Research article

Folate- and glucuronate–functionalization of layered double hydroxides containing dysprosium and gadolinium and the effect on oxidative stress in rat liver mitochondria

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ABSTRACT

Zinc/aluminum layered double hydroxide (LDH) particles were prepared by alkaline precipitation in the presence of dysprosium and dysprosium/gadolinium cations. The particles formed were stable against exchange reactions with folate or glucuronate ions since these organic ions exclusively functionalized the external surface of the layered double hydroxides. While the dysprosium derivatives reached magnetization susceptibilities between 2.06 × 10⁻⁵ and 2.20 × 10⁻⁵ cm³/g, the samples simultaneously containing dysprosium and gadolinium decreased to a range between 1.08 × 10⁻⁵ and 1.73 × 10⁻⁵ cm³/g. This last sample was tested as a magnetic resonance imaging contrast agent and demonstrated a reduction in T₁ and T₂ relaxation times in a linear dependence with the LDH concentration. The oxidative stress assays in rat liver mitochondria demonstrated the low toxicity of the composition simultaneously containing dysprosium and gadolinium as well as the functionalization product with glucuronate ions, suggesting the potential of these particles to design alternative MRI contrast agents.

1. Introduction

Layered double hydroxides (LDHs) are materials formed by brucite-like layers, where divalent metal cations are coordinated by six hydroxyl groups, producing octahedrons that share edges [1]. A fraction of the cationic sites is substituted by trivalent cations, resulting in a positive charge excess, which is compensated by hydrated anions. These compounds are represented by the general formula: [M₂⁺,₁M³⁺,ₓ(OH)₂]ₓ⁻₁A⁻ₓ/m.nH₂O, where M²⁺ and M³⁺ are the cations involved, and A is an anion of charge m⁻ [1]. The overall result of this composition is a hydroxylated structure with positive charged layers that retain exchangeable interlayer anions [1].

The anion-exchange property is the most remarkable feature of these structures [2, 3], and organic anions can also be inserted between the layers during the exchange process to produce hybrid materials such as enzyme supports [2] as well as nutraceutical, cosmeceutical, gene, and drug vehicles [3, 4, 5, 6]. Additionally, LDHs present biocompatibility and low toxicity [7]; therefore, these particles are part of the most promising inorganic matrices to prepare materials for biotechnological and biomedical applications [8]. In addition, diverse metal cations can be included in this structure, leading to the development of multifunctional materials. As an example, the presence of Dy³⁺ or other lanthanide cations in the cationic sites, enables contrast enhancement in magnetic resonance images (MRI) [9]. However, if these lanthanide-containing LDHs are proposed for biomedical applications, biological assays are required to determine the possible biological effects produced by the presence of lanthanides, as reports have demonstrated that a change in the metal cation composition could produce harmful effects [10, 11]. Passivation strategies concerning the nanoparticle surface provide a solution to reduce or eliminate toxicity. In the present work, we prepared LDHs containing dysprosium and gadolinium cations to determine the oxidative stress dysregulation caused by the presence of lanthanides in the alternative MRI contrast agent proposed. Additionally, the functionalization with folate and glucuronate ions was tested to determine if the oxidative stress is regulated by these anions, which are also relevant in the design of nanoparticles for biomedical applications. For example, folate-functionalized nanoparticles are prone to recognize and accumulate in cancer cells [12, 13] in comparison to normal cells [14] and could regulate the oxidative stress caused by nanoparticles [11], whereas...
glucuronate reduces toxicity levels by promoting excretion of nanoparticles through the urine [15].

