisfy by setting higher sampling bandwidth and smaller number of samples. The first requirement is too simplistic in vivo: organs and tissue structures are complicated and it is impossible to find a whole slice with a homogeneous distribution of the same pool of CEST protons. However, it is still possible to identify sufficiently small but clinically relevant volumes where the distribution of the endogenous CEST agent can be assumed to be constant: e.g. in homogeneously-appearing tumors.

In MR spectroscopy, the PRESS pulse sequence is capable of encoding voxel-localized spectrum using three mutually orthogonal slice-selective RF pulses. This property is employed in UCEPR to acquire voxel-based continuous Z -spectrum at the desired localization. Fig. 1 shows the pulse sequence of UCEPR, combining UFZ with PRESS-based localization. Note that since the CEST encoding and the voxel encoding are independent of each other, similar localized sequence can be obtained via combination of UFZ with STEAM(36). The three excitation pulses (90° – 180° – 180°) are used for localizing a volume of interest (VOI). A CEST-saturation RF pulse (gray box in Fig. 1) is applied concurrently with the CEST-encoding gradient G_C (dash line in Fig. 1). Following, is the PRESS localization sequence. Finally, the decoding read-out gradient G_r (dash line in Fig. 1) is applied during data acquisition. In contrast to the non-localized UFZ, the acquired Z -spectrum bandwidth is dependent on the position and the geometry of VOI, as discussed below.

The field-of-view (FOV) along the readout direction, FOV_r , is determined by the sampling bandwidth BW_r , and the read-out gradient G_r :

$$FOV_r = BW_r / (\gamma G_r), \quad [1]$$

where G_r is the strength of G_r . In the case of non-localized UFZ, the CEST bandwidth BW_C is determined by the ratio of the saturation and readout gradients, as well as BW_r (30):

$$BW_C = \gamma G_C FOV_r = BW_r G_C / G_r, \quad [2]$$

where G_C is the strength of G_C . Thus, BW_C can be set through the parameters G_C , G_r and BW_r . This is in stark difference from the standard CEST experiment where the bandwidth of a Z -spectrum is defined by the frequency range covered by the RF saturation offsets. In UCEPR the FOV_r covers the VOI (gray cube in Fig. 2a) that is defined by the PRESS localization. The VOI geometry is: L_r , the length of VOI along G_r , and x_0 , the center position of the VOI. Since the acquired signal is generated from the VOI only, the effective (i.e. useful) CEST bandwidth generated by the combination of UFZ with PRESS is:

$$BW_C^{\text{eff}} = \gamma G_C L_r, \quad [3]$$

and the frequency offset range is $[\gamma G_C(-x_0 - L_r/2), \gamma G_C(-x_0 + L_r/2)]$. When the RF saturation is placed exactly on the water resonance, $\omega_s = 0$, the situation depicted in Fig. 2b may arise if the position of the VOI is not taken into account. Here, the effective Z -spectrum occupies only a part of the encoded Z -spectrum, thus resulting in a low sampling efficiency. The dashed line represents the Z -spectrum without localization and the solid line represents the Z -spectrum from the selected VOI. Furthermore, if $x_0 \neq 0$ (i.e. the center of VOI is not at the gradient isocenter), the center of the acquired Z -spectrum may not correspond to saturation of the water line. Such a Z -spectrum may not be suitable for CEST data analysis, especially for the asymmetry analysis. This situation can be corrected by: (i) shifting the saturation frequency ω_s to “align” the saturation profile with the center of the VOI and (ii) shifting the reference acquisition frequency F_0 to “align” the acquired Z -spectrum with the center of the receiver bandwidth (Fig. 2c).

Thus, the frequency of the saturation RF pulse is set to $\omega_s = \omega(x_0) = \gamma G_C x_0$, and the acquisition frequency offset is $f = \gamma G_r x_0$ (Fig. 2c). The resulting effective CEST bandwidth BW_C^{eff} is still $\gamma G_C L_r$, but the frequency range is now optimized to $[-\gamma G_C L_r/2, \gamma G_C L_r/2]$. To enable most efficient acquisition, the saturation bandwidth should be approximately equal to or just a little bit higher, than the effective CEST bandwidth: $BW_C^{\text{eff}} \approx BW_C$.

