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● *Technical Note*

AGAROSE AS A TISSUE EQUIVALENT PHANTOM MATERIAL FOR NMR IMAGING

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Phantoms for evaluation of nuclear magnetic resonance (NMR) imaging systems were made from water-based agarose gels, according to a standard procedure herein described. Copper sulfate ($CuSO_4$) was included in the gels to further affect their proton relaxation characteristics. The proton relaxation rates of each batch of gel are dependent on the concentrations of agarose and copper ions in it, with *T1* depending more on copper than on agarose, and *T2* depending more strongly on agarose than on copper. The wide range of *T1* and *T2* which can be covered, and the stability and physical characteristics of the agarose gel material make it well-suited for phantom use.

Keywords: **Agarose, NMR imaging, Phantom.**

INTRODUCTION

Tissue equivalent materials have been very useful for calibrating and checking imaging machines. A variety of materials have been used for this purpose with NMR imaging systems. They include aqueous solutions of copper, manganese or nickel ions[1,2]; H_2O/D_2O mixtures,[3] Vaseline,[1,2] glycerol,[4] animal hide gels,[4,5] mineral oil,[5] and agarose gels.[5,6] Although these materials are useful, they are not tissue equivalent because their relaxation times T1 and T2 cannot match the ranges of T1 and T2 found in body tissue.

For solutions of copper ions that have T1 values equivalent to tissue, the corresponding T2 values are too long. The opposite situation applies to agar gels which typically have T2 values much shorter than T1.[7] It has been suggested[5] that by mixing paramagnetic ion salts and agar gels, a wide range of T1 and T2 values can be produced by appropriately varying the concentrations of the ion and the agar. In particular, agarose gels with added copper sulfate can be used to produce phantom materials with tissue equivalent values of both T1 and T2.[6] Deuterated water can be used to vary the proton density as well.[3]

This report describes the method of preparing the agarose gel phantoms and indicates the range of relaxation times achievable at 5 and 60 MHz. These frequencies were chosen because they correspond to those of our 0.12 and 1.4 tesla imaging systems.

PREPARATION OF THE GELS

Agarose (Sigma Chemical Corp. Type 1, #A-6013) is used in concentrations from 0.5 to 4.0% (w/v). Concentrations lower than 0.5% will not produce a firm gel. The dry agarose is weighed out in a plastic weighing boat and then dissolved in distilled, deionized water. Tap water might contain metal ions which lower the relaxation times. The higher concentrations of agarose (3 to 4%) take up to five minutes to dissolve. After the agarose is dissolved the proper amount of the metal salt is added, either as solid or in solution. Concentrations of copper sulfate in the 0.2 to 4.0 millimolar range are usually used. Manganous chloride in the 0.05 to 1.0 millimolar range or nickel chloride from 0.5 to 10 millimolar can also be used.

On a hotplate with magnetic stirring, (VWR Dyla-Dual, #58849-001) the agar solution is heated until it turns from cloudy to clear, a sign that the agarose has made the transition from the sol state to molecular solution. The transition takes place at the boiling point, which at medium heat and stir settings, takes ten to twenty minutes to reach. After the visible transition, the solution is heated an additional ten minutes to

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ensure that the transition is complete. The hot solution is then poured into a vial or other mold and allowed to solidify into a gel. The permanent container is completely filled and sealed to prevent evaporative loss or exchange of D_2O for H_2O.

To make gels of several different copper ion concentrations from a single batch of hot agarose, the agar solution is prepared in a concentration higher than the desired final concentration. The additional water needed to reach the appropriate agarose concentration is placed in each vial, along with the desired amount of copper sulfate. The solutions must be mixed well when the hot agar is added. The agar concentration in individual gels prepared from a single batch can similarly be varied.