2. Methodology

2.1. Layered double hydroxides synthesis

LDHs were synthesized by the precipitation method. Considering 2.000 g of Zn(NO$_3$)$_2$•6H$_2$O as the reference (6.72 × 10$^{-2}$ mol of Zn), the salts Al(NO$_3$)$_3$•9H$_2$O, Dy(NO$_3$)$_3$ and Gd(NO$_3$)$_3$ were weighed to prepare the nominal composition $Zn_{2.5}Al_{1}(OH)_{7}(NO_3)$, $Zn_{2.5}Al_{0.75}$Dy$_{0.25}$(OH)$_{7}$(NO$_3$)$_3$, and $Zn_{2.5}Al_{0.75}Dy_{0.12}$Gd$_{0.12}$(OH)$_{7}$(NO$_3$)$_3$. The reaction products were labeled Al-N03, AlDy-N03 and AlDyGd-N03, respectively. The salts were dissolved in 60 mL of distilled water, and then the reaction vessel was covered with a plastic lid adapted with entrances for the pH electrode and the burette. The vessel was purged with nitrogen. Ammonium hydroxide was dropped with the burette to adjust to pH = 8. The formed suspension was stirred for 24 h, and afterwards, the powder was separated by precipitation, washed with water, and dried at 70 °C.

The LDHs were also subjected to a functionalization process by dispersing 0.50 g of LDH in 10 mL of a 2.50 × 10$^{-2}$ M solution of folic or glucuronic acid, previously adjusted to pH = 8 to promote ionization to folate and glucuronate. The suspensions were stirred for five days under a nitrogen atmosphere; afterwards, the powders were decanted, washed with water, and dried at 70 °C. The sample labels changed from –N03 to –Fol or –Gluc depending on the functionalization in each folate or glucuronate solution.

2.2. Characterization

The X-ray diffraction patterns were acquired with a Panalytical-Empyrean diffractometer operated with Cu Kα radiation (0.15418 nm), produced with 45 kV and 40 mA. The samples were analyzed with steps of 0.02° and exposed for 40 s to the X-ray beam. The infrared (IR) analysis was performed with the reflectance mode using a Thermo Scientific spectrometer, model NICOLET iS5 iD5 ATR. Spectra were recorded in a range between 550 and 4000 cm$^{-1}$ with a resolution of 4 cm$^{-1}$ and accumulation of 16 scans. Magnetization loops of powdered samples were determined using a Quantum Design VersaLab Vibrating Sample Magnetometer (VSM), by sweeping magnetic field intensities at room temperature in a range of -30 kOe to 30 kOe. Hence, the mass magnetic susceptibilities $\chi_m$ were obtained by computing the slopes of the magnetization curves with linear fits to the experimental data. The longitudinal (T1) and transversal (T2) relaxation times of LDH aqueous dispersions were collected using a 7.0 T Bruker 70/16 scanner at room temperature at the National Laboratory for Magnetic Resonance in Medicine (Conacyt, UNAM, UAQ, CIMAT). The resulting T1 and T2 values were recorded at concentrations between 0.08 and 0.40 mg/mL and plotted as 1/T1 or 1/T2 vs. concentration of LDH.

2.3. Biochemical assays

Sprague Dawley rats were obtained from the animal facility of Centro de Investigación Biomédica de Occidente (CIBO, México). The rats were handled following the international guidelines on the ethical use of animals and the Mexican guidelines, found in the “Norma Oficial Mexicana NOM-062-ZOO-1999”. The experimental procedures were approved by the local Animal Ethics Committee (Guadalajara, Jalisco, Mexico). Rats were killed by decapitation, and their livers were immediately exposed to anterior thoracotomy and removed. Livers were rinsed with a cold saline solution and homogenized with a teflon-on-glass homogenizer Potter-Elvehjem in 35 mL of cold SHE buffer (250 mM sucrose, 1 mM ethyleneglycoltetraacetic acid and 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid at pH 7.4). The homogenate was centrifuged at 600 xg for 5 min at 4 °C. The pellet was discarded, and the supernatant was centrifuged at 8,000 xg for 10 min at 4 °C. The foamy layer at the top of the supernatant was removed to isolate a mitochondrial pellet, which was washed with a SHE buffer containing 0.1% fatty acid-free serum albumin. Finally, it was re-suspended in SHE buffer and stored at –80 °C until use. Protein concentration was determined by the Lowry method, using bovine serum albumin as standard [16].

We used LDHs disaggregated in normal sterile saline solution as a vehicle and were exposed to high-frequency sound waves for 10 min just before its use. LDHs at three concentrations (20, 100 and 200 μg) were added to 1.5 mL of mitochondrial suspensions and incubated for 1 h with occasional agitation in an ice-water bath. Mitochondrial suspensions without LDHs incubated with vehicle solution were used as controls. Then, mitochondrial suspensions were immediately used to perform the oxidative stress assays.

