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ABSTRACT

Several molecules have been exploited for developing MRI pH sensors based on the chemical exchange saturation transfer (CEST) technique. A ratiometric approach, based on the saturation of two exchanging pools at the same saturation power, or by varying the saturation power levels on the same pool, is usually needed to rule out the concentration term from the pH measurement. However, all these methods have been demonstrated by using a continuous wave saturation scheme that limits its translation to clinical scanners. This study shows a new ratiometric CEST-MRI pH-mapping approach based on a pulsed CEST saturation scheme for a radiographic contrast agent (iodixanol) possessing a single chemical exchange site. This approach is based on the ratio of the CEST contrast effects at two different flip angles combinations (180°/360° and 180°/720°), keeping constant the mean irradiation RF power ($B_{avg\ power}$). The proposed ratiometric approach index is concentration independent and it showed good pH sensitivity and accuracy in the physiological range between 6.0 and 7.4.

Keywords: CEST; MRI; pH; iodinated contrast media; train pulses; pulsed-CEST; contrast media; radiographic agents;

Highlights:

- A novel ratiometric approach based on a pulsed saturation scheme is proposed
- This approach can be applied to molecules possessing a single proton pool
- A good pH accuracy can be obtained in the physiological range (pH 6.0-7.4)

1. Introduction

Chemical Exchange Saturation Transfer (CEST) is an innovative MRI contrast mechanism that can detect molecules with exchangeable protons upon saturation with selective radiofrequency pulses [1-3]. Exchanging proton pools include endogenous protons (amide, hydroxyls), as well as exogenous ones belonging to added diamagnetic or paramagnetic agents [4-13]. Several applications have been reported, including the assessment of ischemic acidosis [14], tumor detection [4, 15, 16], cell tracking [17-19], proteins structural properties [20-22], metabolites [23, 24], redox potential [25, 26], gene expression [27, 28] and enzymatic activity [29]. In particular, great attention has been dedicated to design agents able to map tissue pH [30-33]. In this context, a good example is represented by iopamidol, a clinical approved x-ray contrast agent possessing two types of amide protons whose different exchange rate has been exploited to set up a ratiometric approach for imaging tissue pH [34-37]. Similar results have been obtained with the related iopromide agent [38] or with imidazole-based pH sensors [39]. The above method relies on the presence of two exchangeable pools in order to exploit the ratiometric approach for a concentration independent measure of pH [40]. Recently, another x-ray agent containing only one mobile amide proton pool, iobitridol, was used to image tumor pH in vivo by ratioing the CEST effects resulting from the application of radiofrequency (RF) pulses of different power [41]. In general, the reported CEST studies relied on the application of a continuous wave (CW) irradiation scheme, consisting of a long off-resonance rectangular RF irradiation pulse. A major drawback of this irradiation scheme is represented by the high specific absorption rate (SAR) that limit the translation of the preclinical procedures to commercial human MRI scanners. Conversely, the pulsed-CEST imaging scheme addresses the hardware and SAR concerns by exploiting repetitive short RF pulses as irradiation scheme [42-49]. This saturation scheme is commonly applied at clinical level for amide proton transfer imaging [50-53]. Recent studies have shown that pulsed CEST contrast comprises both saturation and rotation effects (arising from an oscillating component). Consequently, the repeated rotation of the spin magnetization provides a complementary contribution to the decrease of the bulk water signal following the

chemical exchange [54]. This separation of rotation vs saturation transfer-effects in pulsed CEST experiments was dubbed chemical exchange rotation transfer (CERT). Moreover, it was found that pulsed CEST contrast as a function of the flip angle (θ) is dependent on the chemical exchange rate (k_{ex}) of the exchanging mobile proton pool. Gochberg and colleagues have exploited these properties using the ratio of contrast at multiple θ values for assessing chemical exchange rate of endogenous amide and amine protons by keeping constant the transmitted B_1 amplitudes ($B_{avg\ power}$) at different flip angles [55].

Here, we demonstrate the application of a double-angle ratiometric approach on the clinical approved x-ray contrast agent, iodixanol, possessing only one amide proton pool (Fig. 1), for the generation of a new pH-responsive CERT contrast agent. The proposed method, called ratio of pulsed RF angles (RPA), is based on the ratio of CERT contrast at two different θ values by keeping $B_{avg\ power}$ constant. The influence of different $B_{avg\ power}$ levels, duty cycle, temperature, concentration and θ values under a pulsed CEST sequences was also evaluated.

