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Effect of Gd and Dy Concentrations in Layered Double Hydroxides on Contrast in Magnetic Resonance Imaging

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Abstract: In this work, we explore the synthesis of layered double hydroxide (LDH) particles containing different molar ratios of Gd^{3+} and Dy^{3+} cations. A single crystalline phase was obtained for $Zn_{2.0}Al_{0.75}Gd_{0.125}Dy_{0.125}$ -LDH and $Zn_{2.0}Al_{0.5}Gd_{0.25}Dy_{0.25}$ -LDH, and their efficiency as contrast agents was evaluated by T1- and T2-weighted magnetic resonance imaging (MRI). Both GdDy-LDHs exhibited longitudinal relaxivity (r_1) higher than a commercial reference. The highest contrast in the T1 mode was achieved with the $Zn_{2.0}Al_{0.75}Gd_{0.125}Dy_{0.125}$ -LDH, which contained the lowest concentration of lanthanides; this efficiency is related to the lowest amount of carbonate anions complexing the lanthanide sites. On the contrary, the best contrast in the T2 mode was achieved with $Zn_{2.0}Al_{0.5}Gd_{0.25}Dy_{0.25}$ -LDH. $Zn_{2.0}Al_{0.75}Gd_{0.125}Dy_{0.125}$ -LDH and $Zn_{2.0}Al_{0.5}Gd_{0.25}Dy_{0.25}$ -LDH presented r_2/r_1 ratios of 7.9 and 22.5, respectively, indicating that the inclusion of gadolinium and dysprosium into layered structures is a promising approach to the development of efficient bimodal (T1/T2) MRI contrast agents.

Keywords: layered double hydroxides; gadolinium; dysprosium; MRI contrast

1. Introduction

Cancer is a primary public health problem worldwide, and effective cancer treatment depends on timely detection. Magnetic resonance imaging (MRI) is a non-invasive diagnostic modality that allows the detection of soft tissue disorders using non-ionizing radiation [1]. In this technique, the water protons that constitute the tissues are excited by the application of a magnetic field and radiofrequency energy. The contrasts observed in the images show the differences in the water proton relaxation times in healthy and abnormal tissues [2,3]. The variation in proton density between tissues is small, but the use of contrast agents improves the MRI sensitivity and specificity for an accurate diagnosis [4].

Gadolinium chelates, such as Gadovist and Magnevist, are widely used as MRI contrast agents [5]. The paramagnetic susceptibility of Gd^{3+} induces a prolonged magnetic moment that decreases the longitudinal relaxation time (T1) of tissue water protons generating a positive contrast, that is, brighter images. Dysprosium is another lanthanide that has been explored as a MRI contrast agent. The high magnetic moment of Dy^{3+} reduces the transverse relaxation time (T2) of the water protons, generating a negative contrast [6–8]. The combination of Gd^{3+} and Dy^{3+} chelates, T1 and T2 agents, respectively, has been studied for the differentiation of cardiac anomalies, precise delineation of liver necrosis and the visualization of ischemic intestine using a double contrast technique [6].

Chelation of lanthanides decreases the toxicity of free ions, but also restricts the access of water molecules to their inner coordination sphere [8]. The stabilization of Gd^{3+} and Dy^{3+} into

the layered double hydroxides (LDH) structure has been another strategy explored to reduce the toxicity of lanthanides without compromising their MRI contrast effect [9]. LDHs are anionic clays constituted of divalent (M^{2+}) and trivalent (M^{3+}) cations hexacoordinated to hydroxyl groups. The M^{3+} cations generate an excess positive charge on the layers, which is compensated by interlayer anions. The immobilization of Gd^{3+} [10–12] and Dy^{3+} [13,14] in LDH structures by partial isomorphic substitution of M^{3+} allows the obtaining of biocompatible nanomaterials with possible theragnostic applications [9].

On the other hand, particle size and the positive charge density of LDHs, which is generated by their capacity for anion exchange and the diffusion of surface-adsorbed anions into the environment, favor interaction with cell membranes and promote more efficient internalization [15]. Furthermore, the basicity of LDHs confers selective release of lanthanides under the slightly acidic conditions of the tumor microenvironment. In this way, the controlled administration and selective biodistribution of LDHs could reduce the effective dose of contrast agents and their toxicity in healthy tissues [15,16]. In this paper, we describe the synthesis and characterization of GdDy-doped LDHs as well as the influence of lanthanides concentration on MRI contrast.