2.4. Oxidative stress

End-products of lipid peroxidation (LPO) were measured by a colorimetric method using a LPO cuvette-based assay kit from Oxford Biomedical Research, Inc. (Oxford MI, USA). Each measurement was conducted four times. The kit contains a chromogenic reagent (N-methyl-2-phenylindole) which reacts with the end products of lipid peroxidation (malondialdehyde and 4-hydroxynonenals) at 45 °C, yielding a stable chromophore with maximal absorbance at a wavelength of 586 nm. Absorbance was measured using a Benchmark Plus Microplate Spectrophotometer System, 110/230 V by Bio-Rad, and sample analysis was made using the MPM 5.1 PC Software.

Nitric oxide metabolites (nitrite – nitrate) were estimated as an index of nitric oxide production. The method for nitrite and nitrate levels is based on the Griess reaction, where samples are previously deproteinized with sulfosalicylic acid [17]. The assay was conducted with 20 μL of samples and mixed with 100 μL of 0.8% (w/v) VCl$_3$ solution dissolved in 1 M H$_2$PO$_4$, 50 μL of 0.8% (w/v) sulfanilamide dissolved in 1 M H$_2$PO$_4$, and 50 μL of 0.2% (w/v) N-(1-naphthyl)ethylendiamine. The suspension was incubated at room temperature for 45 min. The absorbance of the samples was read at 550 nm [17].

Formation of carbonyl groups as a result of amino acids oxidation were quantified using 200 μL of the homogenate and mixed with 500 μL of 10 mM 2,4-dinitrophenylhydrazine dissolved in 2 M HCl. The mixtures were incubated for 1 h at room temperature, then 333 μL of trichloroacetic acid was added, and the suspension was centrifuged at 14,000 rpm for 20 min. The resulting pellet was washed three times with 1mL of ethanol/ethyl acetate (1:1) solution. The supernatant was discarded, and the pellet was mixed with 600 μL of guanidine hydrochloride and incubated for 15 min at room temperature. The absorbance of the samples was read at 370 nm in a BIORAD Benchmark Plus spectrometer [18].

Membrane fluidity was measured through the excimer-to-monomer fluorescence intensity ratio (Ie/Im) of the probe 1,3-diprylpyren (DPyP) incorporated into the membranes. For this assay, a mixture of 0.25 mg of protein and 0.11 μmol DPyP dissolved in 2 mL of 10 mM Tris-HCl buffer (pH 7.8) was incubated in darkness at 4 °C for 3 h. The fluorophore was excited at 329 nm, and the monomer and excimer fluorescence intensities were read at 379 and 480 nm, respectively. Then, the intensity ratio (Ie/Im) was calculated. Membrane fluidity was expressed as fluorescence intensity ratio of excimer and monomer DPyP (Ie/Im ratio), where high Ie/Im ratio indicates a high membrane fluidity. Fluorescence was measured at 24 °C on an LS50B Perkin Elmer fluorescence spectrometer. Fluorescence corrections obtained from readings of samples without DPyP were applied to all fluorescence values [19].

The hydrolytic activity of ATCase was determined by quantifying the amount of inorganic phosphate released from ATP. The reaction mixture contained 125 mM KCl, 3 mM MgCl$_2$, 20 mM 3-
morpholinopropanesulfonic acid (MOPS)-KOH (pH 8.0), 2.5 mM ATP, and 0.1 mM ethylene glycol tetraacetic acid (EGTA). The mitochondrial homogenate (15 μL) was added to the reaction medium (0.5 mL) and incubated at 40 °C for 5 min. The reaction was stopped by adding 200 μL of 30% (w/v) trichloroacetic acid. Then, the suspension was centrifuged at 3500 xg for 10 min, and 200 μL of the supernatant was transferred to a clean tube and mixed with 200 μL of 3.3% ammonium molibdate solution and 200 μL of 10% iron sulfate solution. The absorbance of the samples was read at 660 nm in a BIORAD Benchmark Plus spectrometer.