2. Materials and Methods

2.1 Numerical Simulation

Simulated pulsed CEST-MRI was generated using Matlab (Mathworks, Natick, MA, USA) using the modified Bloch-McConnell equations [45, 56, 57] for a three pool model (water, hydroxyl and amide protons labeled as w, b and s, respectively) with a field strength of 7T. Pulsed saturation was modeled using the discretization method, with each Gaussian pulse divided into 64 steps and the spin evolution was modeled assuming a constant B_1 amplitude within each step. The transverse magnetization was set to zero at the end of the inter-pulse period to represent the dephasing caused by crusher gradients, whereas the longitudinal magnetization relaxed toward equilibrium [44].

The variables in the model were set according to the range of values calculated from fitting Z-spectra obtained from phantom #1 (40 mM iodixanol in phosphate buffer solutions titrated in the pH range 5.5-7.9) at 37°C with CW saturation at several irradiation powers (1, 2 and 3 μT for 5s) in the range

±10 ppm with steps of 0.1 ppm. Specifically, the following variables were fixed to previously published values [58, 59]: longitudinal relaxation time, T_{1w}= 4.0s, T_{1b} = 1.0s, T_{1s}= 1.0s; T_{2w} = 1.5s, T_{2b} = 0.8s, chemical shift ω_b = 0.8 ppm, ω_s =4.3 ppm; or to experimental conditions: amide proton ratio (f_s) = 0.00145 (40mM*4/110M), hydroxyl proton ratio (f_b) = 0.0033 (40mM*9/110M). The following parameters were solved from numerical fitting: exchange rates for amide (k_{ex}) and hydroxyl groups (k_{wb}) and T_{2s} for each pH value.

A range of parameter values were simulated for pulsed CEST-MRI: FA (θ) varied from 45° to 900° with intervals of 15°, T_{1w} (3.0-3.7-4.4 s), T_{2w} (1.5-2.0-2.5 s), T_{1s} (1.0-2.0-3.0 s), T_{2s} (10-20-30 ms), f_s (0.007-0.0011-0.0018), k_{ex} (21-47-108-150 Hz), dc was set at 30% and 50%.

2.2 In vitro

2.2.1 Phantom Preparation

Three sets of phantoms were prepared by dissolving iodixanol (Visipaque®, GE Healthcare) in different media. A phantom containing several vials of 40 mM iodixanol in phosphate buffered solution were pH titrated between 5.5 and 7.9 and used for calculating the chemical exchange rates under CW irradiation and for the CERT experiments under Gaussian-train irradiation scheme. A second phantom was prepared by dissolving iodixanol in phosphate buffer solution at pH = 7.2 at different concentrations (2.5-5.0-10.0-20.0-40.0 mM) to investigate the concentration independence of the proposed ratiometric approach. A third phantom was prepared by dissolving 40 mM iodixanol in reconstituted human plasma (Seronorm Human, SERO AS ASKER, Norway) at several pH values (6.3, 6.7, 7.0, 7.4) to mimic *in vivo* conditions with the presence of several proteins and metabolites at physiological concentrations.

2.2.2 Magnetic Resonance Imaging

Pulsed-CEST experiments were acquired on a 7T Bruker Avance 300 scanner (Bruker BioSpin, Ettlingen, Germany) equipped with a micro 2.5 MICWB 30 mm quadrature (1H) imaging probe. Z-

spectra were acquired sampling the frequency offsets from -10 ppm to 10 ppm, with step size of 0.1 ppm. The pulsed-CEST scheme exploited a series of Gaussian irradiation pulses for the saturation part and a single-shot (with centric encoding) fast spin-echo imaging readout. After each pulse, crusher gradients (with alternating sign) were applied to spoil residual transverse magnetization. Each irradiation pulse had duration τ_P, flip angle θ, interpulse delay τ_D and the pulse train repetition (PTR) is given by $\tau_P + \tau_D$. $B_{avg\ power}$ levels were set to be 0.5, 1.0 and 2.0 μT with different values of duty cycle (dc) of 50% and 30% for a total irradiation time of 5 s. To test the predicted angular dependence, 15 values between 45 and 900° were acquired for each $B_{avg\ power}$ level and dc conditions.

