Analysis Protocol for the Quantification of Renal pH Using Chemical Exchange Saturation Transfer (CEST) MRI

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Abstract

The kidney plays a major role in maintaining body pH homeostasis. Renal pH, in particular, changes immediately following injuries such as intoxication and ischemia, making pH an early biomarker for kidney injury before the symptom onset and complementary to well-established laboratory tests. Because of this, it is imperative to develop minimally invasive renal pH imaging exams and test pH as a new diagnostic biomarker in animal models of kidney injury before clinical translation. Briefly, iodinated contrast agents approved by the US Food and Drug Administration (FDA) for computed tomography (CT) have demonstrated promise as novel chemical exchange saturation transfer (CEST) MRI agents for pH-sensitive imaging. The generalized ratiometric iopamidol CEST MRI analysis enables concentration-independent pH measurement, which simplifies in vivo renal pH mapping. This chapter describes quantitative CEST MRI analysis for preclinical renal pH mapping, and their application in rodents, including normal conditions and acute kidney injury.

This publication is based upon work from the COST Action PARENCHIMA, a community-driven network funded by the European Cooperation in Science and Technology (COST) program of the European Union, which aims to improve the reproducibility and standardization of renal MRI biomarkers. This analysis protocol chapter is complemented by two separate chapters describing the basic concepts and experimental procedure.

Key words Chemical exchange saturation transfer (CEST), Magnetic resonance imaging (MRI), pH, Rats, Mice, Iopamidol, Kidney, Contrast agents, pH imaging

1 Introduction

Chemical exchange saturation transfer (CEST) MRI provides a sensitive means to image microenvironment properties such as tissue pH, temperature, metabolites, and enzyme activities via dilute labile protons [1–12]. Endogenous CEST MRI has been increasingly adopted for imaging a host of disorders including acute ischemic stroke [13–22], tumor [23–27], and epilepsy [28, 29]. In addition, CEST MRI contrast agents have been developed for exogenous CEST imaging that may provide more sensitive

CEST detection which is specific to the administered agents [30–34]. This is because the labile proton exchange rate and chemical shifts can be designed/preselected to optimize these for exogenous CEST MRI contrast [35–38]. There has been an emerging library of iodinated-based CEST agents such as iopamidol, iobitridol, iopromide, and iodixanol, which have been approved by the U.S. Food and Drug Administration (FDA) for computed tomography (CT) head and body imaging applications [39–47]. Such FDA-approved iodinated contrast agents are promising for translational CEST imaging to characterize renal dysfunction, diagnose regional kidney injury before symptom onset, and help guide treatment before irreversible damage [48–50].

Because the CEST MRI effect depends on not only pH-dependent exchange rates but also on the labile proton ratio, relaxation rates and experimental conditions such as magnetic field strength, saturation power, and duration, CEST-weighted MRI contrast is often complex [51–55]. As such, it remains challenging to quantify CEST MRI toward tissue indices such as absolute pH and/or total protein concentration. Persistent progress has been achieved toward simplified and quantitative in vivo pH mapping [56–61]. In particular, ratiometric CEST MRI refers to a specific type of CEST MRI analysis that takes multiple CEST measurements, the ratio of which normalizes common confounding factors such as tissue labile proton concentration and relaxation effects, therefore enabling quantitative in vivo CEST mapping [42, 56, 62–67]. Although pH imaging is more straightforward at high magnetic fields ($B_0 \geq 7$ T) due to the large frequency shift difference between labile and bulk tissue water protons, it is important to extend pH MRI to magnetic fields such as 3 and 4.7 T [68, 69]. Due to the complexity of the source of the MRI CEST signal, multiple approaches have been established for a better quantification of the CEST contrast [54, 60]. In this chapter, we describe variant ratiometric pH CEST MRI analysis techniques, image down-sampling expedited adaptive least-squares (IDEAL) fitting algorithm, smoothing splines interpolation algorithm and use of iodinated CEST agents for mapping renal pH in vivo [50, 70].

This analysis protocol chapter is complemented by two separate describing the basic concepts and experimental procedure, which are part of this book.

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2 Materials

2.1 Software Requirements

2.1.1 Essential Tools

1. Matlab/Python: The method described in this chapter requires MATLAB (MathWorks, Natick, MA, <https://www.mathworks.com/products/matlab.html>) for data analysis of applying fitting models and measuring ratiometric. Because Matlab functions used in the data processing can also be implemented in python (<https://www.python.org/>), python can be used instead.
2. An image processing software (e.g., Image J, we recommend using Fiji, which is ImageJ with a wide range of plugins already included, <https://fiji.sc/>, open-source), as a practical tool for the image quality check or to measure SNR.

