N-AcetylLaspartic Acid Phantom for Proton Magnetic Resonance Spectroscopy at 1.5 T and 3 T

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Abstract. Proton Magnetic Resonance Spectroscopy (MRS) phantoms are an important tool for quality assurance and reliability. Some of the contents are not easily available in some countries due to some company policies. We built a phantom using N-Acetyl-DL-aspartic acid as a substitute for N-Acetyl-L-aspartic acid for proton magnetic resonance spectroscopy of human brain at 1.5 T and 3 T. To quantify the B0 homogeneity, phase and magnitude images of a commercial phantom were also acquired with a standard gradient echo sequence. Spectra obtained were corrected and dismissed critical chemical shift due to inhomogeneities. Spectra numerical simulations at 1.5 T and 3 T were performed using a free jMRUI and point resolved spectroscopy sequence for various times of echo. In vitro single-voxel spectra were obtained with the phantom prototype and a commercial phantom using the substitute acid and the same pulse sequence and magnetic field magnitudes as before. Simulated and in vitro spectra showed a very good concordance and majority of metabolites were readily identified for both fields. Spectra acquired with the phantom prototype complied with those quality control criteria for clinical use for both field strengths. This approach offers an alternative way to conduct clinical magnetic resonance spectroscopy.

1. Introduction

Proton (1H) MRS can reliably determine the concentration of individual metabolites to be used as a complementary tool for diagnosis and investigation of human diseases using animal models [1]. The versatility of the technique derives from the sensitivity of the NMR signal to a large variety of molecular-level parameters associated with the chemical environments of nuclear spins [2]. 1H MRS can be performed either using a single voxel (one-dimensional) technique or multi voxel (two-dimensional) technique.

Since the introduction of MRS as a probe for metabolic studies [3], this analytical tool has evolved into a clinical methodology in routine studies of the brain, heart and other organs to add diagnostic value [4–11]. MRS can provide metabolic and physiological information about the human body using several metabolites, such as 1H, 31P, 19F, 13C, 23Na. 1H MRS is mainly used in biomedical sciences because of the high sensitivity of the 1H nucleus, the near 100% availability of this isotope, and the abundant presence of this nucleus in most metabolites.

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A review on the physical principles and applications can be found in [2], and a special issue on MRS has been published in NMR in Biomedicine in 2006 [4]. The usage of hyperpolarized (HP) 13C agents for 13C MRS applications of animal models has also been investigated [12]. The advent of magnetic resonance imagers with higher magnetic fields than 3 T for human applications, has boosted the use of other nuclei besides 1H [13].

A major problem for proton magnetic resonance spectroscopy (1H MRS) is that there is no consensus regarding the reliability of absolute or relative concentrations found in different regions of interest. Specifically, there is a need for an MRS/MRI phantom suitable for quality assurance purposes, but also for testing imager performance and evaluating newspectroscopy techniques and sequences. MRS/MRI phantoms have been used in the past for quality assurance and reliability with specific metabolites and different regions of interest [14-20]. To the best of our knowledge, some of these contents are no longer manufactured due to certain commercial policies and replaced by other products. This is the case of the N-Acetyl-L-aspartic acid, which is no longer available in some countries. This is important since NAA is the most abundant amino acid in the brain, it resonates in an uncluttered portion of 1H MR spectra [21-25], and it can be accurately quantified. In this paper, we developed a phantom using the N-Acetyl-DL-aspartic acid as a substitute for the N-Acetyl-L-aspartic acid in 1H MRS experiments at 1.5 T and 3 T. These are the most popular field strengths in the clinical environment, so the majority of the 1H MR spectroscopy studies published are based on these field strengths. This approach may prove a useful tool for quality assurance of 1H MRS experiments.

2. Methods

2.1. Phantom prototype

To manufacture our brain phantom, 8 different brain metabolites in physiological relevant concentrations were used and summarized in Table 1. These metabolites and their concentrations were particularly chosen because they have been routinely observed in brain MRS using clinical MR imagers [26-27]. As a replacement for the N-Acetyl-L-aspartic acid, we used N-Acetyl-DL-aspartic acid. Their bi-dimensional structures and molecular formulas are in Fig. 1. All chemicals were purchased from Sigma-Aldrich Quimica, Mexico (Sigma-Aldrich Quimica, DF, Mexico). We also used the same concentrations as in Table 1, but no gadolinium as a buffer at this time. All metabolites were put in a 20 cm sphere made out of plastic with similar dimensions as the standard spherical phantom GE MRS-HD manufactured by General Electric (General Electric Company, Milwaukee, WI, USA). Fig 1 shows a photo of our spherical phantom prototype.