RELAXATION TIMES OF THE AGAROSE-COPPER SULFATE GELS

The measurements of relaxation times of the samples were carried out on an NMR spectrometer that was tuned to either 5 or 60 MHz. The T1 value was determined by using an inversion-recovery pulse sequence and measuring T-null, from which T1 is calculated. The T2 value was determined by fitting an exponential curve to the envelope of the spin echoes resulting from a Carr-Purcell-Meiboom-Gill pulse train. The precision of these measurements is estimated to be about five percent, and the values for plain copper solutions agree to within five percent with the values reported by Morgan and Nolle.[8]

Since we are interested in producing a material with both T1 and T2 relaxation times equivalent to those of tissue, it is convenient to plot the relationship between T1 and T2 as concentration of the agarose and copper sulfate changes. Table 1 compares T1 and T2 values measured at 5 and 60 MHz for rabbit tissue with T1 and T2 values for plain agarose and copper solutions. Figure 1 is a T1–T2 plot of the 5 MHz data in Table 1. The plot of the 60 MHz data is similar. Figure 1 shows the extent of the mismatch of the relaxation times. Note that the values for the tissues fall between the solutions of pure agarose and pure copper sulfate.

Figure 2 is a log-log plot of T1 and T2 at 5 MHz for pure solutions of agarose and copper sulfate and of mixtures of agarose and copper sulfate. Dashed lines connect points of equal agarose or copper concentration. The upper right point corresponds to pure distilled deionized water. It can be seen that by selecting an appropriate percentage of agarose and concentration of copper sulfate a mixture with a T1 and T2 equivalent to a selected tissue can be produced. Again, a similar plot can be produced at 60 MHz. Note how T1 depends mostly on copper concentration and T2 depends mostly on agar concentration.

These graphs are not intended to imply a functional relationship between T1 and T2 but rather to act as nomograms allowing the selection of an appropriate

Table 1. Relaxation times in milliseconds of selected rabbit tissues and phantom materials. Means and standard deviations of three samples.

Material	5 MHz		60 MHz	
	T1	T2	T1	T2
Psoas Muscle	366 (19)	25 (2)	820 (36)	52 (6)
Renal Cortex	285 (37)	39 (8)	593 (9)	67 (10)
Renal Medulla	764 (25)	87 (26)	1180 (22)	123 (12)
Perirenal Fat	177 (36)	92 (12)	201 (5)	149 (10)
Blood	605 (50)	145 (9)	1140 (54)	250 (18)
$CuSO_4$ – 0.5mM	676 (12)	606 (12)	1469 (54)	1260 (58)
$CuSO_4$ – 1 mM	411 (8)	351 (11)	1002 (20)	970 (20)
$CuSO_4$ – 2 mM	206 (18)	181 (12)	564 (6)	557 (19)
$CuSO_4$ – 4 mM	116 (5)	100 (4)	320 (4)	298 (10)
Agarose 0.5% W/V	1481 (151)	240 (15)	2743 (71)	278 (43)
Agarose 1% W/V	1309 (106)	106 (26)	1790 (43)	89 (2)
Agarose 2% W/V	1122 (98)	56 (16)	1590 (50)	54 (4)
Agarose 4% W/V	1000 (92)	23 (9)	1390 (84)	27 (3)