For pulsed-CEST imaging, $B_{avg\ power}$ can be calculated by using the following equation [60]:

$$B_{avg\ power} = \sqrt{\frac{1}{PTR}\int_0^{PTR} B_1^2\,dt} = \sqrt{\frac{p_2}{dc}} \cdot \frac{\pi\theta}{180 \cdot \gamma \cdot p_1 \cdot PTR} \qquad [1]$$

Where $B_{avg\ power}$ is the field strength of a continuous wave irradiation with the same average power as the pulsed-CEST, p_1 is the ratio of the average amplitude to the maximum amplitude of the irradiation pulse, p_2 is the ratio of the average of the square of the amplitude to the square of the maximum amplitude of the irradiation pulse and γ is the gyromagnetic ratio of the proton (with units rad s^{-1} T^{-1}). For the Gaussian pulse used in our experiments p_1 and p_2 are equal to 0.418 and 0.299, respectively. Images were acquired with the following parameters: field of view = 30 mm x 30 mm, matrix size = 64 x 64, slice thickness = 4 mm, echo time = 3.5 ms, repetition time = 10 s, two averages. The experiments were performed at 21±1 °C and at 37±1 °C.

2.2.3 CEST image analysis

All Z-spectra were analyzed using custom-written scripts in Matlab (Mathworks, Natick, MA, USA) and interpolated by smoothing splines for B_0 inhomonogeneity correction [61]. CEST contrast named

Saturation Transfer (ST) was quantified at a specific offset of interest (i.e. $\Delta\omega$= +4.3 ppm) using the asymmetry analysis:

$$ST = \frac{S_{-\Delta\omega} - S_{+\Delta\omega}}{S_0} \qquad [2]$$

Where $S_{\pm\Delta\omega}$ is the water signal intensity in the presence of a saturation pulse at offset $\pm\Delta\omega$ and S_0 is water signal intensity in the absence of a saturation pulse.

A new ratiometric index (dubbed ratio of pulsed RF angles or RPA) is calculated as the ratio of the CEST ST contrast at two θ values according to equation 3:

$$RPA = \frac{ST_{\theta 1}}{ST_{\theta 2}} \qquad [3]$$

where $ST_{\theta 1,2}$ represents ST obtained at two selected flip angles (θ_1 and θ_2) by keeping $B_{avg\ power}$ constant.

3. Results

Z-spectra were acquired with CW irradiation on 40 mM iodixanol samples at several pH values (5.5-7.9) and fit to a three-pool exchange model by simultaneously fitting three different B_1 irradiation powers (Fig. S1), giving exchange rates for the amide mobile protons in the range 10 to 850 Hz (Table S1).

Fig. 2 show the simulated CEST contrast for a 40 mM iodixanol solution as a function of θ, showing the amide rotation effects with characteristic relative maximum and minimum peaks at 180°, 540° and 360° and 720°, respectively (Fig. 2a). The shape of the oscillation is dependent only on k_{ex} (Fig. 2a) and is not affected by changes in concentration (f_s), T_{1w}, T_{2w}, and T_{1s}, but only slightly on T_{2s} (Fig. 2b-f). Fig. 3a gives the simulated and the experimental CEST contrast as a function of θ at pH of 6.7, 37°C, showing a good correspondence between expected and measured CEST contrast values. The experimental RPA values measured by ratioing CEST contrasts at 180°/360° at four different pH values are close to the simulated values (Fig. 3b, B_{avg_power} of 1μT and dc of 50%). To mimic *in vivo*

conditions, iodixanol was dissolved in human serum and the measured RPA curve showed marked pH dependence even in presence of other exchangeable protons (Fig. 3c).

Fig. 4a reports the observed CEST contrast at 4.3 ppm as a function of flip angle with a B_{avg_power} of 2 μT and dc 50% (T=21°C, B_0=7T) for several pH values, showing the expected angular signal dependence that oscillates as cos(θ) with a maximum close to 180°. The shape and the magnitude of the resulting CEST contrast oscillation depend on the proton exchange rate k_{ex}. Being k_{ex} base-catalyzed, for increasing pH values an increase of the CEST effect is observed. In addition to k_{ex}, the oscillation is also dependent on $B_{avg\ power}$ and on the applied duty cycle. In fact, the same angular dependence was observed upon decreasing the irradiation power to 1 μT (Fig. 4c), keeping constant the dc to 50%, although the observed CEST contrast appears significantly reduced for all the investigated pH values. When the pulsed sequence was applied with a dc of 30%, an increase in the contrast was observed if compared to dc of 50% and the same irradiation $B_{avg\ power}$ (Fig. 4b and 4d).