### Table 1. Summary of components and their concentrations used to build our N-Acetyl-L-aspartic Acid prototype phantom for brain H1 MRS. CAS No. stands for Chemical Abstract Service number (www.cas.org accessed 9 March 2016).

| Substance name                  | Nomenclature     | Concentration [mM] | CAS No.     |
|---------------------------------|------------------|--------------------|-------------|
| Potassium phosphate mono basic  | KH2PO4           | 50.0               | 7778-77-0   |
| Sodium hydroxide                | NaOH             | 56.0               | 1310-73-2   |
| N-Acetyl-DL-aspartic acid       | NAA              | 12.5               | 2545-40-6   |
| Creatine hydrate                | Cr               | 10.0               | 6020-87-7   |
| Choline chloride                | Cho              | 3.0                | 67-48-1     |
| Myo-inositol                    | ml               | 7.5                | 87-89-8     |
| L-glutamic chloride             | Glu              | 12.5               | 6106-04-3   |
| DL-lactic acid                  | Lac              | 5.0                | 16891-53-5  |

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2.2. Imaging and spectroscopy experiments
Before acquiring proton spectra with our phantom, magnitude and phase images were acquired using the following acquisition parameters: TR/TE = 4.62ms/1.118ms, Flip angle = 30°, slice thickness = 10 mm, matrix size = 256 × 256, Number of Experiments = 1, Field of View = 25 cm x 25 cm.

Since spectroscopy experiments heavily depend on the good uniformity of the main field (B₀) produced by the magnet, it is necessary to experimentally measure it. The phase difference method was used together with a General Electric phantom (40 cm diameter, weight = 11.50 kg, model 2135650 REV5 and filled with NiCl₂H₂O, H₂O) to quantify the homogeneity of B₀ [28]. To acquire phantom phase images, a standard gradient echo sequence with two TE values was used for both imagers. With the image data, the field homogeneities were calculated using the phase difference map technique [29]:

\[ \gamma \Delta TE \delta B_0 = \delta \phi \] (1)

where \( \gamma \) is the gyromagnetic constant, TE [ms] is the time of echo, \( B_0 [T] \) is the magnetic field flow, and \( \phi \) [radians] is the phase. The reference value was at the center of the field of view.

The size of the miss registration of the chemical shift is also an important parameter to measure. To do this the following equation was used:

\[ \Delta x = \frac{\Delta \omega V}{BW_{pulse}} \] (2)

where \( \Delta x [mm] \) is the miss registration, \( \Delta \omega \) is the chemical shift between the reference metabolite (NAA) and the metabolite of interest, \( V [mm^3] \) is the excitation volume size, and \( BW_{pulse} [Hz] \) is the bandwidth of the slice selection RF pulse. In our experiments: \( BW_{pulse}= 4.88 \text{ Hz at 1.5 T (63.87 MHz for protons)} \) and \( BW_{pulse}= 9.76 \text{ Hz at 3 T (127.73 MHz for protons)} \) with a volume, \( V = 1 \text{ mm}^3 \).
Single voxel 1H MRS experiments were also conducted with the following acquisition parameters: TE = 144 ms, and TR = 1500 ms, voxel = 20 x 20 x 20 mm3, 128 acquisitions for both cases and the PRESS sequence. A TE of 114 ms is particularly important because it helps to generate a flatter base line and consequently, a more precise quantification of NAA, Cr, and Cho. All imaging and proton spectroscopy experiments were run at 1.5 T and 3 T using a standard clinical gradient echo sequence on Sigma imagers (General Electric Company, Milwaukee, WI, USA) and an 8-channel coil array. The water signal is suppressed because it masks the signal from the other metabolites.

Shimming was performed applying an automatic second order shimming and according to the standard procedures provided by the manufacturing company for both MR imagers. Remaining shimming problems, line broadening and non-Lorentzian character of the peaks may still be present after correcting for eddy currents.