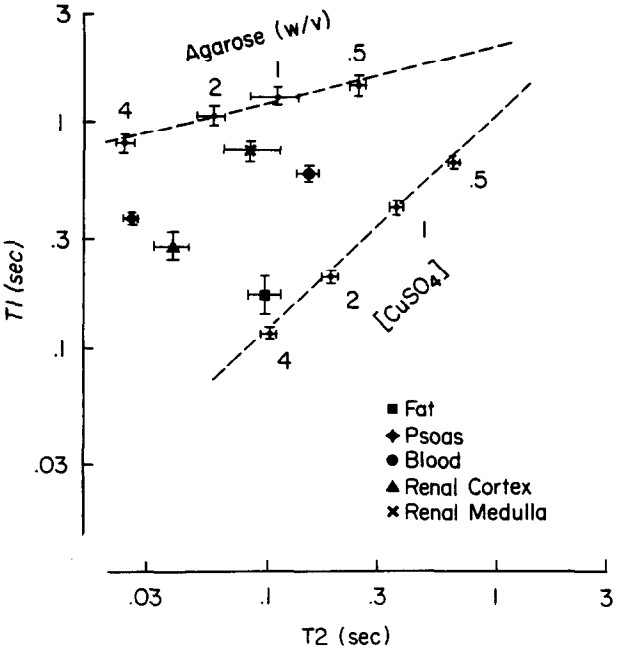

Fig. 1. T1 and T2 values for agarose in concentrations of 0.50 to 4.0% weight/volume; copper sulfate from 0.50 to 4.0 millimolar; and for selected rabbit tissues measured *in vitro*.

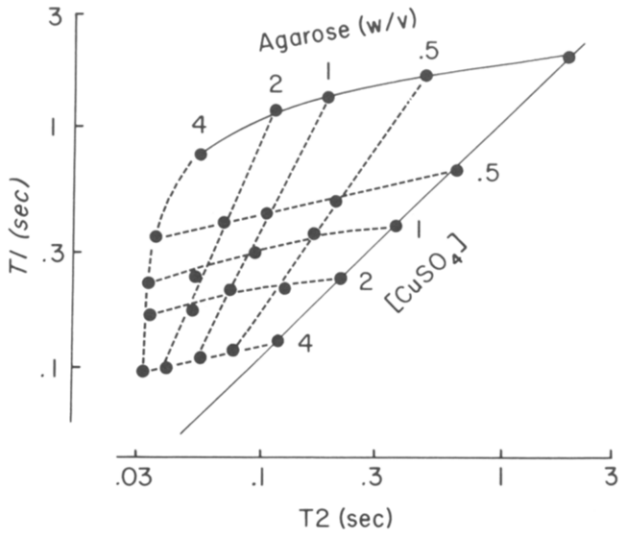

Fig. 2. T1 and T2 values for mixtures of agarose gel and copper sulfate. Agarose concentrations from 0 to 4.0% weight/volume; copper concentrations from 0 to 4.0 millimolar. Dashed lines connect points of equal agarose or copper concentration.

concentration of agarose and copper sulfate to simulate tissue T1 and T2. Since T1 and T2 values vary with frequency and often vary with different instrumentation, it is best to make up calibration graphs like this one for one's own equipment and conditions.

Figure 3 is an inversion-recovery spin echo image of an array of tubes containing agarose and copper sulfate. It was made on our laboratory NMR imaging system operating at 1.4 Tesla, using an inversion-recovery pulse sequence with repetition time (TR) of 4000 msec, inversion time (TI) of 1000 msec, and echo time (TE) of 10 msec. Figure 4 is an image of the same array of tubes made using a partial saturation spin echo sequence with repetition time of 2000 msec and echo time (TE) of 100 msec. Note how the differing sensitivities to T1 and T2 of the two different sequences are shown by the images of the phantom.

STABILITY OF AGAROSE GELS

The main disadvantage of the agarose gels is that if the tubes are not completely filled and tightly sealed, they tend to deteriorate over time. When the vials are properly filled and sealed, fungal or bacterial growth is rare, even without addition of preservatives such as formaldehyde or sodium azide. Pouring boiling agarose into the vials naturally sterilizes them. The most common problem is breakdown of the gel and loss of water. When this occurs, droplets of water form on the walls of the tube. We have, however, kept gels for over a year in glass tubes sealed with rubber stoppers or in plastic tubes sealed with Parafilm without a change in their relaxation times.