The Z-spectra generated with a pulsed CEST irradiation scheme for 40 mM iodixanol solutions titrated in the pH range 5.5-7.9 are shown in Fig. S2. The calculated CEST contrast angular dependence is shown in Fig. 5. A similar oscillating shape is obtained as a function of the flip angle even at higher temperature, hence higher exchange rates, and the CEST contrast magnitude is strongly pH-dependent. A decrease of the irradiation $B_{avg\ power}$ from 2 μT to 0.5 μT corresponds to a marked reduction of the CEST contrast effect (Fig. 5a, 5c and 5d). When exploiting a dc of 30% a similar angular dependence of the CEST contrast was measured (Fig. 5b).

The proposed ratiometric approach relies on the ratioing of the relative intensities at different flip angles as a function of pH (Fig. 6). By ratioing the ST effects observed at flip angles of 180° and 360°, with a constant $B_{avg\ power}$ of 1 μT, RPA (averaged over a ROI placed on each sample) showed a good pH response for pH values from 6.0 to 7.4 (Fig. 6a). Moreover, RPA values calculated upon using a $B_{avg\ power}$ of 0.5 μT yielded an analogous pH response. Good pH sensitivity was obtained also from RF flip angles of 180° and 720° at both the $B_{avg\ power}$ of 1 μT and 0.5 μT (Fig. 6b). Since the capability to measure accurately pH values is dependent on both the attainable CEST contrast as well

as on the pH responsiveness, a $B_{avg\ power}$ of 1 μT and a dc of 50% were chosen, since higher $B_{avg\ power}$ (2 μT) provided lower pH responsiveness (Fig. S3), whereas dc of 30% provided lower CEST contrast (Fig. 5b).

Using the relationship between RPA values and experimental pH determined from Fig. 6, pixel-wise pH maps were derived for the pH phantoms (Fig. 7e with flip angles 180°/360° and Fig. 7f with 180°/720°). Calculated pH values from the obtained maps are plotted as a function of pH-meter measurements (Fig. 7g and 7h). The observed good correlation ($R^2 = 0.998$, $P<0.0001$ and $R^2 = 0.996$, $P<0.001$, respectively) attests the accuracy of the RPA-based interpolation vs pH.

To demonstrate the concentration independence of the method, a series of phantoms at different iodixanol concentrations (in the range 2.5-40 mM) were prepared, with pH titrated 7.2. The RPA values were observed to be constant when ratioing the ST contrast at the two flip angles of 180° and 360° (Fig. 8a, slope = 0.0026 and 0.0011 for $B_{avg\ power}$ of 1 and 0.5 μT, respectively). A robust stability as a function of concentration was obtained also when ratioing the ST contrast obtained at the two flip angles of 180° and 720° (Fig. 8b, slope = -0.0011 and -0.0008 for 1 and 0.5 μT, respectively). Only at 2.5 mM iodixanol concentration the measured CEST contrast was not enough for the calculation of the RPA value. These data demonstrate that this ratiometric approach can measure pH despite a substantial difference in iodixanol concentration, with all regression slopes not significantly different from zero.

4. Discussion

In this study, we report a new ratiometric approach for pH determination based on the transfer of the oscillation of the solute magnetization to the bulk water signal by applying a pulsed-CEST sequence. In contrast to the approach of using endogenous amide groups as investigated by Gochberg and colleagues [55], herein we exploit an exogenous molecule that can potentially provide multiple information related to the extracellular pH and to its extravasation, hence tissue perfusion [59, 62].

Most of the ratiometric approaches for MRI pH mapping have been applied to DIACEST agents (e.g. iopamidol, iopromide, iobitridol) with relatively fast exchange rates (ca. 1-3 KHz at pH 7.4) for exploiting higher CEST contrast and efficiency upon a continuous wave RF irradiation [59]. Within this approach, one may broaden the investigation to exogenous molecules possessing even slower exchange rates, an exclusive field that was limited to endogenous mobile proteins and peptides. Moreover, a low-power pulsed saturation scheme can generate CEST signal, thus facilitating clinical translation [63-65].