2. Materials and Methods

2.1. Synthesis of LDH

LDHs with different concentrations of GdDy were prepared by the precipitation method. Briefly, 40 mL of salt solution containing $Zn(NO_3)_2 \cdot 6H_2O$ (Meyer, Mexico), $Al(NO_3)_3 \cdot 9H_2O$ (Meyer, Mexico), $Gd(NO_3)_3 \cdot xH_2O$ (Sigma-Aldrich, USA) and $Dy(NO_3)_3 \cdot xH_2O$ (Sigma-Aldrich, Missouri) was titrated with 6% NH_4OH (Meyer, Mexico) under vigorous stirring in an environmental air atmosphere. The M^{2+}/M^{3+} molar ratio (where $M^{3+} = Al^{3+} + Gd^{3+} + Dy^{3+}$) in this mixture was equal to 2:1 (Table 1). The final pH of the solution was adjusted to 10 and the resulting suspension was stirred for 24 h at room temperature. Finally, the obtained precipitate was washed three times with deionized water and dried at 40 °C.

Table 1. Amount of metal salts used in the synthesis.

Sample	Metal Salts, g (mmol)			
	$Zn(NO_3)_2 \cdot 6H_2O$	$Al(NO_3)_3 \cdot 9H_2O$	$Gd(NO_3)_3 \cdot xH_2O$	$Dy(NO_3)_3 \cdot xH_2O$
$Zn_{2.0}Al_{1.0}$	1.190 (4)	0.720 (1.92)	-	-
$Zn_{2.0}Al_{0.75}Gd_{0.125}Dy_{0.125}$	1.190 (4)	0.562 (1.50)	0.116 (0.25)	0.087 (0.25)
$Zn_{2.0}Al_{0.5}Gd_{0.25}Dy_{0.25}$	0.595 (2)	0.188 (0.50)	0.113 (0.25)	0.102 (0.29)
$Zn_{2.0}Al_{0.25}Gd_{0.37}Dy_{0.37}$	0.595 (2)	0.094 (0.25)	0.170 (0.37)	0.141 (0.40)
$Zn_{2.0}Gd_{0.5}Dy_{0.5}$	0.595 (2)	-	0.225 (0.50)	0.174 (0.50)

2.2. Characterization of Materials

X-ray diffraction (XRD) patterns were recorded on a PANalytical Empyrean diffractometer using Cu K α radiation at 40 kV and 20 mA. Fourier transform infrared (FT-IR) spectra were collected in a Thermo-Scientific Nicolet iS5 iD5 ATR spectrometer with 32 scans and a resolution of 2 cm^{-1} . The metallic composition was determined via inductively coupled plasma optical emission spectroscopy (ICP-OES) (Varian, Vista-MPX CCD) simultaneously. Thermal analysis was carried out using a Perkin-Elmer STA 6000 analyzer under a flowing nitrogen atmosphere and a rate of 10 °C min^{-1} . Magnetic resonance imaging (MRI) analyses were performed on a Bruker BioSpec 70/16 scanner with a 7.0 T magnetic field and a pulse sequence RARE-VTR. The T1-relaxivity measurements were acquired using the following parameters: repetition time (RT) = 40, 100, 200, 500, 1000, 3000, 5000 and 7500 ms; echo time (ET) = 11.7 ms; field of view (FOV) = 40 × 24 mm^2 ; matrix = 134 × 78; and slice thickness = 2 mm. For the T2-relaxivity measurements, the parameters were RT = 2000 ms, ET = 36 echoes, 40–1440 ms with a separation between echo times of 40 ms.

3. Results and Discussion

3.1. Characterization of LDH

The white solids obtained in the reactions were analyzed by XRD. As shown in Figure 1a–c, the diffraction patterns of samples containing Al = 1.0, 0.75 and 0.50 presented typical reflections of hydrotalcite-like compounds according to International Centre for Diffraction Data (ICDD) card number 48-1023 (Figure 1). Additional reflections were not observed in these XRD patterns, suggesting the formation of the pure LDH phase. The lower full width at half maximum (FWHM) of reflections in the samples with Al = 0.75 and 0.50 indicate that this concentration increased the crystallinity.