Implementing the corrections mentioned above, the frequency resolution Δ_C in the UCEPR Z -spectrum is determined by the number of samples N , acquired during the readout:

$$\Delta_C = \frac{G_C}{G_r} \frac{BW_r}{N} = \frac{BW_C}{N}. \quad [4]$$

Thus, through parameters BW_r , G_C , G_r , and N , UCEPR can achieve Z -spectroscopy with different bandwidth and resolution.

METHODS

UCEPR was tested in phantoms and in vivo in healthy human volunteers ($n=2$). All experiments were performed on a 3T scanner using a 32-channel phased array head coil (Achieva, Philips Medical Systems, The Netherlands). All of the data were processed using custom routines in Matlab (MathWorks Inc., Natick, MA). To evaluate the performance of UCEPR, it was compared with standard CEST imaging (CEST-I) and CEST spectroscopy (CEST-S). The CEST protocols and some of Z -spectrum properties of UCEPR, CEST-I, and CEST-S in phantoms and in vivo are summarized in Table 1.

Phantom experiments

Phantoms containing iopamidol (Isovue-M 300, Bracco Diagnostics Inc, Italy) with pH ranging from 6.0 to 8.0 were prepared. Iopamidol is a clinically accepted X-ray contrast agent and contains two amide proton pools (with chemical shifts 4.2 ppm and 5.5 ppm) with

different pH-dependent exchange rates that can provide accurate pH measurement in the 5.5–7.4 range (37). Five iopamidol samples with various pH values (6.0, 6.5, 7.0, 7.5, and 8.0) were put into five separate 25 mL tubes. Thus, each tube possessed an agent with a unique exchange rate and with a homogeneous CEST response. All of the tubes were placed in a container filled with water. All measurements were conducted at room temperature ($\sim 22^\circ\text{C}$).

A VOI with dimensions $13 \times 30 \times 13 \text{ mm}^3$ was placed inside each tube and a UCEPR Z -spectrum was acquired from each tube. $\mathbf{G}_\text{r}$ and $\mathbf{G}_\text{c}$ were along the AP direction. The length of VOI along $\mathbf{G}_\text{r}$ was $L_\text{r} = 30 \text{ mm}$. Single-volume PRESS with symmetric echo acquisition was used. The acquisition bandwidth $BW_\text{r} = 4000 \text{ Hz}$, and $G_\text{c}/G_\text{r} = -2.0/3.0 \text{ mT/m}$, resulting in $FOV_\text{r} = 31 \text{ mm}$ by Equation [1]. Thus, the FOV_r covered the VOI: $FOV_\text{r} - L_\text{r} = 30 \text{ mm}$. According to Equation [3], the encoded effective CEST bandwidth for the Z -spectrum was $BW_\text{c}^\text{eff} \approx 2560 \text{ Hz}$ ($= 20 \text{ ppm}$). In addition, F_0 was shifted to correspond to the center of VOI, thus the water peak was at the center of acquired Z -spectrum. Therefore, the obtained Z -spectrum possessed symmetric frequency offsets within the range $[-1280, 1280] \text{ Hz}$. The typical number of acquired samples (N) was 64 and therefore the Z -spectrum resolution was 41.7 Hz ($\approx 0.3 \text{ ppm}$) by Equation [4]. For this N , $\text{TR/TE} = 3000/51 \text{ ms}$ and the number of signal averages (NSA) was 8, leading to a total scan duration of 48 s for one localized Z -spectrum. The saturation RF irradiation was composed of a train of 10 hyperbolic secant-shaped pulses, each with 49.5 ms duration with inter-pulse delay of 0.5 ms (total saturation duration of 500 ms). The root-mean-square B_1 used in the saturation train was $1.05 \mu\text{T}$ to $2.11 \mu\text{T}$. Different B_1 , N , and NSA were compared, as discussed in the results. Depending on the NSA , 2-, 4-, and 8-step phase cycling was employed for UCEPR (38).

Similar parameters were used for the standard CEST spectroscopy with PRESS (CEST-S). Here, the number of points in the Z -spectra was 63 in the range $[-1280, +1280] \text{ Hz}$. Thus, the total number of scans was 64 (63 + reference scan) and total time was 192 s. PRESS localization parameters were the same for CEST-S and UCEPR. No additional B_0 inhomogeneity correction (e.g. WASSR) was utilized for UCEPR and CEST-S.

