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$Co_xZn_{x-1}Fe_2O_4$ NANOPARTICLES FERRITE SERIES AS MAGNETIC RESONANCE IMAGING CONTRAST AGENTS

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Contrast agents are used in magnetic resonance imaging to influence the magnetic relaxation process so that lesions, structures or vessel – otherwise would poorly identifiable – can be visualized. The most commonly used media are the paramagnetic contrast agents, which have their strongest effect on the T1, by shortening T1 relaxation time in tissues where they accumulate. To this purpose a lot of *in vitro* and *in vivo* studies on ferrite nanoparticles and iron oxides have been conducted. In our study a series of $Co_xZn_{x-1}Fe_2O_4$ ferrites, with x= 0; 0.2; 0.4; 0.6; 0.8; 1 were obtained by sol-gel method. Ferrite nanoparticles were dispersed in agarose gels and scanned with a medical MRI APERTO (Hitachi, Japan) device (0,4 Tesla). Basic T1 and T2 weighted sequences were obtained. Images were analyzed using a commercial program, Spin Software, to highlight the influence of particles on image contrast by attributing different colors to different intensity of pixels. For small values of x, contrast was enhanced in T2 weighted sequences while for higher values of x a positive T1 contrast was shown.

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1. Introduction

Magnetic resonance imaging (MRI) is a technique used for imaging benign or malignant tumors. This technique is based on the relaxation of magnetic nuclear moments in a high intensity magnetic field (0.2-3T) [1].

When subject to a magnetic field, the magnetic moments will align on 2 energy layers (E_1 - low energy and E_2 - high energy, $\Delta E=\gamma\hbar B_o$). Most of the moments will align on the E_1 level. When a radiofrequency pulse sequence is applied two relaxation fenomena occur – transverse and longitudinal, which are characterized by their respective relaxation times [2].

The contrast in such imaging techniques is given by different relaxation times. For many tissues contrast is similar in plain images. To increase tissue discrimination contrast agents have been used [3].

Contrast agents use magnetic nanoparticles in order to influence relaxation times. Gadolinium based agents - paramagnetic and influencing longitudinal relaxation – are commonly used. To be eligible as contrast agents nanoparticles must have certain properties such as: reduced toxicity for both the environment and the patient, a capability to influence one of the relaxation times and a sufficiently long permanence time in the tissue to be examined [4].

The development of new environmentally friendly technologies to obtain new contrast agents is of special interest. To this end we study the properties of materials such as ferrite, iron

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oxides and chelates of rare earth elements. In order to reduce ferrite and iron oxide toxicity, for biomedical applications, they can be inserted into polymeric matrix.

Several examples of investigations of nanoparticles as MRIcontrast agents can be recently found. In 2008 [5] Barcena et al. reported the results of their study on $Zn_xFe_{1-x}O\cdot Fe_2O_3$ (x up to 0,34) hydrophobic ferrites with a mixed spinel structure. They were encapsuled in polymeric micelae to decrease Zn toxicity. Mixtures of different concentrations were made and then scanned with a Varian INOVA machine. For comparison purposes different dillutions of Feridex® with a concentration of 2,1 µg mL^{-1} were investigated.

In the absence of toxicity tests Wu et al. [6] studied a series of cobalt nanoparticles Co. The nanoparticles were synthesized on the matrix of dendrimers using sodium borohydride asa reducing agent. Different concentrations were dispersed in water and scanned with MRI. Co nanoparticles show positive contrast in T1 sequence which makes them eligible for use in MRI.

This study focuses on the magnetic properties and the image contrast produced by the as obtained ferrite nanoparticles. The biocompatibility of the ferrite nanoparticles will be the subject of a future study.

2. Experimental details

Ferrite nanoparticles with the following formula $Co_xZn_{1-x}Fe_2O_4$ were obtained by sol-gel method with self combustion and then dispersed in agarose gels with identical concentrations. Eight phantoms were prepared with agarose gel by homogeneously 1280 ml of distilled water, 0,35 g NiCl, 37,5 g agar, 6,25 g NaCl and 0,3125 g azyde and splitting then into 8 equal quantities. The composition was poured in a polyethylene recipient with a cylindrical shape. For 6 out of the phantoms $Co_xZn_{1-x}Fe_2O_4$ ferrite, with x = 0, 0.2, 0.4, 0.6, 0.8, 1 was added (0,5 g/mL). For another phantom 0,1 ml of standard Gadolinium chelates contrast agent was added (Multihance, Bracco, Italy) [7] and the last one was used as a blank witness (M).