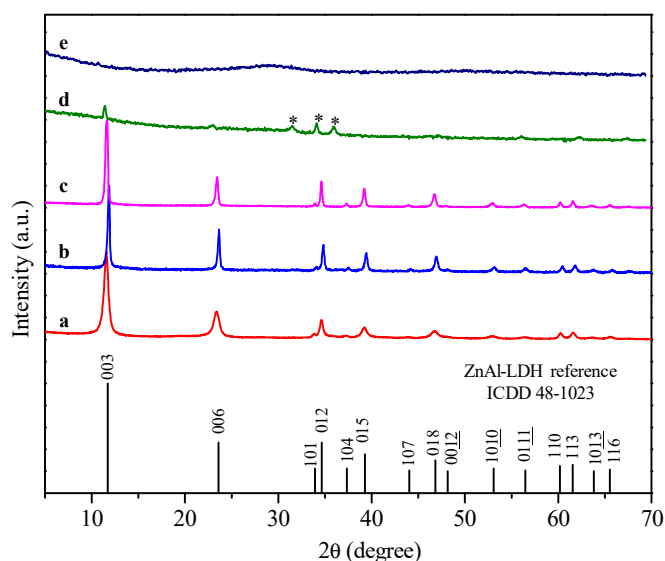


Figure 1. XRD pattern of the samples: (a) $\text{Zn}_{2.0}\text{Al}_{1.0}$, (b) $\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$, (c) $\text{Zn}_{2.0}\text{Al}_{0.5}\text{Gd}_{0.25}\text{Dy}_{0.25}$, (d) $\text{Zn}_{2.0}\text{Al}_{0.25}\text{Gd}_{0.37}\text{Dy}_{0.37}$ and (e) $\text{Zn}_{2.0}\text{Gd}_{0.5}\text{Dy}_{0.5}$.

Meanwhile, the XRD pattern of the sample with Al = 0.25 contained reflections of an LDH structure (Figure 1d) and also signals of ZnO (ICDD card number 36-1451) indicated with asterisk in Figure 1. Although the nominal $\text{M}^{2+}/\text{M}^{3+}$ molar ratio was maintained, the low Al concentration and the addition of the large Gd^{3+} and Dy^{3+} cations did not promote the formation of the layered structures, generating ZnO from the remaining Zn cations. Finally, the pattern of the sample where Al = 0 (i.e., with the highest content of Gd^{3+} and Dy^{3+}) corresponded to an amorphous phase (Figure 1e). Therefore, a content of Al = 0.50 or more favored the formation of LDH structures with the highest Gd^{3+} and Dy^{3+} content, allowing the successful incorporation of Zn cations into the layered structure.

According to the X-ray diffraction patterns, the three LDHs had a rhombohedral 3R symmetry. The first two reflections corresponded to the (003) and (006) planes, respectively. The basal spacing (d_{003}) corresponded to the distance of two layered units and included the thickness of a layer (4.8 Å) and the interlayer region. Then, by calculating the interlayer space, the size and orientation of the intercalated anion could be determined. The interlayer space equal to 2.9 Å in the obtained LDHs suggested the intercalation of carbonate anions with its molecular plane parallel to the layers [17]. The LDHs were prepared from metal nitrates without using a CO_2 -free atmosphere, promoting the intercalation of carbonate anions formed in situ due to the dissolution of atmospheric CO_2 during synthesis. Several experimental conditions for the synthesis of GdDy-LDH intercalated with nitrates were explored as these anions favored the incorporation of interlayer water molecules [18], which could improve contrast on MRI. However, the $\text{Zn}_{2.0}\text{Al}_{0.5}\text{Gd}_{0.25}\text{Dy}_{0.25}$ -LDH was only obtained with interlayer carbonate anions.

The XRD analysis of the LDHs obtained also provided information related to unit cell parameters. The lattice parameter a indicates the distance between cations (M-M) within the layers, which does not depend on the intercalated anion. For LDHs with 3R symmetry, the parameter a was equal to two times the spacing of the (110) reflection, assigned to the first peak of the doublet at around 60° (2θ), then, $a = 2d_{110}$. The lattice parameter c is closely related to the basal spacing (d_{003}) depending on the degree of hydration, size and orientation of the intercalated anion. The value c was three times the distance corresponding to the reflection of the (003) plane, $c = 3d_{003}$. Compared to ZnAl-LDH, the low concentration of Gd^{3+} and Dy^{3+} cations in the LDH structure did not significantly modify the lattice parameters a and c (Table 2); these observations were in agreement with reported values for lanthanides-doped LDH [19,20].

Table 2. The lattice parameters and basal spacing of LDH prepared.

