Possibilities for layered double hydroxides (LDH) nanotubes in biological applications

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Keywords: layered double hydroxides, rare earth, nanomedicine

Abstract

Luminescent plate-like nano-LDH and nanotubular LDH anion exchangers composite were assembled from inorganic oligo-layered walls LDHs containing rare earth elements. This high-surface area and micro and mesoporous structure facilitates interaction with different species, providing significant potential for hosting sensitizers, dendrimers or nanoparticles. As both the interlayer space and the mesopore are accessible, these materials offers great opportunities for catalysis, sensors and transport of biologically active materials.

Introduction

Layered double hydroxides (LDH) are materials formed by the alternating stacking of metallic and anionic layers with the chemical formula $[M_{1-x}^{II}M_x^{III}(OH)_2]^{x+}A^{n-}$ (Figure 1). These lamellar materials have attracted the attention of the scientific community for the versatility of their composition and the variety of possible morphologies, making them suitable for several applications.

Figure 1. Structure of a LDH containing Zn and Al in its metallic layers and a generic anion in its interlamellar regions.

More specifically, the viability of the use of LDHs in biomedical applications has been demonstrated in the last decade mainly due to characteristics such as low cytotoxicity¹⁻³ and permeability in some types of cell walls^{3,4}. In addition, anionic molecules of biological interest such as DNAs, RNAs or bioactive drugs can be adsorbed on the surface of the LDHs or

even protected between the lamellae, suffering release in the intracellular environment².

One of the major problems when trying to use the interlamellar spacing of LDHs for the intercalation of molecules is the restriction imposed by the very structure of the material layers, limiting the molecule size that can be intercalated. Two alternatives have been proposed in the literature to overcome this limitation, necessarily by obtaining LDHs with nanometric dimensions. First the delamination of this material in sheets of double hydroxides^{3,4} or alternatively, as proposed by our group, the structuring of the LDHs in the form of nanotubes which has been shown to considerably increase their surface area and, consequently, their adsorption capacity.

The high-surface area presented by the LDH nanotubes and their easily accessible cylindrical mesopores described by Moraes et al.⁵ offers a new class of lamellar materials, where both the symmetrical spacings and the central mesopore are able to perform different functions. In fact, while the mesopore (central cavity of the tube) is accessible for the adsorption of large molecules, the spacing between the layers of hydroxides remains available for the intercalation of anionic antenna molecules that can enhance the luminescent properties of the nanotubes making them suitable for applications as biological markers.

Methods and Results

In this work, we propose the study of biological viability for both planar nano-LDHs and LDHs in the form of nanotubes.

Initially, the plate-like LDH was prepared according to Kovanda et al.⁶. For the ZnAlEu LDH, a 10 mL metal

solution containing the precursors $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ and $\text{Eu}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ was prepared and added to 200 mL of 10^{-4}M NaOH (base solution) using a syringe at a ratio of 10 mL/h. All the solutions were prepared with Millipore water. The pH is kept at 8 during the synthesis by using a system of a syringe pump - Perfusor® Space B|Braun and a Titrino 702 SM. In order to obtain the nanotubular morphology with walls formed by lamellar double hydroxides containing Eu^{3+} ions, the base solution was replaced by a new solution containing P123-BASF micelles and the anionic sensitizer 1,3,5-benzenetricarboxylic acid (BTC). Figure 2 shows a transmission electron microscopy image of the ZnAlEu LDH nanotubes; their outer diameter and length are estimated to be approximately 20nm and 100nm, respectively.

Figure 2. TEM image of ZnAlEu LDH nanotubes.

In addition to the ZnAlEu LDH nanotubes, we also synthesized ZnAl and MgAl planar LDHs by quick dripping coprecipitation method, as described by Xu et al.⁷. In this method, a 10mL metal solution containing the precursors (for ZnAl LDH: $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$; for MgAl LDH: $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$) was added directly into a solution of 0.15M NaOH under stirring. Different of the traditional synthesis, the metal solution is added under just 5 seconds instead of slowly dripped. Although the synthesis of MgAl planar LDH appears to have formed a stable LDH, the rapid dripping method for Zn and Al precursors does not appear to form the desired LDH, probably forming a solution containing zinc oxide. Such hypotheses must be confirmed by X-ray diffraction measurements.