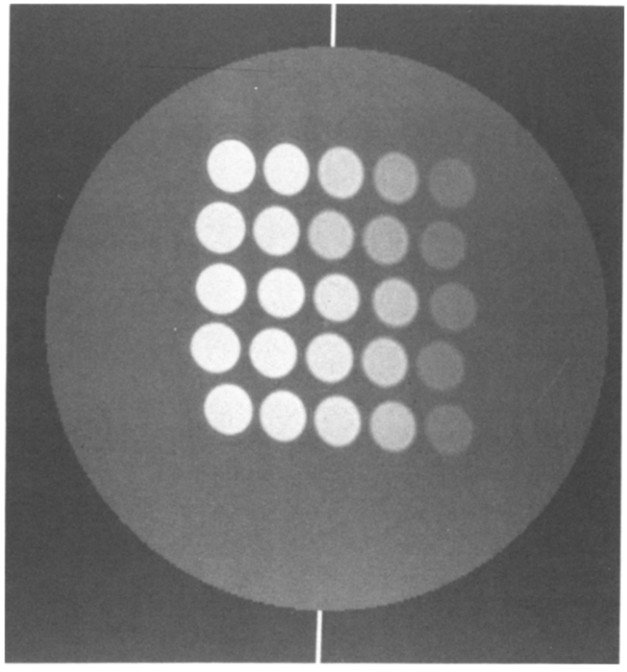

Fig. 3. A "T1 weighted" image at 1.4 tesla of an array of tubes containing agarose-copper sulfate mixtures. The rows from top to bottom contain 0, 0.50, 1.0, 2.0 and 4.0% agarose. The columns from right to left contain 0, 0.50, 1.0, 2.0, and 4.0 millimolar copper sulfate. The relative intensities of each sample in the array were not significantly changed by inverting the array in the magnet.

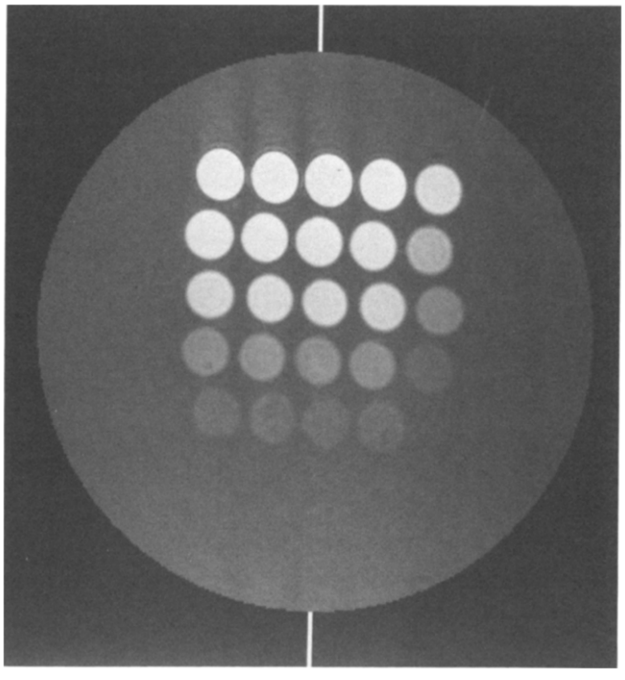

Fig. 4. A "T2 weighted" image at 1.4 tesla of an array of tubes containing agarose-copper sulfate mixtures. The rows from top to bottom contain 0, 0.50, 1.0, 2.0 and 4.0% agarose. The columns from right to left contain 0, 0.50, 1.0, 2.0, and 4.0 millimolar copper sulfate. The relative intensities of each sample in the array were not significantly changed by inverting the array in the magnet.

CONCLUSIONS

The range over which T1 and T2 can be varied in copper-agarose gels, and the ease with which such variation can be achieved makes this material ideal for NMR phantom construction. The physical properties of the gel are also ideal. The hot gel can be poured into molds of any shape, while the solidified gel can be cut with a knife. The relaxation properties of the gel are also reproducible and stable, two important qualities.

Spin-density can be varied by the substitution of D_2O for H_2O in the gel. The effects of D_2O on proton relaxation in the gel are complicated, and cannot be fully explained at this time.

We believe that copper-agarose gels are well-suited for NMR imaging phantoms. A comparison of *in vitro* and *in vivo* imaging using different pulse sequences is now in progress. The use of tissue-equivalent phantoms made of this material will allow the standardization and calibration of T1 and T2 measurements made *in vivo*, and improve quality control in NMR imaging.

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