

Self-assembled Nanomaterials Type Layered Double Hydroxides Antibiotics Used as Drug Delivery Vectors

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Due to their biocompatibility, nanomaterials type layered double hydroxides (LDHs) development has attracted many researchers attention especially in medical area using them as drug delivery vectors. Their efficiency is due to the drug controlled release from the interlayer space of LDHs matrices. The controlled drug delivery characteristic is due to the reduction of drug concentration variations and maintains the desired level of drug concentration for long time. Furthermore, this action limits adverse effects and reduces the therapy duration, enhancing bactericidal activity and leading to an effective treatment. This work highlights the synthesis and characterization of hybrid structures type LDHs- tobramycin having application as antibacterial factor against *Pseudomonas aeruginosa*. The obtained self-assembled structures were morphologically characterized in order to point out the drug-interlayered products. Results indicate superior properties of MgAlLDHs and ZnAlLDHs behaving as host for a wide variety of molecules generally and, in our case, for tobramycin as guest.

Keywords: nanomaterials, layered double hydroxides, morphology characteristics, drug delivery vectors, antibiotics, bactericidal activity

Using nanocarriers for drug transport can be improved considerably pharmacokinetics and therapeutic efficacy, respectively while limiting the side effects [1]. The most appropriate nano-vectors should encapsulate the doses of drugs remaining stable under physiological conditions. These systems must be protected from degradation during the delivery in the human body and release the drug intracellularly; nanoparticles surface should remain stable and after biodegradation, the breakdown products should be biocompatible.

In last few years, significant progress has been made in order to tailor nano-carrier targeting moieties, for therapy and diagnosis [2-6].

Systemic antibiotic administration can cause side effects because of high drug concentration that influence pharmacokinetic profiles. Inorganic matrices type layered double hydroxides can successfully be used for drug delivering and release complex systems designed to solve these problems [7]. More over these nanosystems slowly release their intercalated molecules in order to maintain antibiotic concentrations at optimal levels for long time. Drug nanovectors surface can be modified to target infections caused by pathogenic microorganisms and minimize side effects [8-11].

Layered double hydroxides can be good candidates as unique injectable drug nanoreseviors due to their blood biocompatibility properties. Previous *in vivo* studies regarding anticancer drugs intercalated into interlayer space showed that these intercalated molecules are released slowly in a controlled path into blood fluids (pH = 7.4) exhibiting good blood clearance in comparison with free drugs [12].

Although it is well known that some LDHs occur naturally, last years have gain an explosive development in the controlled synthesis of new LDHs type nanomaterials for use in clinical therapy [13].

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Figure 1 show LDHs belonging to the class of anionic lamellar compounds formed from brucite-like sheets possessing positively charge with an interlayer space containing anions and water molecules as counterbalancing negatively charge.

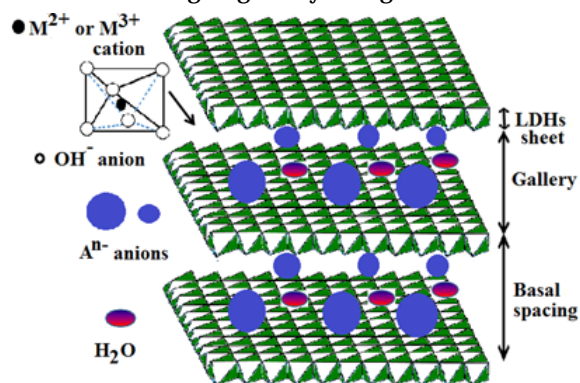
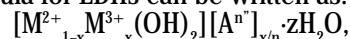


Fig. 1. Schematic representation of LDHs

Metal cations located in the centers of shared octahedral have vertices that contain hydroxide ions connecting to form infinite two-dimensional lamella.

General formula for LDHs can be written as:



where part of M^{2+} representing metal cations (e.g.: Mg^{2+} , Zn^{2+} and Ca^{2+}) are replaced by M^{3+} cations (e.g.: Al^{3+} , Ga^{3+} , Fe^{3+} and Mn^{3+}); A^{n-} is an inorganic or organic anion (e.g.: CO_3^{2-} , NO_3^- , Cl^- , SO_4^{2-} , or RCO_2^- , and x is the mole fraction of M^{3+} [14-19].