All phantoms were sonicated by using an ultrasound bath and were introduced in the microwave oven for 3 minutes at 800 W.

Cooling was achieved at room temperature for 24 hours resulting a solidified gel (Fig.1).

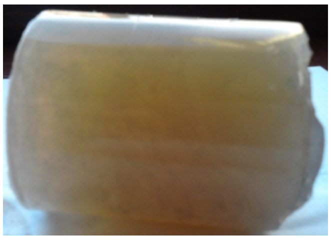

Fig. 1. Agarose gel phantom with ferrite nanoparticles.

Phantoms were imaged with an Aperto MRI scanner (Hitachi, Japan). This device has a permanent magnet with 0,4 T field strength. A head coil using a specific protocol design was used. Phantoms were arranged in 2 rows inside the coil (Fig 2). Due to the cilindrical shape and the phantoms arrangement, we used coronal sections, the resulting image being in an axial plane.

Fig. 2. Arrangement of the phantoms inside the coil.

To scan these phantoms, standard MRI sequences were used - T1- and T2-weighted with their respective relaxation times. Parameters are shown in Table 1.

Table 1. Fiel of view (FOV), repetition time (TR), echo time (TE), signal-to-noise ratio (S/N, SNR).

Sequence	**FOV**	**TR (ms)**	**TE (ms)**	**SNR**
T1	220	194	13	4
T2	220	2500	100	4

3. Results and discussion

The structural characteristics and the magnetic properties were studied using X-ray diffraction (XRD) and vibrating sample magnetometer techniques (VSM).

The variation of the $Co_xZn_{x-1}Fe_2O_4$ series magnetization depending on the applied field (maximum 10 kOe) was studied at room temperature. Average crystallite dimensions, saturation magnetization and coercitive field values are shown in Table 2. An increase in the Co concentration increases the value for the saturation magnetization reaching a maximum for the x = 0.8 sample, while the coercitive field has the lowest value for the same sample. This behaviour is due to microstructural factors and to the influence of the magnetic Fe^{3+} and Co^{2+} cations in the B-site which contribute to the increase of the total magnetization.

Table 2. Crystallite size (D), saturation magnetization (Ms) and coercive field (Hc)

x	D (nm)	Ms (emu/g)	Hc (Oe)
0	67		
0.2	67	4.31	
0.4	49	44	15
0.6	48	77	54
0.8	67	87	174
1	68	80	563

Contrast agents based on paramagnetic ions are the most extensively used. They have a positive magnetic susceptibility. The magnetic moments orient themselves along the magnetic field. The interactions between the magnetic moments and the tissue are of two types: 1. the interactions between water molecules and the paramagnetic ions - scalar relaxations which are given by the total number of molecules linking to the ions and 2. the dipol-dipol interaction between the protons and the paramagnetic ions. Paramagnetic ions are used as T1 contrast agents [8]. Superparamagnetic ions are used as T2 contrast agents. They induce inhomogeneities in the magnetic field resulting in an offset of the magnetic moments. This effect shortens the T2 relaxation time increasing the brightness of T2-weighted images.

MRI scans are shown in Fig 3 and 4.

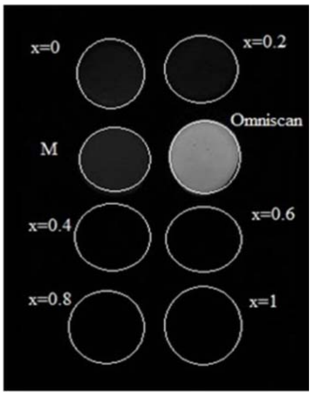

Fig. 3. T1-weighted images of phantoms.

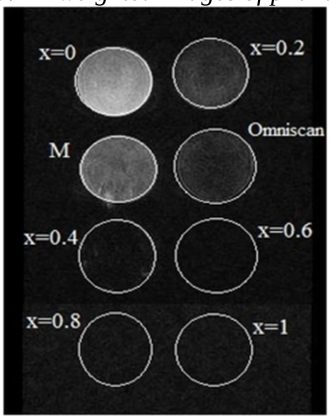

Fig. 4. T2-weighted images of phantoms.

Fig. 3 shows that in T1-weighted images of phantoms with contrast agent (Omniscan) have a positive contrast. This is in agreement with the literature data [9 - 14] as it is well known that gadolinium chelates have a positive T1 contrast. Gadolinium chelates are more extensively used beacause of their smaller toxicity effects on the body compared to pure Gadolinium.