Sample	a (Å)	c (Å)	d_{003}
$Zn_{2.0}Al_{1.0}$	3.095	23.1	7.70
$Zn_{2.0}Al_{0.75}Gd_{0.125}Dy_{0.125}$	3.087	22.53	7.51
$Zn_{2.0}Al_{0.5}Gd_{0.25}Dy_{0.25}$	3.096	23.01	7.67

The reaction products were also characterized by Fourier transform infrared spectroscopy (FT-IR). The FT-IR spectra of compounds with Al = 1.0, 0.75 and 0.50 presented characteristic bands of hydrotalcite-type compounds (Figure 2a–c). The broad band centered at 3410 cm^{-1} corresponded to O-H stretching of layer hydroxyl groups, as well as the adsorbed and intercalated water molecules. The broad band at 3000 cm^{-1} was attributed to stretching of O-H groups hydrogen bonded to intercalated carbonate anions [21], while the band at 1640 cm^{-1} was related to H-O-H bending of physically-adsorbed water. The broad bands at around 750 , 600 and 550 cm^{-1} were due to the metal-OH (M-OH) translation mode [19,21]. For GdDy-LDHs, this band was more intense and was slightly shifted to higher frequencies than that for the ZnAl-LDH. The changes in the bands M-OH suggest the occurrence of GdDy-induced small modulations, which has also been observed for other lanthanide-doped ZnAl-LDHs [19]. For samples $Zn_{2.0}Al_{0.25}Gd_{0.37}Dy_{0.37}$ and $Zn_{2.0}Gd_{0.5}Dy_{0.5}$, the bands in the range from 1000 to 525 cm^{-1} did not correspond to M-OH vibrations for LDH structures (Figure 2d,e), as shown in their XRD patterns.

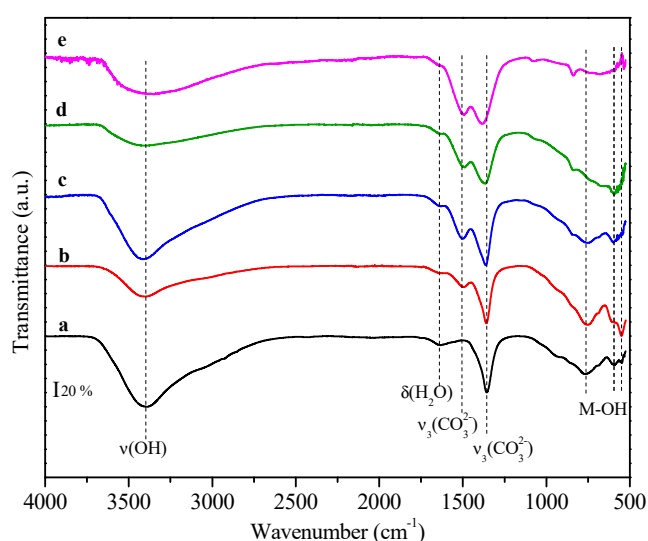


Figure 2. FT-IR spectra of the samples: (a) $Zn_{2.0}Al_{1.0}$, (b) $Zn_{2.0}Al_{0.75}Gd_{0.125}Dy_{0.125}$, (c) $Zn_{2.0}Al_{0.5}Gd_{0.25}Dy_{0.25}$, (d) $Zn_{2.0}Al_{0.25}Gd_{0.37}Dy_{0.37}$ and (e) $Zn_{2.0}Gd_{0.5}Dy_{0.5}$.

The presence of the interlayer carbonate was also corroborated by FT-IR. The band at 1360 cm^{-1} was attributed to ν_3 stretching of the interlayer carbonates in a symmetric environment. The interactions of carbonates with other species decreased the symmetry of the anion from D_{3h} to C_{2v} or C_s , breaking the double degeneration of the ν_3 stretching mode [21,22]. In the spectra of the GdDy-LDHs, an additional band was observed at around 1500 cm^{-1} due to the splitting of ν_3 mode of the anion (Table 3), evidencing the interactions between carbonates and the lanthanides. As shown in Figure 2, the higher lanthanide content increased the intensity of the band located at 1500 cm^{-1} , indicating a larger amount of lower-symmetry carbonate in the interlayer space of $\text{Zn}_{2.0}\text{Al}_{0.5}\text{Gd}_{0.25}\text{Dy}_{0.25}$ -LDH. The change in the symmetry of carbonate could be due to the formation of a mono- or bidentate carbonate-metal complex [23]. Based on experimental data, Nakamoto [22] proposed that the separation of ν_3 vibration bands ($\Delta\nu_3$) indicates the coordination mode of carbonate. In GdDy-LDHs, the $\Delta\nu_3 \approx 140\text{ cm}^{-1}$ (Table 3) suggested a monodentate coordination, since $\Delta\nu_3 > 300\text{ cm}^{-1}$ is characteristic of bidentate carbonate metals [22]. Unlike transition metals, lanthanide cations have large coordination numbers, generally from 8 to 10 [24]. Therefore, carbonate anions and water molecules can complete the inner coordination sphere of Gd^{3+} and Dy^{3+} .