Standard CEST imaging was also performed (CEST-I). The imaging parameters were: single shot Turbo Spin Echo (TSE) acquisition, $FOV = 220 \times 220 \text{ mm}^2$, in-plane matrix size = 112×112 , pixel resolution = $1.96 \times 1.96 \text{ mm}^2$, axial slice thickness = 10 mm. The saturation parameters were the same as in UCEPR and CEST-S. Similar to CEST-S, 63 points in the range $[-1280, +1280] \text{ Hz}$ were collected to form Z -spectrum. WASSR was used for B_0 inhomogeneity correction with total saturation length of 100 ms, intensity of $0.26 \mu\text{T}$, and a total of 41 points covering range of $[-200, +200] \text{ Hz}$. Thus, the total number of scans (CEST-I + WASSR) was 106 and total scan time 330 s (see also Table 1).

First-order pencil-beam (PB) volume shimming was employed in CEST-S and UCEPR and global first-order PB-volume shimming was employed in CEST-I. CEST-I was processed on the pixel-by-pixel basis. CEST-I and CEST-S data were processed using interpolation to 631 points. UCEPR data was used without additional interpolation. In CEST-I, to facilitate comparison between imaging and spectroscopy, the ROIs were placed over the specific

iopamidol sample similar to the VOI used in CEST-S and UCEPR. ROIs mean values were calculated to generate the Z -spectrum. In CEST-S, to allow comparison between standard spectrum and UCEPR, the intensity at zero frequency in each PRESS spectrum was used to generate the Z -spectrum.

In vivo experiments

In vivo imaging and spectroscopy of the brain were collected in two healthy volunteers, under the approval of the local IRB, after an informed consent was obtained. The VOI with dimensions $10 \times 15 \times 10 \text{ mm}^3$ was placed in the white matter with $L_T = 10 \text{ mm}$, and $\mathbf{G}_C$ was along the HF axis. The CEST saturation RF train was the same as in the phantom experiments. The acquisition bandwidth $BW_T = 1600 \text{ Hz}$ and $G_C/G_T = -3.0/3.0 \text{ mT/m}$, resulting in $FOV_T = 12.5 \text{ mm}$ ($> L_T$) and $BW_C = 1600 \text{ Hz}$. Therefore, the effective bandwidth of the Z -spectrum was $BW_C^{\text{eff}} \approx 1280 \text{ Hz}$ ($= 10 \text{ ppm}$), as per Equation [3]. The number of acquired samples was 64 covering the range of $[-800, +800] \text{ Hz}$ and therefore the resolution was 25 Hz ($\approx 0.2 \text{ ppm}$), as per Equation [4]. $TR/TE = 3000/75 \text{ ms}$ and $NSA = 8$, leading to total scan duration of 48 s. No additional B_0 inhomogeneity correction was used.

CEST-S data were collected using the same parameters with the same VOI and same shimming methods. The number of points in the Z -spectra was 41 in the range $[-640, +640] \text{ Hz}$. In CEST-I, the imaging parameters were: multi-shot TSE acquisition with echo train length $ETL=16$, $FOV = 200 \times 200 \text{ mm}^2$, the in-plane matrix size $= 112 \times 112$, pixel resolution $= 1.79 \times 1.79 \text{ mm}^2$, transverse slice thickness $= 5 \text{ mm}$, also 41 points in the range $[-640, +640] \text{ Hz}$, $TE/TR = 7/3000 \text{ ms}$. In order to correct B_0 inhomogeneities in CEST-I, WASSR data acquired with the saturation range $[-320, +320] \text{ Hz}$ and the weak saturation pulse $B_1 = 0.13 \text{ } \mu\text{T}$, duration $= 100 \text{ ms}$.

Second-order PB-volume shimming was employed by CEST-S and UCEPR and global first-order PB-volume shimming was employed by CEST-I. All of the in vivo Z -spectra were filtered by a 3-point median filter to decrease signal variation. All offsets were fitted using a 12th-order polynomial (15,39). To maintain the same resolution across different CEST methods, all fitted curves were calculated using the experimental offsets. Slightly different processing was used for in vivo data vs phantom data: filtering and fitting in former vs interpolation in later. This was done because: (i) the SNR and CEST effects are significantly smaller in vivo than in phantoms and (ii) this processing was used in the in vivo data reported earlier (see e.g. refs. (15,39)).