Statistical results were expressed as the mean values ±SD. Each sample was assayed with four replicates and the data were compared by analysis of variance (ANOVA); when the analysis indicated a significant difference, the means were compared with the Student Newman-Keuls and Bonferroni post-hoc test. Values were considered significant at P < 0.05.

3. Results and discussion

3.1. Particle characterization

The X-ray diffraction patterns were analyzed taking the pristine Al–NO3 LDH as reference. The profile of this compound matched that of the card number 22–0700 from the International Centre for Diffraction Data (ICDD), which belongs to an LDH structure with a hexagonal unit cell belonging to the R-3m space group. The reflection at the lowest angle in this profile corresponds to the basal distance of the layered structure. The profile was maintained when dysprosium and gadolinium were added, as noted in the diffractogram of AlDy-NO3 and AlDyGd-NO3 in Figure 1. In these patterns, even the basal reflection occurs at the same angle (9.9 °, 2theta), indicating that the orientation of the nitrate ions is the same. However, when the AlDy–NO3 and AlDyGd–NO3 compounds...
were treated with folate and glucuronate solutions, the basal reflection shifted to 11.5°, 2theta (dotted line in Figure 1), indicating a slight reduction in the basal space from 8.9 to 7.7 Å, which is commonly associated with the re-orientation of interlayer nitrate ions from a perpendicular to parallel position [20,21]. Therefore, the LDH structures were not intercalated with folate nor glucuronate ions.

The infrared spectra of the LDH Al–NO3 and AlDy-NO3 (Figure 1) presented the same profile composed by bands from O–H, NO3- and M-O stretching movements at 3400, 1345 and 590 cm⁻¹, respectively, and from water molecules bending at 1630 cm⁻¹ [22,23]. This similarity was also observed in the spectrum of the AlDyGd-NO3 compound (Figure 1), indicating that the insertion of Dy and DyGd maintains the regular LDH composition. Although environmental CO2 can be captured during the synthesis of LDH [24], the content of this anion would be low and would not affect the intercalation of folate and glucuronate anions.

The spectra of the folate functionalization products (AlDy-Fol and AlDyGd-Fol) are formed by signals from vibrational modes of C=O (1690 cm⁻¹), N–H (1607 cm⁻¹), cis-phenyl (1410 cm⁻¹), phenyl ring (1500 cm⁻¹), C-O (1300 cm⁻¹), and C-N (1188 cm⁻¹) corresponding to folate ions (Figure 1) [13, 25], whereas signals of the COO⁻ (1587 cm⁻¹) and C-O (1078 cm⁻¹) vibrational modes correspond to glucuronate ions present in AlDy-Glu and AlDyGd-Glu [26, 27]. According to the XRD information, folate and glucuronate were not intercalated in the LDH products, nevertheless, as shown by the spectra, these anions were present, therefore, it is possible to infer that the organic ions only functionalize the external surface of the LDH particles.

On the other hand, magnetization assays were conducted to determine if the magnetic susceptibility is affected by the functionalization with folate and glucuronate ions.

The H-M response of the Al–NO3 compound was negligible, as expected for a compound without paramagnetic atoms (data not shown).

The materials containing dysprosium (AlDy–NO3, AlDy–Fol and AlDy–Glu) presented similar mass magnetic susceptibilities. The results presented in the H-M plot (Figure 1) demonstrated that the paramagnetic values reached were: \(X_{\text{AlDy} \text{NO}3} = 2.06 \times 10^{-5} \text{cm}^3\text{g}^{-1}\), \(X_{\text{AlDy} \text{Fol}} = 2.18 \times 10^{-5} \text{cm}^3\text{g}^{-1}\) and \(X_{\text{AlDy} \text{Glu}} = 2.20 \times 10^{-5} \text{cm}^3\text{g}^{-1}\). These data indicated that a mean increase of 6% occurred after the functionalization of AlDy–NO3 with folate and glucuronate, indicating that susceptibility is mostly retained regardless the functionalization. Regarding the addition of gadolinium, the co-existence of dysprosium and gadolinium cations in the same structure diminished the susceptibility to \(X_{\text{AlDyGd} \text{NO}3} = 1.73 \times 10^{-5} \text{cm}^3\text{g}^{-1}\). Furthermore, the addition of glucuronate clearly diminished this value by up to 3% \(X_{\text{AlDyGd} \text{Glu}} = 1.68 \times 10^{-5} \text{cm}^3\text{g}^{-1}\), whereas the decrease due to folate was of 36% \(X_{\text{AlDyGd} \text{Fol}} = 1.08 \times 10^{-5} \text{cm}^3\text{g}^{-1}\). The decay of susceptibility with the addition of folate indicates that this anion probably modifies the surface of the particles, specifically in proximate sites to lanthanide cations.