2.1.2 Optional Tools

A statistical analysis software (e.g., SPSS, SPSS Inc., Chicago, IL or Prism, GraphPad, USA) as a practical tool for the statistical significance calculation.

2.2 Source Data: Format Requirements and Data Preprocessing

2.2.1 Input Requirements

The CEST images can be retrieved directly from the MRI scanner as raw binary images or in DICOM format (*see Note 1*). To be able to perform CEST analysis different scans are needed:

- Anatomical image (optional).
- Unsaturated image (optional).
- CEST images or Z-spectra.
- CEST images (Z-spectra) before and after contrast agent (CA) injection for in vivo acquisitions.

Information about some scan acquisition parameters is also necessary as is the frequency offsets vector.

2.2.2 Background Removal

In order to avoid the analysis outside the object of interest and to shorten the analysis duration a first segmentation between the background and the imaged object is suggested. Manual thresholding or automatic thresholding (as by the Otsu method) can be easily applied within the Matlab environment.

3 Methods

3.1 Motion Correction

If needed, postprocessing motion correction can be applied to coregister images to correct for motion artifacts.

3.2 Z-Spectra Analysis

Z-spectra can be analyzed as mean contribution inside one or more regions of interest, that can be drawn on an anatomical image reference, or preferentially, by a voxel-by-voxel analysis. Different approaches for Z-spectra analysis will be described in detail.

3.2.1 Multi Pool Lorentzian Fitting

The Z-spectra are numerically described using a multipool Lorentzian model [24, 71–74]:

$$Z(\omega) = 1 - \frac{M_z}{M_0} = \sum_{i=1}^N L_i(\omega) \quad (1)$$

where ω is the frequency offset from the water resonance, N is the total number of proton pools, and L_i is the Lorentzian spectrum of the i th pool. The Lorentzian lineshape is represented by the following equation:

$$L(\omega) = \frac{A}{1 + 4\left(\frac{\omega - \omega_0}{\sigma}\right)^2} \quad (2)$$

where A , ω_0 , and σ are the amplitude, center frequency, and linewidth of the i th saturation transfer effects, respectively.

1. Map B_0 inhomogeneity with water saturation shift referencing (WASSR) using external acquired B_0 maps, by field maps or by interpolation procedures looking to the minimum of the Z-spectrum (internal B_0 mapping) [75–79].
2. Shift CEST MRI Z-spectrum based on the field inhomogeneity, per voxel, for correction.
3. Normalize the Z-spectra (i_z) by the signal without RF irradiation (M_0).
4. Fit the Z-spectrum using a five pool Lorentzian model (two pools for iopamidol amide groups at 4.3 and 5.5 ppm, one for bulk tissue water (0 ppm), and two pools for the hydroxyl groups at 0.8 and 1.8 ppm) [80, 41]. Representative multipool Lorentzian fitting is described in Fig. 1.

3.2.2 Image Down-Sampling Expedited Adaptive Least-Squares (IDEAL) Fitting Algorithm

1. Initially down-sample the B_0 field inhomogeneity-corrected CEST images to one or a few pixels and calculate the global Z-spectrum by averaging the Z-spectra of all voxel within an ROI to substantially improve the signal-to-noise ratio (SNR) for the numerical fitting.
2. Set the boundaries to be between 1% and 100 times of the initial values for the amplitude and linewidth of each chemical pool, with their peak frequency shift within ± 0.2 ppm of the initial chemical shift. The relaxed boundary constraints ensure that the initial fitting provides a reasonable estimation of the multiple Lorentzian pools under the condition of sufficiently high SNR.
3. Fit the down-sampled image exploiting.
4. Resample the CEST images to 2×2 matrix size.