Table 3. Position of the bands assigned to the ν_3 stretching modes of the carbonate anion.

Sample	$\nu_3(\text{CO}_3^{2-})\text{ cm}^{-1}$, (intensity, %)	$\Delta\nu_3$
$\text{Zn}_{2.0}\text{Al}_{1.0}$	1356	-
$\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$	1359 (86), 1505 (40)	146
$\text{Zn}_{2.0}\text{Al}_{0.5}\text{Gd}_{0.25}\text{Dy}_{0.25}$	1363 (99), 1502 (63)	139

The incorporation of lanthanides in LDH was confirmed by inductively coupled plasma optical emission spectroscopy (ICP-OES). The analysis of $\text{Zn}_{2.0}\text{Al}$ -LDH revealed a $\text{M}^{2+}/\text{M}^{3+}$ molar ratio equal to 1.3:1, which is lower than the expected value (2:1). On the contrary, the molar fractions in Table 4 indicate that the content of metals in $\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$ was quite close to the nominal amounts used in synthesis. Likewise, ICP-OES analyses suggest that the addition of a higher concentration of Gd^{3+} and Dy^{3+} in the reaction mainly promoted the partial isomorphic substitution of Zn^{2+} by the lanthanide cations, which can be explained by Goldschmidt's rules of ionic substitution [25]. Shannon-effective ionic radii of Zn^{2+} , Al^{3+} , Gd^{3+} and Dy^{3+} were 0.88, 0.67, 1.08 and 1.05 Å, respectively [26]. The ionic radius of lanthanide favors the substitution of zinc cations due to a greater similarity with its ionic radii compared to aluminum. Furthermore, this substitution was possible since the charge difference did not exceed the unit [25]. In all the preparations, the zinc and other remaining ions were removed by washing with deionized water.

Table 4. Molar fractions of metal cations in the samples.

Sample	Molar Fraction ¹ (Nominal)				Molar Fraction ¹ (Experimental)			
	Zn	Al	Gd	Dy	Zn	Al	Gd	Dy
$\text{Zn}_{2.0}\text{Al}_{1.0}$	0.67	0.33	-	-	0.57	0.43	-	-
$\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$	0.67	0.25	0.04	0.04	0.68	0.25	0.04	0.03
$\text{Zn}_{2.0}\text{Al}_{0.5}\text{Gd}_{0.25}\text{Dy}_{0.25}$	0.67	0.17	0.08	0.08	0.41	0.28	0.17	0.15

¹ Molar fraction = M_x/M_{total} , where M_x = Zn, Al, Gd or Dy; and M_{total} = Zn + Al + Gd + Dy.

The influence of lanthanides on the thermal stability of LDHs was studied by TGA (Figure 3). Thermal decomposition of the GdDy-LDHs occurred mainly in four stages, which are typical for hydrotalcite-like compounds [27]. Once the mass losses associated with each stage were not well-defined transitions, the first derivative of the TGA curves was calculated to determine the temperature of each step, and these data are reported in Table 3. The first mass loss occurred at temperatures below 100°C and was related to the removal of physisorbed water, which constituted less than 5% of the initial

mass of the analyzed LDHs. The second mass loss was observed between 100 and 200 °C, and it was attributed to the elimination of interlayer water and partial dehydroxylation of the layers [19]. In this step, $\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$ -LDH loses 16% of its initial mass, a greater amount compared to 12.8% and 10.2% for $\text{Zn}_{2.0}\text{Al}$ -LDH and $\text{Zn}_{2.0}\text{Al}_{0.5}\text{Gd}_{0.25}\text{Dy}_{0.25}$ -LDH, respectively (Table 5). According to Posati et al. [19], the different thermal behavior of the LDHs is due to the ability of the lanthanide ions to coordinate interlayer water molecules to complete the coordination vacancies. Coordinated water loss occurs at temperatures below that observed for interlayer hydrogen-bonded water. Therefore, dehydration promotes dehydroxylation and the collapse of the GdDy-LDHs structures [19,20].