The capability of magnetization is useful to demagnetize neighboring fields in medical imagenology based on magnetic resonance. The evidence is given by the reduction of longitudinal (T1) and transversal (T2) relaxation times influenced by AlDy-Gd–NO3. This compound was selected for the MRI assays once it simultaneously contains dysprosium and gadolinium cations as active contrast agents for T2 [15,28] and T1 [28,29] MRI modes, respectively. The T1 and T2 plots in Figure 1 show that the reciprocal values of the relaxation times tend to have a linear dependence with the LDH concentration, thus demonstrating the capability to use them as contrast agents in MRI analysis. Although the correlation coefficients are 0.85 and 0.87, the linear trend is clear, and the deviations could be related to the instability of aqueous suspensions.

### 3.2. Bioassays in liver mitochondria

Mitochondria provides ATP to cells but also produces reactive oxygen species (ROS) as a result from disturbed oxidative phosphorylation [30], ROS they also regulate apoptosis at the mitochondrial level [31, 32]. Dysregulations in the oxidation and consumption of the ROS rate are related to alteration in the mitochondrial membrane structure and functioning, the experiments in this section aim to evaluate the changes produced in the oxidative stress markers and Mg-ATPase activity in live mitochondria by the LDH nanoparticles.

Mitochondria are the main site where adenosine triphosphate (ATP) is synthesized by ATP synthase. ATP synthase is the enzyme that uses adenosine diphosphate, inorganic phosphate and electrochemical gradient of protons through the internal mitochondrial membrane as substrates. The enzyme can function as Mg-ATP synthase (ATP synthesis) or Mg-ATPase (ATP hydrolysis). The activity of the Mg-ATP synthase is inhibited by ROS [33], and oxidative stress is another factor which could contribute to the increase of Mg-ATPase under pathological conditions [34].

The mitochondrion generates reactive oxygen species (ROS) when the respiratory process is inhibited [30]. On the other hand, the oxidative stress arises when the organism is not capable enough to regulate the over-production of ROS and this stress induces structural and functional failures in the cell membrane, for example, ROS readily react with double bonds of membrane phospholipids producing lipid peroxidation and then the membrane fluidity is modified as well as the physiological functions [35].

Malondialdehyde (MDA) and 4-hydroxynonenal (HNE) appear as end products of the lipid peroxidation, and they are capable to react with proteins, thus enabling the interaction with metal cations and intensifying the oxidative phenomena [36]. The stable products of protein oxidation contain carbonyl (CO) groups from ketones and aldehydes, and therefore, they are used as biomarkers of oxidative stress [37].

Nitrites and nitrates formation is another reliable indicator of dysregulation in the oxidation process, these are endogenous products from metabolism in human fluids. The presence of nitrogen oxides is associated to a reduction of the oxidant capacity [38].

Figure 2 shows that the lipoperoxide content in mitochondria treated with Al–NO3 is similar to that of untreated mitochondria (control) and that AlDyGd-Fol significantly increases these levels. Furthermore, the highest concentration of AlDy–NO3 and AlDyGd–NO3, as well as all concentrations of AlDy–Fol and AlDyGd–Glu, present a significant increase in lipoperoxides in relation to untreated mitochondria. Changes are considered significant when \(P < 0.05\).