Fig. 1 Multipool Lorentzian fitting of representative CEST Z-spectra from a 40 mM iopamidol solution vial with a pH of 7.0. CEST Z-spectrum obtained from under (a) $B_1 = 1 \mu\text{T}$ and (b) $2 \mu\text{T}$ under body temperature. A five-pool Lorentzian model was used to describe the CEST spectrum [Adapted with permission from *Magnetic Resonance in Medicine* 2018 (A generalized ratiometric chemical exchange saturation transfer (CEST) MRI approach for mapping renal pH using iopamidol, Volume: 79, Issue: 3, Pages: 1553–1558, DOI: <https://doi.org/10.1002/mrm.26817>)]

5. Take the initial values for the fitting of each voxel of the resampled image from the results of the previous image with lower spatial resolution.
6. Set the boundary constraints relatively loose albeit narrower than the initial fitting, to be 10% and ten times of the initial values.
7. Use a nonlinearly constrained fitting algorithm with twofold overweighting applied for Z-spectra between 4.0 and 5.8 ppm to increase the fitting accuracy of Iopamidol CEST effects at 4.3 and 5.5 ppm.
8. Resample the CEST images, 4×4 , 8×8 , 12×12 , 24×24 until the original resolution of 48×48 or voxel-wise multipool Lorentzian fitting and repeat from **step 5** until you get the desired final resolution.
9. Figure 2 shows the flowchart of the IDEAL fitting algorithm.

3.2.3 Smoothing Splines Interpolation

The cubic smoothing splines estimate the interpolating function f , minimizing the following expression, linearly composed by two parts:

$$p \sum_{j=1}^N |y_j - f(x_j)|^2 + (1 - p) \int |D^2 f(t)|^2 dt \quad (3)$$

The first addend represents the mean square error between data y_j and the interpolating cubic spline $f(x_j)$ calculated in x_j measure

Fig. 2 Illustration of the Image Downsampling Expedited Adaptive Least Squares (IDEAL) CEST analysis algorithm. **(a)** Flow chart of data processing steps of the IDEAL fitting algorithm. **(b)** The intermediate fitting results of iteratively less downsampled images from a two-compartment CEST phantom [Adapted with permission from *Scientific Reports*. 2017 (Quantitative chemical exchange saturation transfer (CEST) MRI of glioma using Image Downsampling Expedited Adaptive Least-squares (IDEAL) fitting, Volume: 7, Issue: 1, Pages: 84, DOI: <https://doi.org/10.1038/s41598-017-00167-y>)]

points. The second term consists of the integral of the squared second-order derivative of f and is a measure of function flexibility. p is a smoothing parameter and determines the relative weight you would like to place on the contradictory demands of having

f be smooth ν having f be close to the data. Its value ranges between 0 and 1. For $p = 1$, the curvature constraint is nullified, f passes for all data points and converges to the interpolating spline, while, at the other extreme, for $p = 0$, f curvature is minimized and f results in a linear least square fit.

1. Normalize pre- and postinjection Z-spectra to the maximum intensity value of the free water signal, generally corresponding to the most distant offset or to the unsaturated image (M_0) [79].
2. Interpolate each voxel Z-spectra data with a cubic spline function paying action to regularization factor selection (*see Note 2*) and ignoring the background pixels/voxels (in Matlab use `csasp` function).
3. Find in the fitted spectra the absolute minimum corresponding to bulk water frequency offset (in Matlab use `fmin` function).
4. Use the minimum position (corresponding to water peak shift from zero) to correct B_0 inhomogeneity shifting the frequency offsets vector.
5. Save minimum position in a matrix to construct the B_0 shift map.

To exclude noisy data points from the analysis a filtration step is suggested:

6. Construct R^2 matrix evaluating the distance of the interpolating function to data in each pixel.
7. Define a R^2 threshold (in our application R^2 ranges between 0.97 and 0.99).
8. Ignore pixel for which the R^2 value is lower than the threshold.

Smoothing spline algorithm workflow is described in Fig. 3.

3.3 CEST Quantification

After having fitted the Z-spectra by smoothing splines or Lorentzian fitting, CEST contrast quantification can be evaluated and ratio-metric values (ratio between two CEST contrast quantifications to remove the concentration effect) can be calculated.

3.3.1 CEST Ration Calculation by Asymmetry Analysis

The CEST ratio (CESTR) is calculated by asymmetry analysis:

$$\text{CESTR}(\omega) = \frac{M_z(-\omega) - M_z(\omega)}{M_0} \quad (4)$$

where ω is the labile proton chemical shift from the bulk water resonance (for Iopamidol $\omega = 4.2$ ppm and 5.5 ppm). For the in vivo images, contrast was calculated by subtracting contrast after CA injection from the contrast before the injection at the different frequency offsets.