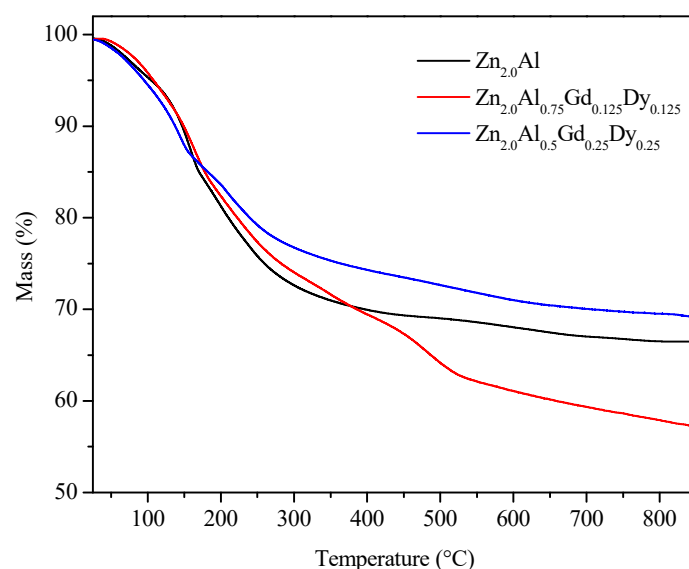


Figure 3. TGA curves of the LDH prepared.

Table 5. TGA data of the sample LDH structures.

Sample	Temperature Range (°C) and Mass Loss (%)			
	1st Loss	2nd Loss	3rd Loss	4th Loss
$\text{Zn}_{2.0}\text{Al}_{1.0}$	25–100 (4.4)	100–200 (12.8)	200–450 (13.7)	450–850 (2.6)
$\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$	25–80 (2.6)	80–210 (16.1)	210–560 (19.4)	560–850 (4.7)
$\text{Zn}_{2.0}\text{Al}_{0.5}\text{Gd}_{0.25}\text{Dy}_{0.25}$	25–100 (4.3)	100–180 (10.2)	180–440 (11.7)	440–850 (4.6)

Additional mass losses, from 200 to 850 °C, were associated with complete dehydroxylation and decarbonation, generating the largest mass loss in all samples [28]. These mass losses occurred in different temperature ranges, which could be due to the diverse interactions between metal cations and carbonate anions [20], and the formation of mixed metal oxides [27]. In particular, LDH exhibited a significant mass loss around 500 °C, suggesting a greater amount of H_2O and CO_2 molecules removed during the dehydroxylation and decarbonization reactions, respectively [19,28].

3.2. MRI Contrast Effect

Relaxometry was assessed in T1- and T2-weighted MRI images as a function of lanthanides concentration in aqueous suspensions of $\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$ -LDH and $\text{Zn}_{2.0}\text{Al}_{0.5}\text{Gd}_{0.25}\text{Dy}_{0.25}$ -LDH. The T1-relaxivity plots of both GdDy-LDHs showed a linear correlation between the Gd^{3+} concentration and the longitudinal relaxation rate (Figure 4). The enhanced contrast was assigned to the presence of Gd^{3+} since the positive contrast ($1/T_1$) increased proportionally to the Gd^{3+} concentration in the aqueous suspensions. The slope from this graph was defined as the longitudinal relaxivity (r_1) and indicated the efficiency of a T1 contrast agent. The r_1 values of $\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$ -LDH and $\text{Zn}_{2.0}\text{Al}_{0.5}\text{Gd}_{0.25}\text{Dy}_{0.25}$ -LDH were 7.18 and 3.71 $\text{mM}^{-1}\cdot\text{s}^{-1}$,

respectively, which suggest that both GdDy-LDHs exhibited a higher contrast effect than Gadovist ($r_1 = 2.24 \text{ mM}^{-1} \cdot \text{s}^{-1}$), a clinically-approved T1 contrast agent. In this Gd complex, the coordination of organic ligand only allowed a site of water binding to the inner sphere of Gd^{3+} . GdDy-LDH possibly allowed more access of water molecules to the first coordination of the Gd^{3+} , reducing the T1 of the water protons (Figure 5).