The pulsed-CEST contrast curves of iodixanol as a function of pH showed similar characteristic feature points at 180°/360°/540° and 720° in comparison to endogenous amide groups. On the contrary, the CEST contrast ratio calculated from different flip angles showed a different relationship with k_{ex} between endogenous and iodixanol-derived amide protons. In fact, the CEST contrast ratio calculated at three θ values for the endogenous amide groups (dubbed CCR in [55]) showed a monotonic function that increases for slow exchange rates with a $B_{avg\ power}$ of 1.0 μT and then decreases for higher exchange rate. In contrast, with iodixanol we observed only a constant decrease of our ratiometric index (RPA, calculated as the ratio at two different flip angles) at all the investigated pH values. A similar relationship was observed also when exploiting the same CCR metric approach for the iodixanol data (Fig. S4), therefore this behavior is likely dependent on the higher exchange regime of iodixanol amide protons in comparison to the endogenous ones.

The pH relationship of the ratiometric index was found to be dependent on the applied $B_{avg\ power}$ and duty cycle, therefore a $B_{avg\ power}$ of 1.0 μT and a duty cycle of 50% were chosen, which gives good pH sensitivity. In addition, the proposed ratiometric RPA index displays robust solute concentration independence in the investigated pH, $B_{avg\ power}$ and θ values.

Several papers have recognized the advantages of exploiting the ratiometric approach to remove the concentration term. In particular, CEST-MRI pH sensing agents need to rule out the concentration term for an accurate measurement of the pH values. Since Ward and Balaban seminal work, only molecules possessing multiple chemical exchange sites with different frequencies offsets have been

considered as CEST-based pH sensing agents [66]. This approach was applied for both DIACEST and PARACEST molecules obeying to the conditions of multiple proton pools, such as iopamidol and Yb-HPDO3A for assessing pH in several tissues [67-69]. Remarkably, an expansion of this approach has been obtained based on the irradiation of a single exchanging pool at different RF saturation powers, hence potentially transforming every CEST molecule into a pH responsive contrast agent [41, 70]. Notably, the pH-dependence of the chemical shift of a single water exchange CEST peak has been proposed as a novel pH-imaging approach following a PARACEST agent administration [71, 72]. While all these methods used long duration and/or high power CW saturation scheme, the herein reported approach is based on a pulsed CEST sequence that is easily translatable to clinical MRI scanners owing to the reduced SAR limitation and amplifier restriction due to shorter pulse duration. In addition, in contrast to the ratiometric approach based on different B_1 power levels, we propose a completely new ratiometric index with a constant $B_{avg\ power}$ by varying the irradiation flip angle θ. The proposed ratiometric method requires only one exchanging pool and it covers a broad pH range, similar to that achieved with conventional ratiometric pH MRI approaches. The pH sensitivity index ΔR_{pH}, measured as the difference of the ratiometric index between pH values of 6.0 and 7.4, was found to be between 1.4-1.7 (with $B_{avg\ power}$ of 0.5-1.0 μT and dc 50%), slightly lower than those attainable with the iopamidol- or iopromide-based ratiometric approaches (ΔR_{pH} = 2.8 and 2.7 for iopamidol and iopromide, respectively), but higher to that attainable with the Yb-HPDO3A PARACEST agent (ΔR_{pH} = 1.1) [41]. Furthermore, the present method, described in this paper using a x-ray agent characterized by only one mobile amide proton pool, may be applied as well in the presence of two exchangeable pools (as in the case of iopamidol), with the advantage of a double independent estimation of pH and thus, in principle, of a higher reliability. More importantly, radiographic agents, owing to their high safety profile, have already been demonstrated for assessing pH values at clinical level [73, 74].

R2.11

Ratiometric approaches usually require multiple full Z-spectra acquisition that results in longer overall acquisition time than single acquisition and could be more prone to motion artifacts, although

fast acquisition approaches have already been developed [75-77]. Conversely, the proposed approach can quantify the CEST contrast by the irradiation at only two flip angles, hence resulting in shorter acquisition times.

The proposed approach relies on irradiation train pulses at different θ, hence B_1 inhomogeneities may affect the accuracy of this procedure, but others have shown that the ratiometric approach is relative robust to B_1 errors [55] and B_1 inhomogeneities are not an issue at animal scanners. On the other hand, in whole-body-scanners severe B_1 inhomogeneities may appear with fluctuations up to +-50% [78]. However current developments in interpolation approaches of repeated scans with different effective B_1 and in parallel transmission techniques will also allow mitigation of B_1 inhomogeneities in the near future and make the presented approach also translatable in the high field imaging in humans [78, 79].