Al–NO3, AlDy–NO3, AlDyGd–NO3 increase the nitrite-nitrate concentration in relation to the control (Figure 2). This increase is also observed with AlDy–Glu, AlDy–Fol and AlDyGd–Fol at the highest concentration, whereas AlDyGd–Glu does not affect the nitrite-nitrate formation.

Protein carbonyl group formation was increased with respect to the control when mitochondria were treated with AlDy–NO3 and AlDy–Glu at all concentrations, with Al–NO3, AlDy–Fol and AlDyGd–Fol at the highest concentrations, and with AlDyGd–Glu at the lowest concentration (Figure 2).

Mitochondrial membrane fluidity is strongly increased by treatments with Al–NO3, AlDy–NO3, AlDy–Fol and AlDy–Glu. The increase is also observed with AlDyGd–Fol at the highest concentration, whereas AlDyGd–Glu does not modify the fluidity at any concentration (Figure 2).

ATPase activity is significantly increased by AlDyGd–NO3 and AlDy–Glu. This increase is attenuated in treatments with Al–NO3, AlDy–NO3, AlDy–Fol and AlDy–Glu. The AlDyGd–Glu does not affect this activity (Figure 2).

All the above data demonstrate that the LDH composed by Al–NO3, AlDy–NO3 and AlDyGd–NO3 does not present a relevant increase in liperoxidation; nonetheless, they increase the levels of nitric oxide metabolites, carbonyl formation in proteins, ATPase activity, and membrane fluidity. These effects do not change when the particles are functionalized, for example with AlDy–Fol, AlDy–Glu and AlDyGd–Fol - except in nitric oxide, reduced levels were observed. In addition, the DyGd–Glu compounds do not significantly modify the levels of liperoxidation, nitric oxide metabolites, carbonyl formation in proteins, membrane fluidity, and ATPase activity – except for the medium concentration.
An overall trend with functionalization is that folate in AlDy-Fol also increases the oxidative stress compared with the parent AlDy-NO3. Also in AlDyGd-Fol, the same alteration occurs (except for nitrites and carbonyl formation in protein). Regarding glucuronate functionalization in AlDy-Gluc, this compound affects the oxidative stress marker in the same manner of the parent AlDy-NO3. On the contrary, the oxidative stress presents the same levels as the control with the AlDyGd-Gluc compound. In other words, the inclusion of gadolinium and the passivation with glucuronate diminishes rat liver mitochondria damage. These biochemical results must be complemented with further assays in living organisms to confirm the reduction of toxicity, a recent report indicates that drug-loaded LDH produce histological damage in liver [39], although, if the biological model is different, the consideration must be taken into account.

4. Conclusions

Both layered double hydroxides with AlDy and AlDyGd were obtained by alkaline precipitation and functionalized with folate and glucuronate ions. Although nitrate is easily exchanged in LDH particles, the functionalization conditions used in this study did not replace nitrate ions; results indicated that the folate and glucuronate ions only covered the external surface of the particles.

All the LDH materials presented an intense magnetization due to the allocation of Dy(III) and Gd(III) cations, this magnetic response was not affected by the functionalization, except the magnetization of AlDyGd, which slightly decreased after functionalization with folate. The oxidative stress assays in rat liver mitochondria demonstrated the low toxicity of the AlDyGd-NO3 composition, especially the functionalization product with glucuronate ions. This behavior, along with the demonstrated MRI contrast, reveals the potential of AlDyGd-NO3 particles to design alternative MRI contrast agents.

Declarations

Author contribution statement

Cecilia Sánchez Juménez, Karina Nava Andrade, Ana Laura Briones Torres: Performed the experiments; Analyzed and interpreted the data.