RESULTS

Z-spectroscopy in phantoms

Fig. 3a shows the UCEPR signal acquisition in the iopamidol sample with pH 6.0, where S_{sat} is CEST intensity with saturation, and S_0 is the reference signal without saturation (i.e. the profile of VOI). S_{sat} and S_0 were very similar at the edges of the spectra, both decreasing as they reached the edges of VOI (recall $FOV_T > L_T$). The UCEPR Z -spectrum was obtained after normalization of S_{sat} by S_0 , as shown in Fig. 3b. Due to noise, S_{sat} was slightly higher than S_0 at some points at the edge of BW_C where the signal was very weak. Fig. 3c shows

UCEPR Z -spectra for all of the iopamidol samples using identical pulse sequence and parameters. Fig. 3c indicates that UCEPR can successfully distinguish different CEST effects in iopamidol solutions with different pH values. In addition, the two peaks at 4.2 ppm and 5.5 ppm (generated by the two amide proton pools in iopamidol) were observable. A broad inflection of the CEST spectrum was also observed at the position of the $-OH$ proton pool, around 1.2–2 ppm.

Fig. 4 shows validation of UCEPR against standard CEST imaging and spectroscopy. There was a good agreement between the Z -spectra acquired in different ways for the same sample. To be consistent with UCEPR, the range of frequency offsets and Z -spectrum resolution were set to be the same (see methods and Table 1).

UCEPR characterization

To study some of the salient features of the UCEPR method, Z -spectra were acquired using various parameters. Z -spectra acquired with different saturation B_1 strengths is shown in Fig. 5a. Just like in standard CEST, larger B_1 had led to stronger CEST effects and a broader water peak. However, as expected, all peak positions in the Z -spectra remained at the same frequency offsets. The effect of Z -spectra acquired with different NSA was studied in Fig. 5b. As expected, higher NSA led to less noise and a more stable signal. Z -spectra with $NSA = 2, 4$, and 8 were all very similar, showing cancellation of signal instabilities, while the Z -spectrum with $NSA=1$ still presented observable intensity variations. While less NSA led to less scan time (6, 12, 24, and 48 s for NSA of 1, 2, 4, and 8, respectively), the additional increase in SNR that came with the increased NSA may be beneficial when computing the small CEST effects in vivo. The presence or absence of phase cycling as well as increasing TR to 10 s (data not shown) did not influence the results. Though some sort of “steady-state” effect cannot be completely ruled out, we hypothesize that increased NSA , leading to better SNR, potentially eliminated noise related instabilities in UCEPR.

The effect of the number of sampling points (N), which determines the resolution of the Z -spectrum through Equation [4] is shown in Fig. 5c. Here, $N = 32, 64$, and 128 had led to resolution of 0.6, 0.3 and 0.15 ppm in the Z -spectrum, respectively. When more sampling points were acquired in UCEPR, a longer sampling time was required, leading to a longer TE if the acquisition bandwidth was kept the same. Here, in the case of constant $BW_r = 4000$ Hz, TE's were 43, 51, and 67 ms for $N = 32, 64$, and 128 , respectively. As seen from the Fig. 5, all three cases presented very similar Z -spectra.

The effects of various readout bandwidth BW_r and gradient G_r in acquiring the Z -spectra are shown on Fig. 5d. According to Equation [1], if BW_r is changed, G_r needs to be changed proportionally to produce the same FOV_r . Here, $G_r = 3.0, 4.5$ and 6.0 mT/m corresponding to $BW_r = 4000, 6000$, and 8000 Hz. When higher BW_r was used in PRESS, a shorter acquisition time was required, leading to a shorter TE with the same number of sampling points. Here, for $N = 64$, TE's were 51, 46, and 43 ms when $BW_r = 4000, 6000$, and 8000 Hz, respectively. A longer TE will reduce the intensity of signals which may affect the CEST contrast. However, after normalizing by the non-saturated scan, we observed a good agreement between the Z -spectra with different TE's, as seen in Fig. 5c,d. Aqueous solutions typically have long T_2 's and the signal decrease for increasing TE will be more

pronounced in tissue or in short T_2 samples. However, we anticipate that similar to PRESS, as long as the SNR remains reasonable, normalization will be sufficient to recover correct CEST.

UCEPR Amide Proton Transfer measurements in human brain

Amide Proton Transfer (APT) effects in white matter of healthy human brain were measured by UCEPR. Fig. 6a shows a sagittal slice of human brain and a red box of the selected VOI in the desired location of white matter. In yellow, the shimming box is indicated. The acquired UCEPR intensities S_{sat} and S_0 are shown in Fig. 6b, with Fig. 6c showing the Z -spectrum calculated as S_{sat}/S_0 . The zero-value points at the edges of the spectrum in Fig. 6b had been removed in Fig. 6c. As seen, fully localized Z -spectrum were obtained in vivo in less than a minute, thus opening the possibility for clinical applications of this method.