In the case of phantoms where ferrite was dispersed we can see a decreased contrast compared to Ominiscan.

In T1-weighted images phantoms with x=0, 0.2 shows a moderate contrast. For the other phantoms it is hard to assess the intensity of the different gray tones. These results are in agreement with the results of Wu et al who noted signal modifications when the Co ferrite is used in different concentrations being scanned with a 0,5 T device.

The phantom with Zn ferrite nanoparticles (x=0) in T2-weighted images has positive contrast while the other shows minimal contrast (see Fig. 4). For the Zn ferrite the same results were obained by Barcena et al. [5] the Zn ferrite is well known for laboratory applications in MRI and it shows positive contrast in T2-weighted and negative in T1-weighted images.

For every phantom, during the scanning process, seven images were obtained in each sequence. Fig. 5 shows an example for x=0, x=0,2 and Omniscan. The images keep the signal intensity constant through-out the phantom. No major variations of the intensity of the signal or the composition of the phantom were observed proving that an uniform distribution of the ferrite nanoparticles and contrast agent was achieved by sonnication. That is why Fig. 3 and 4 show only the central slice of the phantom.

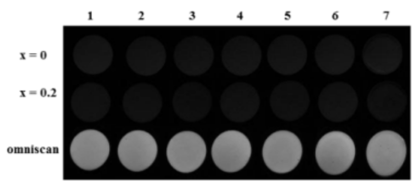

Fig. 5. Omniscan and ferrite with x=0 and x=0,2 in T1-weighted images.

Using the Spin software [15] program we post-processed the image in Fig. 5. The principle of this software is to attribute the red colour to the pixels with the highest intensity and black to those with the lowest intensity as can be seen in Fig. 6.

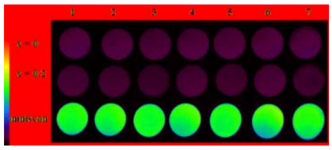

Fig. 6. Post-processing results using with Spin software program.

Using ImageJ [16],we studied the gray intensity depending on the degree of Zn substitution with Co.

To this end the images in Fig.3 and Fig. 4 were loaded into ImageJ. For each image of each phantom an area of 132 mm^2 was selected - the center of the phantom. The mean of the gray value for the selected area was computed. The mean gray value command makes a simple mean of the gray tones in the selected area. For colour images the mean value is calculated by transforming each pixel into the corresponding gray value by using formula:

$$\text{Gray} = (\text{red}+\text{green}+\text{blue})/3 \qquad (1)$$

or this formula:

$$\text{Gray} = 0.299 \times \text{red} + 0.587 \times \text{green} + 0.114 \times \text{blue} \qquad (2)$$

The integrated density was another function from the ImageJ software and represents the sum of the intensities for all the pixels in the selected area.

Fig. 7 shows a visual representation of the dependency between the Co concentration and the mean of the gray tones as well as the integrated density for T1 and T2.

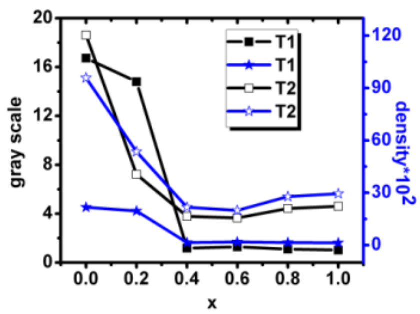

Fig. 7. Integrated density and mean gray dependence with x value from $Co_xZn_{1-x}Fe_2O_4$ formula.

For the Zn ferrite (x=0) the intensity of gray is the highest for both T1 and T2. As the Co concentration increases the intensity of gray diminishes between x= 0-0,4 and for the other vaues of x only small variations of gray intensity can be seen in T2. For T1 the interval x=0,4-1 is almost constant. This can be explained by the magnetic properties of the ferrite inducing an increase of the T2 time - a less bright image.

4. Conclusions

This study investigates the potentiality of $Co_xZn_{x-1}Fe_2O_4$ ferrite nanoparticles to be used as a contrast agent. The ferrite was uniformly dispersed in agarose gels as our images'analysis proved. The images were supplementary processed using Spin and ImageJ softwares. The analysis of the images obtained through MRI shows that the phantom containing the commercially available contrast agent presents positive contrast in T1. The Zn ferrite (x=0) reveals positive contrast in T2. The substitution of the Zn ions to the Co ions reduces the signal in T2-weighted images and leads to a minor increase of the signal in T1-weighted images.

Futher investigations are needed to use the Zn ferrite dopped with Co as a contrast agent in MRI .

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