2.3. Numerical simulations

Single voxel 1H MRS simulations were made using the jMRUI (NMR-Scope: quantum-mechanics spectra simulation algorithm) software tool (V. 4.0, Marie Curie Framework, France) [30], according to our phantoms contents, as mentioned in the previous section. This tool has a Java-based graphical user interface and works in the time domain directly performing operations on the measured free induction decay (FID). Additionally, this package allows easy data file conversation from the major biomedical NMR/clinical MRI systems. All simulations were performed with the PRESS (Point RESolved Spectroscopy) sequence [29], which has become a commodity across the world for clinical MRS of a number of diseases and other applications. This is a volume-selected technique using a multispin echo single shots to obtain spectral data via the selection of a volume. The volume of interest is selected by using frequency-selective RF pulses and field gradients along the three orthogonal axes. Fig. 2 shows the time pulse sequence diagram. A review on spatially-localized MRS sequences can be found in [2]. Spectra were simulated at 1.5 T and 3 T with the following Times of Echo 1500 ms and 35 ms, and 1024 acquisition points for comparison purposes.

3. Results and Discussion

Fig. 3 shows phase maps obtained at the two magnetic fields to investigate the B0 uniformity of both imagers. The uniformity of B0 was experimentally measured giving approximately 5 ppm (1.5 T) and 2 ppm (3 T) using eq (1) and show very good concordance with values published by Dydak and Schar [29]. These uniformity values are usually acceptable for imaging and MRS experiments. The Fig. 3 phase difference maps for both field magnitudes show a linear relation between the phase and B0 as described by eq. (1), allowing us to study the uniformity of the imager static field, B0. These maps showed a good field uniformity for the 1.5 T imager. However, the 3 T imager has a poor uniformity but within the acceptable values for MR spectroscopy experiments demand a better B0 field uniformity for good quality and reliable spectra [2, 13, 26-27].
Phantom images were also acquired at 1.5 T and 3 T with clinical pulse sequences and shown in Fig. 4.a) and.b). The B1 uniformity profile of Fig. 4.c) also shows a reasonable homogeneity of the coil array. The signal-to-noise ratio (SNR) values were also computed for both field intensities giving approximately 40 (1.5 T) and 55 (3 T). The performance of the coil arrays is acceptable for imaging experiments.

**Figure 3.** Difference phase maps of both clinical MR imagers obtained from using the commercial phantom are shown in a) and b). Pixel values were computed using the gyromagnetic constants for 1H at 1.5 T and 3 T.

**Figure 4.** Phantom images acquired at 1.5 T with a clinical MR imager (left) and, uniformity profile obtained using the red line as indicated in the phantom image (right).

Experimental spectra was obtained using the commercial phantom and ours to test their viability to run at both magnetic fields. Fig. 5 shows experimental spectra of both phantoms acquired using a TE = 135 ms at 1.5 T and 3 T. These experimental results have a very good concordance with results already reported in the literature [2]. The chemical metabolites used in our phantom can be readily identified at 3 T: Cho, Cre, Glx, NAA, Ala, Lac and, ml. However, the metabolites Glu (1.5 T: spectral overlap of strongly coupled spin systems) and, ml (1.5 T) were not obtained for both phantoms. Because of the field strength increase, the 3 T in vitro spectra is able to show the metabolite, ml. Spectra acquired using a TE of 35 ms show less noise, but the metabolites, Glx and ml are not clearly identified. The metabolite, P-Cr can not be observed using the same TE regardless of the field strength. This result corresponds very well with abundant literature pointing out that the TE of 144 ms is better than shorter echo times. Something peculiar is the behaviour of Lac in both field strengths, and in the two phantoms.

The Lac peak does not change as in standard spectra acquired at 144 ms. With our results obtained from $B_0$ uniformity, we can assure that our spectra were obtained correctly, so the Lac peak can not be included in the phantom to simulate the brain’s lactate.
Figure 5. Comparison spectra of single voxel 1H MRS acquired with our phantom for both field strengths and two times of echo. Majority of metabolites were readily identified for all cases. The metabolites, P-Cr and mI cannot be observed in the spectra obtained with a TE of 35 ms.

Figure 6. Simulated spectra showing metabolites for 1.5 T (left) and 3 T (right) with two different times of echo (TE); top: 35 ms and bottom: 144 ms. Spectra was edited using the free software tool, jMRIU. Major metabolites can be readily identified for all spectrum.

1H MRS spectrum simulations using jMRI were computed at 1.5 T and 3 T for the TE values above and shown in Fig. 6. All the important metabolites were positively identified for all simulated spectra. These results show a very good concordance with the spectra reported in the literature for the clinical pulse sequences used.