Fermin Pacheco-Moises: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mario E Cano: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Gregorio Guadalupe Carbajal Arizaga: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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References

[1] M. Rajamathi, G.S. Thomas, P.V. Kamath, The many ways of making anionic clays, Proc. Indiana Acad. Sci. 133 (2001) 617–680.
[2] C. Forano, S. Vial, C. Moushy, Nanohybrid enzymes - layered double hydroxides: potential applications, Curr. Nanosci. 2 (2006) 285–294.
[3] S.-J. Choi, Y.-R. Kim, Bioinspired layered nanoclays for nutraceutical delivery system, in: ACS Symp. Ser., 2013, pp. 207–220.
[4] S. Hwang, Y. Han, J. Choy, Intercalation of functional organic molecules with pharmaceutical, cosmeceutical and nutraceutical functions into layered double hydroxides and zinc basic salts, Bull. Korean Chem. Soc. 22 (2001) 1019–1022.
[5] K. Ladewig, Z.P. Xu, G.Q. (Max) Lu, Layered double hydroxide nanoparticles in gene theranostic systems, Mater. Sci. Eng. B 203 (2016) 7–11.
[6] U. Costantino, V. Ambrogi, M. Nocchetti, L. Perioli, Hydrotalcite-like compounds: versatile layered hosts of molecular anions with biological activity, Microporous Mesoporous Mater. 107 (2008) 149–160.
[7] C. Del Hoyo, Layered double hydroxides and human health: an overview, Appl. Clay Sci. 36 (2007) 103–121.
[8] V. Kuthati, R.K. Kankala, C.-H. Lee, Layered double hydroxide nanoparticles for biomedical applications: current status and recent prospects, Appl. Clay Sci. 112–113 (2015) 109–116.
[9] J. Arratia-Quijada, C. Sánchez Jiménez, A. Gurinov, A. Pérez Centeno, I. Ceja Andrade, G.G. Carbajal Arizaga, Dysprosium-containing layered double hydroxides intercalated with biologically active species as an approach for thermosensitive systems, Mater. Sci. Eng. B 203 (2016) 7–12.
[10] S.-J. Choi, J.-H. Choy, Effect of physico-chemical parameters on the toxicity of inorganic nanoparticles, J. Mater. Chem. 21 (2011) 5547–5554.
[11] A.K. Sánchez Lafargue, F.P. Pacheco Moisés, A. Gurinov, G.G. Ortiz, G.G. Carbajal Arizaga, Dual responsive dysprosium-doped hydroxyapatite particles and toxicity reduction after functionalization with folic and glucuronic acids, Mater. Sci. Eng. C 25 (2005) 3095–3064.
[12] A. Virvege, G.G. Carbajal Arizaga, R. Hernandez Gutierrez, A.R. Oaxaca Camacho, J. Arratia-Quijada, Passive targeting effect of Dy-doped LDH nanoparticles hybridized with folic acid and gallic acid on HER293 human kidney cells and HT29 human cells, J. Nanoparticle Res. 20 (2018) 1–10.
[13] K. Kattel, J. Young, W. Xu, H. Gyed, E. Jung, B. Alam, W. Choul, S. Jin, J. Su, Y. Chang, T. Jeong, J. Eain, K. Seek, G. Ho, Paramagnetic dysprosium oxide nanoparticles and dysprosium hydroxide nanorods as T2 MRI contrast agents, Biomaterials 33 (2012) 3254–3261.
[14] S. Murphy, P. Nandha Kumar, P. Poojar, S. Kannan, Simultaneous substitutions of Gd3+ and Dy3+ in β-La3(PO4)2 as a potential multifunctional bio-probe, Mater. Des. 120 (2017) 336–344.
[15] Y.V. Park, M.J. Baek, E.S. Choi, S. Woo, J.H. Kim, T.J. Kim, J.C. Jung, K.S. Chae, Y. Chang, G.H. Lee, Paramagnetic ultrasmall gadolinium oxide nanoparticles as advanced T1 MRI contrast agent: account for large longitudinal relaxivity, optimal particle diameter, and in vivo T1 MR images, ACS Nano 3 (2009) 3683–3699.
[16] S. Murphy, Production of nitric oxide by glial cells: regulation and potential roles in the CNS, Glia 29 (2000) 1–13.