Fig. 3 Representative z-spectra from in vitro (bottom) and in vivo (top) images showing the analysis steps for data normalization, smoothing splines interpolation and B_0 shift correction (from left to right)

1. Calculate CEST contrast according to Eq. 4 for the two pools of Iopamidol (4.2 and 5.5 ppm).

3.3.2 CEST Ratio
Calculation from Lorentzian Fitting

1. Calculate CEST contrast from the amplitude obtained from the Lorentzian fitting according to Eq. 4 for the two pools of Iopamidol (4.2 and 5.5 ppm).

3.3.3 Chemical
Shift-Based Ratiometric CEST Analysis

Because the direct saturation is relatively small at the magnetic field at or above 7 T, the coupling between multiple CEST effects is relatively small, and a ratiometric analysis of two CEST effects obtained under the same RF irradiation power level can be calculated for pH calibration, as

$$R_{ST}(pH) = \frac{CESTR(\delta 1)}{CESTR(\delta 2)} \tag{5}$$

3.3.4 RF Power-based
Ratiometric CEST Analysis

For CEST agent of a single labile proton group, the conventional ratiometric analysis does not apply. It has been shown that the ratiometric analysis can be generalized by taking the ratio of CEST effects obtained under two RF power levels as [42].

$$R_{ST}(pH) = \frac{CESTR(B_{1b})}{CESTR(B_{1a})} \tag{6}$$

If the direct water saturation is not negligible, the saturation effect can be calculated/removed to improve the precision of RF power-based ratiometric analysis [56, 64].

3.3.5 The Generalized RF Power- and Chemical Shift-Hybrid Ratiometric CEST Analysis

When CEST MRI is performed at lower magnetic field strengths ($B_0 < 7.0$ T) or the RF saturation power induces nonnegligible direct saturation effect, the coupling between multiple CEST effects (i.e., CEST and direction water saturation) become nonnegligible. Under such conditions, the routine ratiometric analysis (chemical shift- and RF power-based methods) may be susceptible to the coupling. In addition, the coupling depends on the transverse relaxation rate, which may be the difference between phantom calibration and in vivo experiments. To minimize such confounding coupling effect, the CEST effect can be decoupled with the multipool Lorentzian model, and their ratio is more reproducible and specific to pH. As such, the ratiometric analysis is generalized to a ratio of CEST effects of different chemical shift obtained under different saturation power levels.

$$R_{ST}(pH) = \frac{CESTR(\delta 1, B_{1b})}{CESTR(\delta 2, B_{1a})} \quad (7)$$

1. Calculate the ratiometric values following one of the previously described Eqs. 5–7.

3.4 Set-Up of pH Calibration Curve

To investigate the relation between the R_{ST} value and the pH a calibration is needed. In the following steps the calibration done for Iopamidol on a pH varying phantom is described. Two examples of pH calibration curves obtained at 7 T and at 4.7 T are shown in Fig. 4.

1. Acquire CEST spectra images of the pH varying phantom;
2. Draw the ROI including only the pH compartment;
3. Evaluate mean CESTR at 4.2 and 5.5 ppm inside each compartment;
4. Calculate R_{ST} ;
5. Fit the R_{ST} as function of the titrated pH (usually a polynomial fit of the third order is selected).

It is worth to observe that by decoupling the CEST effects at 4.2 and 5.5 ppm, the generalized ratiometric CEST MRI index provides an extended range of pH measurement at 4.7 T (Fig. 4b). Note that such a calibration experiment is important to ensure that the pH dynamic range is sufficient to cover tissue pH of interest. The polynomial regression enables the derivation of the absolute pH map of renal images.

6. From the calculated ratiometric value derives the pH value according to the used calibration curve.

Fig. 4 Comparison of ratiometric analysis of Iopamidol at high magnetic field of 7 T (a) and at sub-high magnetic field of 4.7 T (b). Note that the routine R_{ST} of 5.5 and 4.3 ppm provides a limited pH MRI range below pH = 7 due to the CEST MRI effect coupling at high pH. In comparison, the modified approach extends the pH imaging range to 7.5 [Adapted with permission from *Magnetic Resonance in Medicine* 2018 (A generalized ratiometric chemical exchange saturation transfer (CEST) MRI approach for mapping renal pH using Iopamidol, Volume: 79, Issue: 3, Pages: 1553–1558, DOI: <https://doi.org/10.1002/mrm.26817>)]