It is known that the APT effect used to image cellular mobile proteins and peptides depends strongly on the RF irradiation power (40). Therefore, three power levels, $B_1 = 1.0, 2.0$, and $3.0 \mu\text{T}$ were tested with UCEPR in vivo. MTR_{asym} for each power level was calculated to quantify APT. APT contrast from healthy brain tissue is fairly weak, reaching only several percent and often negative due to the underlying MT (3). Fig. 7a shows the processed Z -spectra with different saturation power levels. With higher B_1 , the saturation effect was larger, as expected due to the higher degree of direct water saturation. The MTR_{asym} curve for each B_1 was calculated (Fig. 7b) and the value at 3.5 ppm with respect to water resonance was used to quantify the APT effect. Fig. 7c shows that the measured MTR_{asym} at 3.5 ppm increases nearly linear with increasing B_1 . It was also evident that the MTR_{asym} close to 0 ppm behaved erroneously, probably due to direct water effects and the influence of MT (3,6).

To probe the equivalency of UCEPR to the known methods for measuring CEST, in terms of ability to generate similar CEST contrast, a comparison was performed between UCEPR and CEST-I and CEST-S. Fig. 8 shows in vivo Z -spectra and MTR_{asym} spectra for each CEST method using $B_1 = 2 \mu\text{T}$. To acquire a consistent APT effect, the location of the scan slice in CEST-I was the same as the location of VOI in UCEPR or CEST spectroscopy. An ROI at the location of the VOI in UCEPR was selected to form the Z -spectrum from the CEST-I data. The same post-processing method was applied when processing all scanned data. While Fig. 8a demonstrates an overall agreement between the Z -spectra acquired using the three methods, deviations are observed in the calculated MTR_{asym} curves. Several reasons may contribute to the deviations, as discussed in the following section.

DISCUSSION

As shown in phantoms and in vivo, UCEPR is capable of rapid localized Z -spectroscopy. The main underlying assumption is a homogenous distribution of the CEST “agent”. Figs. 3 and 6 demonstrated the acquisition and normalization of the signals in phantoms and in vivo. Various pH values are clearly distinguishable in phantoms, with the UCEPR Z -spectrum preserving the resolvable signature of the different exchangeable protons. Thus, UCEPR, similarly to its non-localized UFZ counterpart, can be applied for a fast scanning of potential

CEST agents. However, in contrast to the non-localized UFZ, more than one sample can be present during scanning and the samples can be arranged in an arbitrary fashion in space.

As Fig. 4 demonstrates, the results of UCEPR are in excellent agreement with the more standard CEST acquisition methods. The specific acceleration factor for UCEPR compared with standard methods would depend on the details of the implementation (e.g. how many points in the Z -spectrum and what sequence used for imaging, as well as how many $NSAs$ used in UCEPR). In the current implementation of the standard CEST imaging and spectroscopy, the UCEPR's time acceleration is six-fold compared with imaging and four-fold compared with spectroscopy (using $NSA = 8$ for UCEPR).

Characterization of the UCEPR acquisition with different saturation and acquisition parameters was demonstrated in Fig. 5. Simplistically, the UCEPR approach can be viewed as dividing the VOI into an N number of sub-samples, each resonating at its own frequency. Thus, a reduction in SNR is expected compared with standard methods. While we observed that $NSA = 4$ in Fig. 5b leads to results almost identical to $NSA=8$, we have continued using $NSA=8$ for consistency. However, if additional acceleration is needed for a specific project, $NSA=4$ or even 2 could be used.

The resolution of the Z -spectrum can be changed by setting a different number of samples, N , as exemplified in Fig. 5c. Increasing N leads to the associated increase in TE, as described in the Results section. Thus, UCEPR is capable to achieve a high-resolution Z -spectrum acquisition by increasing the number of samples, yet without the concomitant increase of the total scan time. This is in contrast with the conventional CEST imaging and spectroscopy, where increasing the Z -spectrum resolution linearly increases the total scan time to accommodate a large number of acquisitions. The ability of UCEPR to generate a continuous, high resolution Z -spectra may prove useful in discerning additional features in the exchangeable protons profiles. While TE increases with increased N , and the raw CEST signal intensity becomes weaker, following normalization, the UCEPR Z -spectra with different resolutions are consistent, without any notable decrease in overall CNR. UCEPR properties were further investigated in Fig. 5d, where different readout bandwidths were used (and different TEs as the result). Again, within the parameters tested, no apparent decrease in CNR was observed.