[17] H.I. Simon, A. Haj-Yehia, F. Levi-Schaffer, Role of reactive oxygen species (ROS) in apoptosis induction, Apoptosis 5 (2000) 415–418.
[18] G. Nishimura, R.J. Proske, H. Doyama, M. Higuchi, Regulation of apoptosis by respiratory cyanochrome c release by respiratory substrates, FEBS Lett. 505 (2001) 399–404.
[19] V. Zhang, O. Marcillac, C. Giolivi, L. Eriesser, K.J.A. Davies, The oxidative inactivation of mitochondrial electron transport chain components and ATPase, J. Biol. Chem. 265 (1990) 16330–16336.
[20] C. Chinopoulos, V. Adam-Vizi, Mitochondria and aging. An overview, Zbl. Physiol. (Leipzig) 105 (1998) 492–500.
[21] S. Hwang, Y. Han, J. Choy, Intercalation of functional organic molecules with pharmaceutical, cosmeceutical and nutraceutical functions into layered double hydroxides and zinc basic salts, Bull. Korean Chem. Soc. 22 (2001) 1019–1022.
[22] S. Hwang, Y. Han, J. Choy, Intercalation of functional organic molecules with pharmaceutical, cosmeceutical and nutraceutical functions into layered double hydroxides and zinc basic salts, Bull. Korean Chem. Soc. 22 (2001) 1019–1022.
[23] K. Ladewig, Z.P. Xu, G.Q. (Max) Lu, Layered double hydroxide nanoparticles in gene and drug delivery, Expert Opin. Drug Deliv. 6 (2009) 907–922.
[24] U. Costantino, V. Ambrogi, M. Nocchetti, L. Perioli, Hydrotalcite-like compounds: versatile layered hosts of molecular anions with biological activity, Microporous Mesoporous Mater. 107 (2008) 149–160.
[25] C. Del Hoyo, Layered double hydroxides and human health: an overview, Appl. Clay Sci. 36 (2007) 103–121.
[26] V. Kuthati, R.K. Kankala, C.-H. Lee, Layered double hydroxide nanoparticles for biomedical applications: current status and recent prospects, Appl. Clay Sci. 112–113 (2015) 109–116.
[27] J. Arratia-Quijada, C. Sánchez Jiménez, A. Gurinov, A. Pérez Centeno, I. Ceja Andrade, G.G. Carbajal Arizaga, Dysprosium-containing layered double hydroxides intercalated with biologically active species as an approach for thermosensitive systems, Mater. Sci. Eng. B 203 (2016) 7–12.
[28] S.-J. Choi, J.-H. Choy, Effect of physico-chemical parameters on the toxicity of inorganic nanoparticles, J. Mater. Chem. 21 (2011) 5547–5554.
[29] A.K. Sánchez Lafargue, F.P. Pacheco Moisés, A. Gurinov, G.G. Ortiz, G.G. Carbajal Arizaga, Dual responsive dysprosium-doped hydroxyapatite particles and toxicity reduction after functionalization with folic and glucuronic acids, Mater. Sci. Eng. C 48 (2015) 541–547.
[30] G.D. Venkatasubbu, S. Ramasamy, G.S. Avadhanli, V. Ramakrishnan, J. Kumar, Surface modification and paclitaxel drug delivery of folic acid modified polyethylene glycol functionalized hydroxyapatite nanoparticles, Powder Technol. 235 (2013) 437–442.
[31] J.-H. Choy, J.-S. Jung, J.-M. Oh, M. Park, J. Jeong, Y.-K. Kang, O.-J. Han, Layered double hydroxide as an efficient drug reservoir for folate derivatives, Biomaterials 25 (2004) 3095–3064.
[32] A. Virvege, G.G. Carbajal Arizaga, R. Hernandez Gutierrez, A.R. Oaxaca Camacho, J. Arratia-Quijada, Passive targeting effect of Dy-doped LDH nanoparticles hybridized with folic acid and gallic acid on HER293 human kidney cells and HT29 human cells, J. Nanoparticle Res. 20 (2018) 1–10.
[33] K. Kattel, J. Young, W. Xu, H. Gyed, E. Jung, B. Alam, W. Choul, S. Jin, J. Su, Y. Chang, T. Jeong, J. Eain, K. Seek, G. Ho, Paramagnetic dysprosium oxide nanoparticles and dysprosium hydroxide nanorods as T2 MRI contrast agents, Biomaterials 33 (2012) 3254–3261.