3.5 In Vivo

Application for pH Mapping

3.5.1 pH Mapping by Lorentzian Fitting or IDEAL Approach

1. Obtain two representative in vivo CEST Z-spectra from a normal rat (or mouse) kidney during Isovue infusion under two RF saturation power levels of 1 and 2 μ T at 4.7 T as shown in Fig. 5.
2. Apply the IDEAL algorithm and perform Lorentzian decoupling to resolve Iopamidol CEST effects at 4.3 and 5.5 ppm.
3. Outline the renal cortex, medulla, and calyx based on T_2 -weighted MRI.
4. Z-spectra are broadened at a higher RF power level due to more prominent direct RF saturation effect. Notably, the CEST effect increases from the cortex, medulla to calyx. This is because pH gradually reduces from the cortex, medulla to the calyx, which causes a persistent reduction in the Iopamidol CEST exchange rate. As such, the saturation efficiency increases when the exchange rate becomes comparable to the saturation field. In addition, the kidney concentrates and excretes Isovue, resulting in a concentration gradient across the kidney.
5. Describe the CEST effect by a six-pool Lorentzian model (i.e., five-pool model plus semisolid macromolecule magnetization transfer (MT) effect in tissue). Corresponding parametric images for CEST contrast and pH mapping in rat kidneys are shown in Fig. 6.

Fig. 5 Demonstration of regional CEST Z-spectra of calyx (a, b), medulla (c, d), and cortex (e, f) at 1 and 2 μT , respectively [Adapted with permission from *Magnetic Resonance in Medicine* 2018 (A generalized ratiometric chemical exchange saturation transfer (CEST) MRI approach for mapping renal pH using lopamidol, Volume: 79, Issue: 3, Pages: 1553–1558, DOI: <https://doi.org/10.1002/mrm.26817>)]

Fig. 6 Demonstration of a renal pH map from a representative rat following Iopamidol injection at 4.7 T. The resolved maps of ST effects at 5.5 ppm (a) and 4.3 ppm (b) were obtained with the IDEAL fitting algorithm, from which the ratiometric map was obtained (c). (d) pH map overlaid on the corresponding T_2 -weighted image show the renal pH gradually decreasing from the cortex, medulla to calyx

3.5.2 pH Mapping by Using the Smoothing Splines Approach

1. Import your scans (anatomical image, pre- and postinjection CEST images) and save them in a matrix.
2. Use cubic spline algorithm to interpolate both pre- and post-injection Z-spectra as described in Subheading 3.2.3.
3. Use cubic spline interpolated Z-spectra to calculate CESTR at specific ω (+4.2 and +5.5 ppm for Iopamidol) or/and B_1 levels before and after the CA injection and save CESTR values in a matrix to construct CESTR maps (in Matlab use `fval` function to evaluate interpolating cubic spline values at ω corrected for calculated B_0 shift).

Fig. 7 Demonstration of a physiologic renal pH map from a representative mouse following lopamidol injection at 7 T. The resolved maps of ST effects at 4.2 ppm (a) and 5.5 ppm (b) were obtained with the smoothing splines algorithm, from which the ratiometric map was obtained (c). (d) pH map overlaid on the corresponding T_2 -weighted anatomical image show the renal pH gradually decreasing from the cortex, medulla to calyx

4. To remove endogenous effects and to isolate contrast agent contribution, subtract postinjection CEST contrast map to preinjection CEST contrast map (Fig. 7a, b).
5. Calculate the ratio map ratioing difference CEST contrast maps obtained at different ω values (Fig. 7c) or/and B_1 levels.
6. Derive pH map from the ratio map using the experimental calibration curve calculated in Subheading 3.4, step 6 (Fig. 7d).

3.6 Representation

The obtained Z-spectra and pH maps can be represented as averaged values in a region of interest (ROI) or as parametric pixel-by-pixel maps. Before the representation, in order to remove residual noise, contrast and pH maps can be filtered, by applying a threshold

corresponding to the measured signal intensities variability of the exploited MRI scanner to discriminate between enhanced and nonenhanced voxels, following CA injection (*see* **Note 3**).

1. Select a noise threshold.
2. Set to 0 or to NaN all pixels inside the map for which the contrast increment is lower than the threshold.
3. Use the anatomical image (or alternatively the first CEST image) to identify and draw one or more ROIs (in Matlab use the `roipoly` function).
4. Create a mask from the ROI(s), a matrix that contains 1 value inside the region of interest and 0 or NaN values outside.