In vivo, increasing N and thus increasing TE may lead to TE times which are prohibitively long (considering that a typical T_2 for the white and gray matter at 3T is on the order of 60 ms and 80 ms, respectively (41)). This situation is similar to standard PRESS. Indeed, the signal decay is governed by PRESS properties. Thus, in tissues with short T_2 or if very large N (high resolution Z -spectrum) is needed, PRESS can be replaced with STEAM (42).

The in vivo performance of UCEPR was demonstrated in the human brain and shown on Figs. 7 and 8. The relationship between the APT effect and saturation power level was investigated in Fig. 7. Large fluctuations are observed close to the zero (water) frequency, especially at lower B_1 . These might be associated in part with the effect of direct saturation of water, increased number of points acquired around zero as well as decreased APT effect

at lower B_1 value. Additionally, we cannot rule out the influence B_0 inhomogeneity, as discussed in more details in the following.

Fig. 8 compared the in vivo results acquired using different techniques. Overall, the agreement of the Z-spectra is good, especially away from zero frequency. However, close to the zero frequency, MTR_{asym} acquired with UCEPR deviated from other methods. There are a number of potential factors contributing to the deviation. First increased number of points around zero; second, slightly lower saturation (Fig. 8a) at zero; third decreased SNR around zero frequency (due to overall signal reduction). The first may lead to the increased Z-spectrum intensity around zero, the second and the third to different MTR_{asym} values (after fitting/interpolation and subtraction is done). Finally, while the selected volume is small and second-order shimming was used, it is still possible that sufficient B_0 inhomogeneity was present, its influence to be discussed in the next paragraph. Despite these differences near zero, the asymmetry curves at higher frequencies, and especially the APT effect at 3.5 ppm, agree well between all three CEST methods.

As already mentioned, a potentially important contributor to the deviations observed between UCEPR and CEST-I and CEST-P is B_0 inhomogeneity. It is well established that CEST is sensitive to B_0 . Most of the works so far focused on the influence of B_0 on the resonance frequency and the necessity to correct for it (vide supra). In UCEPR, B_0 inhomogeneity may add to the encoding gradient, thus producing an overall shift in the Z spectrum, as discussed by Xu *et.al.* (30). Indeed, careful inspection of Fig. 8a indicates slight shift in the minima of UCEPR spectrum vs CEST-I and CEST-S, similar to the shift observed by Dopfert *et.al.* (31). Slightly different shimming and F0 re-adjustment automatically performed by the scanner between the scans may cause the shift. This small shift may lead to increased deviations in MTR_{asym} around zero frequency, as observed. Employing one of the fitting methods described above and re-aligning the minimum of Z-spectrum with zero frequency could potentially eliminate this imperfection.

In addition, as was discussed by Dopfert *et.al.* in Ref. (31), B_0 imperfections exist in all directions, thus potentially complicating ultrafast CEST spectroscopy. It may lead to broadening the spectral lines, thus obscuring or producing an erroneous CEST effect. This effect may also present in CEST-I and CEST-S but generally is not acknowledged in imaging (except generally challenging/impossible CEST imaging in hard to shim areas, e.g. near sinuses). To investigate this point further we have conducted a series of experiments under different shimming conditions. The results are presented in the Supplementary material. To achieve optimal shim, a standard shimming procedure, the PB-shimming, available on commercial Philips scanners, was used. Overall, UCEPR results are very consistent under good shimming conditions. No difference was observed between first or second order shimming, with $MTR_{\text{asym}}(4.2\text{ppm})=41\%$ and $MTR_{\text{asym}}(5.5\text{ppm})=24\pm 1\%$. However, when artificially de-shimmed, the MTR_{asym} measured by UCEPR deviated quickly from the “good” values. To evaluate the shim, FWHH measured by PRESS spectroscopy was employed (the same voxel in PRESS and UCEPR). Perhaps surprisingly, the change in MTR_{asym} was not linear with the increase in FWHH. When the FWHH increased by 30 to 400 %, the MTR_{asym} remained approximately stable around 51% and 31% for 4.2 and 5.5ppm respectively. Finally, when the shimming was so bad the linewidth increased almost

tenfold compared with the “good” shim, the UCEPR data became completely and obviously unreliable. This short study indicates importance of the good shimming for a reliable UCEPR application. At the same time we would like re-iterate its importance for any type of CEST studies.