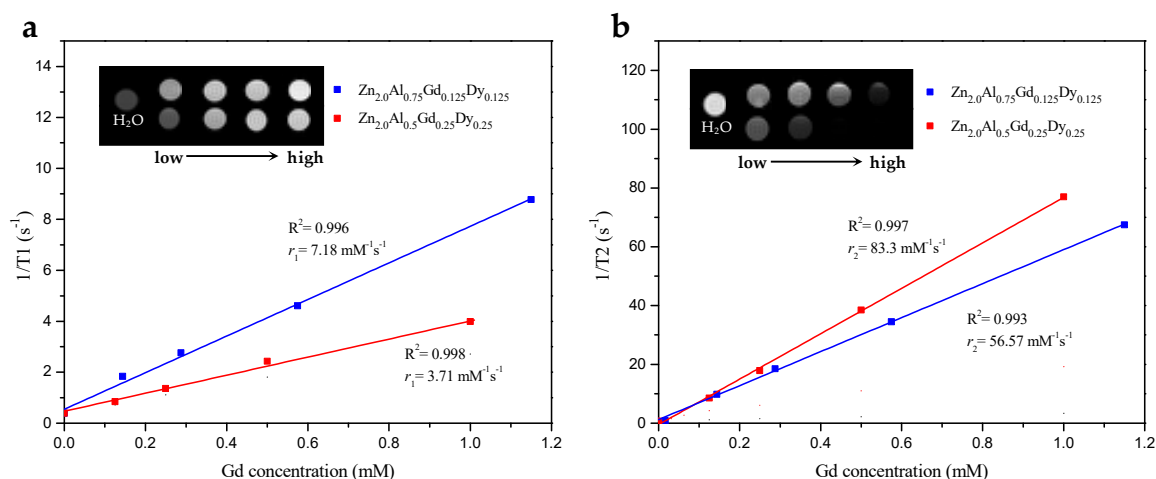


Figure 4. (a) T1- and (b) T2-relaxivity plots of aqueous suspensions of GdDy-LDHs with different Gd^{3+} concentrations, and MRI images.

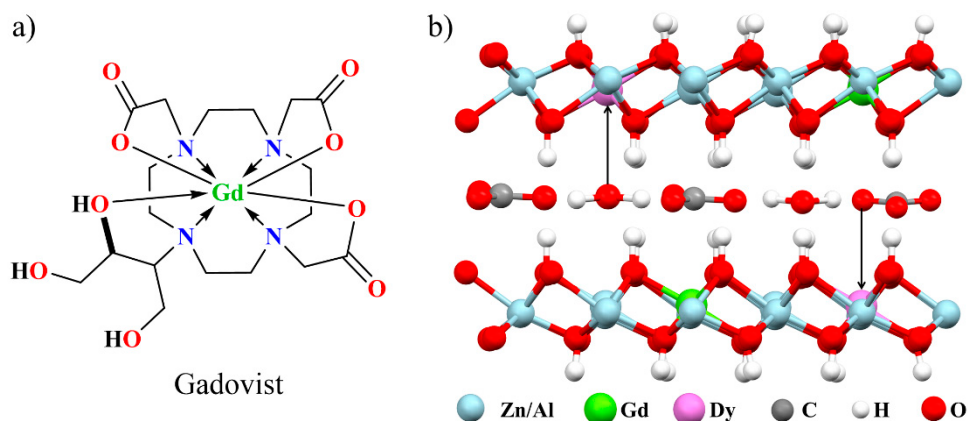


Figure 5. Schematic representation of (a) Gadovist and (b) GdDy-LDH.

An important observation was the larger r_1 value of $\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$ -LDH compared to that of $\text{Zn}_{2.0}\text{Al}_{0.5}\text{Gd}_{0.25}\text{Dy}_{0.25}$ -LDH, i.e., the LDH composed with the lower amount of Gd^{3+} produced higher contrast. The r_1 value of $\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$ -LDH indicates that Gd^{3+} sites were more effective for the contrast, very probably since they were more available for coordination with water molecules. This fact seems to be also related to competition with carbonate ions. As observed in the FT-IR spectrum, the higher content of gadolinium increased the band at 1500 cm^{-1} associated with the amount of carbonate coordinating lanthanides, while the band of the bending mode of water at 1640 cm^{-1} barely changed. The I_{1500}/I_{1640} intensity ratio allowed for comparison of the changes; while the value for $\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$ -LDH was 3.0, it increased to 4.2 in $\text{Zn}_{2.0}\text{Al}_{0.5}\text{Gd}_{0.25}\text{Dy}_{0.25}$ -LDH. These data indicate that $\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$ -LDH contains more interlayer water in agreement with TGA analysis. According to Perez et al. [29], the direct interaction of the carbonate anions with the layer hydroxyl groups is favored by the presence of less water in the layer space. Due to this, the higher interlayer water content in $\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$ -LDH reduces interactions between

carbonate anions and lanthanides, improving the T1 contrast effect. Therefore, the design of an efficient MRI contrast agent does not mainly depend on the amount of lanthanides in the LDH structure.