For representing mean Z-spectra:

5. Calculate mean Z-spectra (pre- and postinjection), ignoring values outside the ROI.

For representing parametric maps:

6. Represent contrast and pH maps overlaid to the anatomical image for having a morphological reference.

Representative mean spectra are shown in Fig. 8, whereas parametric images for CEST contrast quantification and pH values calculated in rat kidneys at 4.7 T and in murine kidneys at 7 T are shown in Fig. 6 and Fig. 7, respectively.

3.7 Quantitative Analysis

Statistics values as mean, median, and standard deviation can be obtained evaluating maps inside different ROI (cortex, medulla, and calyx) to have a quantitative description of the analysis results.

3.8 Results Validation

3.8.1 Evaluation of Analysis Errors

The quality of CEST fitting can be evaluated by the following three methods: (1) coefficient of variation (standard deviation/mean) within ROI, (2) contrast-to-noise ratio (CNR) between the two vials in the phantom study calculated by $CNR = |S_1 - S_2| / \sqrt{(\sigma_1^2 + \sigma_2^2)}$, where S_1, S_2 are the mean values for the two ROIs and σ_1, σ_2 are their standard deviations, and (3) goodness of fit (R^2) maps.

3.8.2 Comparison with Reference Values from the Literature

It has been documented that kidney pH values are heterogeneous, with a gradient from the cortex, medulla to calyx due to filtration and blood volume difference. MRI-based pH imaging reveals significant different pH values among the three layers, with more neutral pH values (7–7.4) for the cortex region, mild acidic pH values (6.6–7.0) for the medulla, and acidic pH values for the calyx (6.3–6.7). Please consider Table 1 for average pH values measured in specific kidney regions.

Fig. 8 Demonstration of regional CEST Z-spectra and contrast of calyx (a, b), medulla (c, d), and cortex (e, f) at 3 μ T. Described CEST contrast became higher from calyx, trough medulla, to cortex, due to pH variation

Table 1
Typical pH values for each specific renal tissue for healthy mice and rats (literature values)

pH				
Inner medulla	Outer medulla	Cortex	Field strength, T	Reference
6.3 $\pm$ 0.45	7.0 $\pm$ 0.29	7.3 $\pm$ 0.13	4.7	[81]
6.6 $\pm$ 0.2	6.85 $\pm$ 0.15	7.0 $\pm$ 0.11	7	[69]
6.5 $\pm$ 0.2	6.8 $\pm$ 0.1	7.0 $\pm$ 0.1	4.7	[80]
6.6 $\pm$ 0.1	6.7 $\pm$ 0.08	6.8 $\pm$ 0.1	7	[49, 50]

4 Notes

1. Data are stored in the 2dseq file for Bruker scanners or in .img file for ASPECT MRI instrumentations. In both cases metadata files (method, acqp and reco for Bruker scanner or dat file for Aspect systems) need to be read for retrieving all the information needed to correctly read the raw binary file. Bruker import file plugins are available in ImageJ for directly opening raw Bruker files (for PV5). Matlab-based scripts for importing Bruker images and for the CEST analysis described in this chapter can be made available upon sending a request to the authors (dario.longo@unito.it; mtmcmaho@gmail.com; pzhesun@emory.edu). More information on the software can be found at the following links: http://www.cim.unito.it/website/research/research_processing.php <http://godzilla.kennedykrieger.org/CEST/>.
2. The choice of the regularization factor plays a key role in calculating CEST spectra and contrast; in particular, a trade-off between the “zero” estimation, noise suppression and peak identification is needed for an optimal choice [78]. Z-spectra and resulting CEST contrast obtained with different p values are shown in Fig. 9: when the regularization factor increases, the flexibility of the interpolating curve increases, yielding more evident peaks, but at the same time the smoothing of the raw data decreases. In our application p ranged between 0.90 and 0.99.
3. Signal variations (or fluctuations), as a measure of scanner instability, can be evaluated by repeating the same CEST acquisition (without saturation) several times (10–100 repetitions) and then evaluating the oscillation (or standard deviation) of

Fig. 9 Z-Spectra (a) and CEST contrast (b) obtained with different smoothing factors. The contrast decreases with a decrease of the smoothing factor value

the average signal along the repetition. Such value can be exploited to set a threshold for evaluating CEST contrast increase following contrast agent injection due to the contrast agent itself and not due to signal oscillation (i.e., it acts as a detection threshold). Signal fluctuation can be expected to be less than 0.2–1% with slight constant increase with the age of the scanner.

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