Compared to the conventional CEST imaging and spectroscopy, UCEPR substantially reduces the scan time. In Table 1, the acquisition times and other parameters of Z -spectrum acquisition in each method can be found, as implemented in our study. It shows that UCEPR possesses advantages in speed of acquisition and increased resolution of Z -spectroscopy. Although the spatial resolution is less than in CEST imaging (i.e. the size of voxel is larger), UCEPR is capable of acquiring Z -spectra with increased temporal and spectral resolution, which may prove useful in additional characterization of lesions whose location is already known or in observation of agents with multiple exchanging groups.

In general, many factors will influence the ultimate timing performance and advantages (or lack thereof) of UCEPR compared with CEST-I or CEST-S. Note that the parameters that we have used here, specifically, the number of frequencies in the Z -spectra and acquisition of additional WASSR data, may not be optimal or minimal and it might be possible to reduce the total conventional CEST-I scan time. However, a brief (non-encompassing) survey of the last two years literature indicated that in vivo human studies at 3T used at least 10 frequencies in the Z -spectra (22,23,43–53). Thus, for most cases UCEPR would be faster or comparable. Additional time could be saved if fitting methods are used instead of WASSR (in our case reducing total CEST-I scan time from 330 s to 215 s and from 510 s to 255 s, for phantom and in vivo experiments, respectively). However, the fitting may be challenging in vivo at 3T where broad lines are present. Moreover, in this case too, good spectral resolution would be required for a reliable fit, potentially increasing the total acquisition time. Thus, UCEPR still could provide faster acquisition with much higher Z -spectral resolution. Finally, if many samples were present simultaneously, UCEPR would have to be repeated for each one of the samples. Depending on how many samples are needed to be scanned, and how are they arranged in space, conventional CEST imaging or 2D ultra-fast Z -spectroscopy (31), may provide faster scan times. In summary, it will be up to the researcher discretion and depend on an application, as to which approach and implementation to choose.

As in any other technique, UCEPR is sensitive to various system imperfections and instabilities. The influence of B_0 has already been discussed, and we would like to note two additional influences here. First, the space-frequency mapping relies on the one-to-one correspondence between gradients values and frequency. The gradients are notoriously non-linear, which will affect this correspondence. However, for the very small FOV used in UCEPR (up to 3 cm) the gradients can still be considered linear, and the effects of non-linearity can be ignored (54). Second, CEST is also sensitive to B_1 value. However, it was shown that CEST effect is not very sensitive to the B_1 inhomogeneity, provided that the field is reasonably homogenous (26). UCEPR collects data from a relatively small volume, compared to imaging, and the assumption of homogenous B_1 should be valid.

Although the results presented here are focused on DIACEST, the method is applicable to PARACEST. The main caveat in translating the methodology to PARACEST is the

requirement that the gradient strength should be high enough to allow the acquisition of the increased bandwidth of the Z -spectrum. However, this should be achievable on standard clinical 3T scanners for PARACEST agents, especially if only the characteristic part of the Z -spectrum is acquired. This study is currently underway and will be presented separately.

CONCLUSIONS

The ultrafast localized CEST-spectroscopy with PRESS (UCEPR) is proposed and demonstrated on a 3T clinical scanner for phantom and in vivo applications. Experimental results show that voxel-based Z -spectrum information at the desired location can be acquired with as little as four scans. This may lead to significant decrease in the scan time in vivo, compared to a conventional CEST imaging. Thus, UCEPR allows fast assessment of suspicious areas and could become an important tool in the clinical setting. Though it does not offer spatial information, it would offer a localized assessment of metabolites or physiology on a time scale unattainable by the standard CEST methods, allowing, for instance, monitoring of dynamic processes. One example, in cases where lesion already identified (e.g. breast or brain cancers) it may offer quick assessment and may be an easier alternative to CEST imaging in a clinical setting. In these cases UCEPR could be used for initial assessment (malignant/non-malignant) as well as in treatment follow-up. Second, it may offer some advantages in hard to shim areas (breast or higher fields), since only localized shimming is required. Third, in ratio-metric quantifications it may offer increased spectral resolution. Finally, it may allow the practical implementation of quantification methods, e.g. Omega-plots (55), in relatively fast fashion. We envision that the sequence will complement imaging and spectroscopy methods used in vivo today.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1. UCEPR pulse sequence. The new added elements, as compared to a standard PRESS, are presented in gray and in dashed line. See the text for more details.