There are some remaining challenges that will be addressed to improve the proposed procedure. First, accurate pH responsiveness requires a suitable local concentration of the detected CEST contrast agent; in particular, obtained preliminary results seem to indicate that iodixanol should accumulate in the organ of interest with concentrations higher than 2.5 mM. However, previous studies have shown that this is feasible even in the tumor extracellular space with an average accumulation of 5-8 mM [59]. In addition, future studies should be addressed to validate *in vivo* the proposed new ratiometric approach for assessing pH.

5. Conclusions

In summary, this study provides a new ratiometric approach for exogenous agents based on a pulsed CEST scheme with multiple irradiation flip angles and constant B_1 amplitude that extends the field of application for CEST-based pH imaging.

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Figures Legends

Figure 1 Chemical structure of the radiographic agent iodixanol.

Figure 2 Simulated CEST contrast (ST%) as a function of θ showing the oscillation component for iodixanol when irradiated with a pulsed gaussian train with constant $B_{avg\ power}$ of 2μT and dc 50%. Simulations were performed for different k_{ex} (a), f_s (b), T_{1w} (c), T_{2w} (d), T_{1s} (e), T_{2s} (f) by using a three-pool model. In panels (b-f) the normalized CEST contrast (normalized ST%) is the CEST contrast (ST%) normalized at θ = 900°.

Figure 3 (a) Simulated (solid line) and experimental (circle) CEST contrast for a 40 mM iodixanol solution as a function of θ (θ = 45°, 90°, 135°, 180°, 225°, 270°, 315°, 360°, 405°, 450°, 540°, 630°, 720°, 810°, 900°, k_{ex} =160 Hz, $B_{avg\ power}$ 2 μT, dc 50%, B_0 7T, 37°C). (b) Simulated (square) and experimental (circle) ratiometric value (RPA) calculated for different titrated pH values (B_0=7T, $B_{avg\ power}$ 1 μT and dc 50%). (c) Calculated RPA curve with iodixanol dissolved in human plasma (B_0=7T, $B_{avg\ power}$ 1 μT and dc 50%, 37°C).

Figure 4 Plot of CEST contrast as a function of θ at T=21°C for a 40 mM iodixanol solution at several pH values in the range 5.5-7.9 for different experimental conditions: (a) $B_{avg\ power}$ 2 μT and dc 50%; (b) $B_{avg\ power}$ 2 μT and dc 30%; (c) $B_{avg\ power}$ 1 μT and dc 50% and (d) $B_{avg\ power}$ 1 μT and dc 30% with a total irradiation time of 5 s (B_0 = 7T~~; T=21°C~~).

Figure 5 Plot of CEST contrast as a function of θ at 37°C for a 40 mM iodixanol solution at several pH values in the range 5.5-7.9 for different experimental conditions: (a) $B_{avg\ power}$ 2 μT and dc 50%; (b) $B_{avg\ power}$ 2 μT and dc 30%; (c) $B_{avg\ power}$ 1 μT and dc 50% and (d) $B_{avg\ power}$ 0.5 μT and dc 50% with a total irradiation time of 5 s (B_0 = 7T~~; T=37°C~~)

Figure 6 CEST contrast ratiometric RPA values as a function of pH for $B_{avg\ power}$ of 1 μT and 0.5 μT and dc 50% with θ ratio of (a) 180°/360° and (b) of 180°/720° (B_0 = 7T; T=37°C; total irradiation time of 5 s).

Figure 7 T_{2w} image of phantom containing 40 mM iodixanol adjusted to the indicated pH values (a), ST maps obtained after pulsed irradiation with θ values of 180° (b), 360° (c) and 720° (d) and corresponding pH maps as determined by the RPA approach by ratioing (e) 180°/360° and (f) 180°/720° θ values with $B_{avg\ power}$ of 1 μT and dc 50%. Calculated pH vs experimental pH by ratioing (g) 180°/360° θ values (R^2 = 0.998, P<0.001) and (h) 180°/720° θ values (R^2 = 0.996, P<0.0001).

Figure 8 Regression analysis between RPA ratiometric values and iodixanol concentration (range 2.5-40 mM) with $B_{avg\ power}$ of 1 μT and 0.5 μT and dc 50%: by ratioing (a) 180°/360° θ values and (b) 180°/720° θ values (B_0 = 7T; T=37°C; total irradiation time of 5 s). All regression lines have slopes not significantly different from zero.