One of the concerns when working with nanomaterials is the tendency of the particles to form aggregates. For some specific applications such as delivery of drugs to biological cells, the dispersion of

the planar nano-LDH or LDHs nanotubes will be fundamental for the success of the incorporation of those particles in the cellular environment⁷. In order to try to disperse the material, a hydrothermal treatment was carried out on the samples suspended in aqueous solution, as described by Xu et al.⁷. In these assays, the LDHs were washed several times with deionized water and resuspended with 40mL deionized water. These suspensions were then loaded into a hydrothermal reactor and submitted to a thermal treatment at 100°C during 4 hours.

After this treatment, the MgAl planar LDH solution became less turbid, indicating that the desired dispersion may have occurred. To confirm this hypothesis, dynamic light scattering (DLS) measurements were conducted in a Malvern Zetasizer Nano equipment, at 21°C and detection at 173°. Tables 1 and 2 show the results of three consecutive DLS measurements for pre and post treatment of the MgAl LDH samples. Figure 3 presents the intensity of scattered light by the particle size distribution for all DLS measurements for pre and post treatment MgAl LDH. The pretreated solution had a greater contribution of micrometric scale clusters and particles with 670-830nm (table 1). This was significantly decreased after the treatment, as can be seen in table 2, where only one measurement presented a small contribution in the micrometric region (figure 3).

Table 1. Sizes obtained by three DLS measurements for pre-treatment MgAl LDH

	Peak1	Peak2	Peak3
Meas. no	Size (d.nm)	Size (d.nm)	Size (d.nm)
1	700(290)	119(44)	5070(840)
2	830(380)	159(63)	5280(730)
3	670(340)	73(34)	5100(820)

Table 2. Sizes obtained by three DLS measurements for post-treatment MgAl LDH

	Peak1	Peak2
Meas. no	Size (d.nm)	Size (d.nm)
1	44(26)	-
2	43(23)	5000(880)
3	38(24)	-

The ZnAlEu LDH nanotubes did not show any visual change after the hydrothermal treatment. The equipment could not perform reliable DLS measurements in this case; the nanotubes may have clustered in micrometric arrangements outside the limits of this technique.

Figure 3. DLS measurements of the scattering light intensity (%) by the distribution of particle sizes of pre and post treatment MgAl planar LDH.

Discussion

The quick dripping coprecipitation method seems to be efficient to form planar MgAl LDH in both micro and nano scales. One possible explanation could be due to the fact that the rapid synthesis does not allow the system to continue growing large flakes, limiting the particle size, unlike the slow dripping of the original preparation method. It is not clear yet why this type of synthesis does not appear to form LDHs in the case of Zn and Al.

The dispersion of LDHs in order to reach nanometric sizes has been the major challenge for subsequent biological application in this project. The hydrothermal treatment appears to have an effect on the planar MgAl LDH, but not on the ZnAlEu LDH nanotubes. As shown in the DLS results of the MgAl samples, the particles size after the hydrothermal treatment decrease substantially assuming a nanometric size (nano-LDH). The production of LDH nanotubes formed by MgAl via rapid synthesis and its subsequent dispersion should be tested in the future. As an alternative for the new synthesis routes, other methods of dispersion will be tested for the already formed ZnAlEu LDH nanotube, such as ultrasound.

The dependence on BTC will also be another challenge to be overcome, since the presence of this molecule seems to be essential for the nanotubes formation⁵. One of the alternatives could be searching for drugs similar to BTC molecule, such as acetylsalicylic acid.

Biological tests should be carried out to determine whether the synthesized material is biologically compatible. Firstly, cytotoxicity tests should be performed to ensure the non-toxicity of the suggested LDH; if the results are positive, trials should be carried out on its stability in cell-like conditions, ability to penetrate cell walls and its release profile. Finally, the luminescent LDH could be tested as a biological marker.

Acknowledgments

We would like to thank CAPES and FAPESP for supporting this project, and the organization of the 18th BWSP for this opportunity. We also would like to thank Fernando M. de Melo for the DLS measurements.

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