FIG. 2.

Z-spectra encoding and acquisition in UCEPR. **a:** The geometry of VOI. The VOI with length L_r along x -axis is located at a position x_0 . **b:** Dashed line: a schematic of *Z*-spectrum acquired using non-localized UFZ with $\omega_s = 0$, solid line: the acquired region of *Z*-spectrum due to PRESS localization. Due to localization, only signal originating from the VOI is non-zero, the rest of the spectrum will be occupied by noise. **c:** Optimized acquisition with RF offset $\omega_s = \gamma G_C x_0$ and acquisition frequency offset $f = \gamma G_r x_0$, thus aligning the *Z*-spectrum with VOI and allowing for best CEST sampling efficiency.

FIG. 3.

UCEPR Z -spectroscopy in phantom (saturation pulse: $B_1 = 1.05 \mu\text{T}$, duration = 500 ms). **a:**

Intensities of UCEPR signal with saturation S_{sat} and without saturation S_0 . **b:** The real Z -

spectrum obtained by S_{sat}/S_0 . **c:** UCEPR Z -spectra can distinguish different iopamidol

samples with different pH values.

FIG. 4.

Comparison of Z -spectra of iopamidol samples acquired by UCEPR, CEST imaging (CEST-I) and CEST spectroscopy (CEST-S). The saturation pulse: $B_1 = 1.05 \mu\text{T}$, duration = 500 ms. Z -spectra of samples with (a) pH 6.0, (b) pH 7.0 and (c) pH 8.0.

FIG. 5. Characterizing UCEPR Z-spectroscopy with different acquisition parameters. **a:** $B_1 = 1.05$, 1.32, 1.58, 1.85, and 2.11 μT . **b:** Number of signal averages 1, 2, 4, and 8. Phase cycling was used for later three values. **c:** Number of acquisition sampling points $N = 32$, 64, 128. **d:** Acquisition bandwidth $BW_r = 4000$, 6000, and 8000 Hz.

FIG. 6.

A UCEPR Z-spectrum acquired in white matter. **a:** A sagittal slice of human brain, with the UCEPR VOI indicated by the red box. **b:** Intensities of UCEPR signal with saturation S_{sat} and without saturation S_0 . **c:** Actual Z-spectrum obtained by S_{sat}/S_0 .

FIG. 7.

UCEPR Z-spectra and MTR_{asymp} spectra of white matter from a healthy subject with different B_1 saturation levels. **a:** UCEPR Z-spectra with $B_1 = 1.0, 2.0$, and $3.0 \mu\text{T}$. **b:** Corresponding localized MTR_{asymp} curves. **c:** MTR_{asymp} at 3.5 ppm versus B_1 .

FIG. 8.

An in vivo comparison of UCEPR, CEST-I, and CEST-S. **a:** Z-spectra acquired with $B_1 = 2$ μ T, and **b:** The corresponding MTR_{asym} curves.

Table 1

Summary of Z-spectrum protocols

	CEST method	Protocol	TE/TR (ms)	Voxel size (mm ³)	Offset Freq. range (Hz)	Num. of points	Total Num. of scans	Total scan time (s)
In phantom	UCEPR	SV, echo, PRESS	51/3000	13.0×13.0×30.0	-1280:1280	64	16	48
	CEST-S	SV, echo, PRESS	51/3000	13.0×13.0×30.0	-1280:1280	63	64	192
	CEST-I	TSE, single-shot	7/3000	1.96×1.96×10.0	-1280:1280	63	64	215
	WASSR	TSE, single-shot	7/3000	1.96×1.96×10.0	-1280:1280	41	42	115
In vivo	UCEPR	SV, echo, PRESS	75/3000	10.0×15.0×10.0	-640:640	64	16	48
	CEST-S	SV, echo, PRESS	75/3000	10.0×15.0×10.0	-640:640	41	42	126
	CEST-I	TSE, ETL=16, SENSE=2	7/3000	1.79×1.79×5.00	-640:640	41	42	255
	WASSR	TSE, ETL=16, SENSE=2	7/3000	1.79×1.79×5.00	-640:640	41	42	255