On the other hand, Dy^{3+} cations produced a negligible T1 contrast effect as they promoted very fast electronic relaxation and lowered the T1 relaxation time. In addition, the slow exchange of water molecules in the inner sphere of dysprosium increased efficiency in transverse relaxivity (r_2), generating a negative contrast [6,7]. T2-relaxivity plots (Figure 4) show the proportional increase of the transversal relaxation rate ($1/T_2$) with Dy^{3+} concentration. The r_2 values of $\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$ -LDH ($56.57 \text{ mM}^{-1}\cdot\text{s}^{-1}$) and $\text{Zn}_{2.0}\text{Al}_{0.5}\text{Gd}_{0.25}\text{Dy}_{0.25}$ -LDH ($83.86 \text{ mM}^{-1}\cdot\text{s}^{-1}$) were significantly higher than spirodiamide, the Dy^{3+} complex most studied as a T2 contrast agent ($r_2 = 0.12 \text{ mM}^{-1}\cdot\text{s}^{-1}$) [30,31]. Currently, Dy-based MRI contrast agents are not commercially available. These relaxometries confirm that T2 contrast increases with lanthanide content in GdDy-LDHs, which is mainly attributed to the high magnetic moment of dysprosium. However, Gd^{3+} content also contributes to T2-weighted MRI since this lanthanide increases $1/T_1$ and $1/T_2$ by roughly similar amounts but is best visualized using T1-weighted images [5,32]. Due to the absence of lanthanides, ZnAl-LDH does not exhibit an MRI contrast.

The relaxivity values indicate the type and efficiency of a contrast agent. According to Caravan [33], T1 agents usually have a r_2/r_1 ratio of 1–2, whereas, for T2 agents, the r_2/r_1 ratio is as high as 10 or more. Based on the MRI analyses, $\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$ -LDH and $\text{Zn}_{2.0}\text{Al}_{0.5}\text{Gd}_{0.25}\text{Dy}_{0.25}$ -LDH are classified as T2 agents with r_2/r_1 ratios of 7.9 and 22.5, respectively. Therefore, the combination of gadolinium and dysprosium mainly enhances T2-weighted MRI and, particularly, $\text{Zn}_{2.0}\text{Al}_{0.5}\text{Gd}_{0.25}\text{Dy}_{0.25}$ -LDH can be applied as an efficient T2 contrast agent.

4. Conclusions

The synthesis of ZnAlGdDy-layered double hydroxide was explored, using a Zn/M^{3+} ratio of 2 ($\text{M}^{3+} = \text{Al} + \text{Gd} + \text{Dy}$) and different molar ratios of Al and lanthanides. $\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$ -LDH and $\text{Zn}_{2.0}\text{Al}_{0.5}\text{Gd}_{0.25}\text{Dy}_{0.25}$ -LDH were obtained as a single crystalline phase. The content of metal cations quantified experimentally for the $\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$ -LDH is in accordance with the nominal content used in synthesis, while for the $\text{Zn}_{2.0}\text{Al}_{0.5}\text{Gd}_{0.25}\text{Dy}_{0.25}$ -LDH, the high lanthanide ion content isomorphically substitutes the Zn cations in the layers. The effect of concentrations of lanthanides in the GdDy-LDHs on contrast in magnetic resonance imaging was studied. The longitudinal relaxivity (r_1) of $\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$ -LDH ($7.18 \text{ mM}^{-1}\cdot\text{s}^{-1}$) and $\text{Zn}_{2.0}\text{Al}_{0.5}\text{Gd}_{0.25}\text{Dy}_{0.25}$ -LDH ($3.71 \text{ mM}^{-1}\cdot\text{s}^{-1}$) suggest that water protons access the Gd^{3+} coordination spheres, promoting better T1-weighted MRI than a commercial MRI agent. $\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$ -LDH exhibited the highest efficiency because the low concentration of lanthanides reduces the interactions between these cations and interlaminar carbonates, increasing the probabilities to coordinate water molecules. Meanwhile, the transversal relaxivity values (r_2) of $\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$ ($56.6 \text{ mM}^{-1}\cdot\text{s}^{-1}$) and $\text{Zn}_{2.0}\text{Al}_{0.5}\text{Gd}_{0.25}\text{Dy}_{0.25}$ -LDH ($83.3 \text{ mM}^{-1}\cdot\text{s}^{-1}$) confirm the high T2 contrast effect exhibited by both GdDy-LDHs, mainly attributed to the inclusion of Dy cations in the layers. The efficiency is proportional to the lanthanides content within the LDH structure. Finally, the r_2/r_1 ratios of $\text{Zn}_{2.0}\text{Al}_{0.75}\text{Gd}_{0.125}\text{Dy}_{0.125}$ (7.9) and $\text{Zn}_{2.0}\text{Al}_{0.5}\text{Gd}_{0.25}\text{Dy}_{0.25}$ -LDH (22.5) reveal that the combination of gadolinium and dysprosium mainly enhances T2-weighted MRI images. Based on this, $\text{Zn}_{2.0}\text{Al}_{0.5}\text{Gd}_{0.25}\text{Dy}_{0.25}$ -LDH can be applied as an efficient bimodal contrast agent.

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