The simulation results at 3 T show a magnitude increment of Cre when compared to the 1.5 T simulated spectrum as a result of the field intensity increased as expected. All spectra have a relatively good resolution compared with the spectra normally generated with the GE phantom at the same field magnitude [2]. The 1.5 T experimental spectra showed a pretty similar behavior to experimental 3 T one and, that the magnetic field intensity do not seem to importantly affect the peak amplitude and resolution. However, a clearly opposite pattern can be observed for the Cho peak between the experimental and simulated spectra. This is probably due to the Cho amount was not the correct one. The Glx peak can not be appreciated on the simulated spectra as opposed to the in vivo spectra for both field magnitudes.

We used the criteria reported Kries to reject or accept the spectra acquired using both phantoms in this research [31]. The Full Width at Half Maximum (FWHM) values of the metabolites: NAA, Cr, Cho and P-Cr are summarized in Table 2. From these results, we can appreciate that all metabolites comply with the quality criteria as mentioned before. These experimental results also satisfy the minimum acceptable metabolite linewidths for pattern recognition MRS as established by the INTERPRET Project and reported by Taylor [4]. Then, all spectra have good quality data assuring that both phantoms are able to produce reliable MRS data for clinical applications.
Table 2. FWHM values for all metabolites in Fig 6 of our phantom prototype and commercial phantom at 1.5 T and 3 T for two different times of echo.

| Metabolite/TE | Phantom prototype 1.5 T [ppm]/[Hz] | Commercial phantom 3.0 T [ppm]/[Hz] |
|--------------|-----------------------------------|------------------------------------|
| NAA          | 0.048/3.036 0.037/2.342            | 0.028/3.618 0.037/4.763             |
| Cr           | 0.048/3.073 0.038/2.420             | 0.028/3.815 0.027/3.461             |
| Cho          | 0.047/3.18 0.048/3.032              | 0.028/3.618 0.037/4.737             |
| P-Cr         | 0.048/3.036 0.038/2.407             | 0.037/4.763 0.037/4.737             |
|              |                                   |                                    |

Table 3. Values of chemical shift miss-registration for both phantoms and the metabolite, NAA acquired with TE= 144 ms.

| Metabolite   | Miss registration [mm] |
|--------------|------------------------|
| NAA          |                        |
| Phantom type | Commercial 3.1 Prototype 3.6 |
|              |                        |

The chemical shift, $Deltaomega$, was computed for the metabolite, NAA acquired with both phantoms at both field strengths. Fig 7 shows spectra highlighting the NAA peaks for all spectra: the spectra acquired at both field strengths show very defined and smooth peak shapes of the NAA metabolite, whereas for the 3 T spectra, the peak shape acquired using the commercial phantom tends to a plateau. Although the peak shape is not drastically altered, this will definitely affect any calculation based on this peak. Red circles shows an artifact mainly reported as unbalanced gradient crusher pulses in combination with $B_0$ gradients (shifted echo and inhomogeneous $B_0$). This implies that adjustments should be done on the gradients to check if eddy currents are causing this artefacts.

The chemical shift miss registration was calculated using eq. (2) and parameters above together with the data from Fig. 7, for the NAA metabolite acquired with TE = 144 ms only. Results for both phantoms are summarized in Table 3. The miss registration values are practically the same regardless of the field strength and the type of phantom, demonstrating that our phantom has a good performance compared to other commercial MRS phantoms. This also implies that both imagers performance allowing one to reliably conduct MRS experiments. At this point, we do not know if the commercial MR imager used in this work, does frequency shift corrections in an automatic fashion, as other commercial scanners do. From these results, we can observe that this spherical phantom may also used for quality control routines ($B_0$ uniformity, eddy currents, RF amplifier, gradient amplifiers, amplifier linearity, coil performance and various noise sources) to assure the optimal spectra under different conditions involving new hardware and software specifically developed for MRS of the brain. This phantom may represent an alternative phantom to N-Acetyl-L-aspartic acid-based commercial phantoms. This is particularly important because the tendency to use MR imagers equipped with greater magnetic field magnets is developing at firm pace [13].
Figure 7. Comparison of spectra acquired with a commercial phantom and our phantom at 1.5 T (top) and 3 T (bottom). Blue and green spectra were acquired with the commercial and prototype phantoms, respectively.

4. Conclusion
Experimental and simulated results using N-Acetyl-L-aspartic acid (NAA) in a brain phantom prototype showed a very good concordance with data reported in the literature, using common clinical pulse sequences for 1H MRS. We demonstrated that replacing the NAA metabolite in the phantom did not affect the spectra. Further investigation should be carried out to determine the reliability of this phantom to routinely perform clinical 1H MRS of the brain.

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Compliance with Ethical Standards
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