Cations ordering influence the charge density of the layered double hydroxides sheets having thus consequences for some physicochemical properties such as structure, reactivity, organization, surface morphology

and exchange capacity of the chemical molecules in the interlayer gallery [20-29].

Drugs confinement between the layers improves long-term stability and storage of active molecules.

The aminoglycoside tobramycin is an antibiotic that treats infections produced by *Pseudomonas aeruginosa* infections. *P. aeruginosa* is an opportunistic pathogen and nosocomial which causes acute infections in planktonic form and chronic infections in biofilm form, respectively. Acute infections, found in burn wounds and surgical sites are often resistant to antibiotic treatment resulting in delayed healing, sepsis and even death [30]. Chronic infections are found in patients with diabetes, in the lungs of patients with cystic fibrosis and chronic obstructive pulmonary disease being susceptible to antimicrobial therapy [31-38].

Incorporation of tobramycin in LDHs matrix gives new opportunities to enhance their stability and to control the drug release. This work presents the results of synthesis and morphology characteristics of LDHs-tobramycin formulation.

Synthesis of LDHs samples

LDHs nanomaterials are synthesized from biocompatible metal salts and can easily be decomposed in the acidic biological environment.

MgAlLDHs and ZnAlLDHs samples were obtained by co-precipitation technique, the simplest and most commonly used method.

A solution containing carbonate salts of Mg^{2+}/Al^{3+} molar ratio of 3/1 as precursors is added to an aqueous solution of 1M NaOH/ Na_2CO_3 were added slowly dropwise maintaining the pH of solution at a constant value of 10. The obtained white precipitate was aged at 70°C for 24 h and centrifuged. The resulting sample was calcined at 600°C for 12 h.

In the same way ZnAlLDHs sample was obtained for a molar ratio of 2/1 and a pH solution of 9.5. The formed precipitate was aged at 60°C for 25 h and the solid was separated by centrifugation. The resulting sample was thermally treated at 550°C for 20 h.

Tob/LDHs nanostructures synthesis

Samples containing tobramycin were intercalated into LDHs nanoparticles by reconstruction method. This method involves removal of the water interlayer and anions by along with dehydroxylation of the layer resulting in the formation of metal mixed oxides and regeneration of LDHs consisting of metal mixed oxide exposure to the water and anions. This procedure leads to regeneration of the pristine layered structure. Finally, anions intercalated into the interlayer space and water reforms the hydroxyl sheets.

Therefore, 1 g of calcined LDHs sample was added to an aqueous solution containing tobramycin under vigorous stirring conditions reconstructing thus the initial structure of LDHs. The obtained samples were aged at 40°C for 20 h, separated by centrifugation, washed with a large amount of deionized water and dried at 30°C.

All samples were physicochemical characterized using FTIR and scanning electron microscopy (SEM) analysis technique.

Results and discussions

FTIR spectra of MgAlLDHs and calcined MgAlLDHs presented in figure 2 a reveals wavenumber at 3450 cm^{-1} for strong and broad H-bonding stretching vibration of the OH groups and interlayer water molecules. At around 1650 cm^{-1} the binding vibration of interlayer water molecules occurs.

Bands belonging to 1360 cm^{-1} , 1120 cm^{-1} and 680 cm^{-1} are characteristic of carbonate anions and caused by asymmetric stretching and bending ways.

Al-O bond vibration at 790 cm^{-1} and the band at around 500 cm^{-1} are typically to brucite structure [39-41].

In case of calcined MgAlLDHs, peaks belonging to carbonate and hydroxyl groups disappeared because thermal treatment at high temperatures removed these groups and water molecules.

For ZnAlLDHs sample, FTIR spectra (fig. 2b) exhibits broad and intense adsorption bands at 3440 cm^{-1} due to O-H bonds vibration and at 1640 cm^{-1} attributed to O-H bending vibration of interlayer water molecules [39-41]. Band indicated at 1350 cm^{-1} implies carbonate species intercalated in layered structure of these nanomaterials.

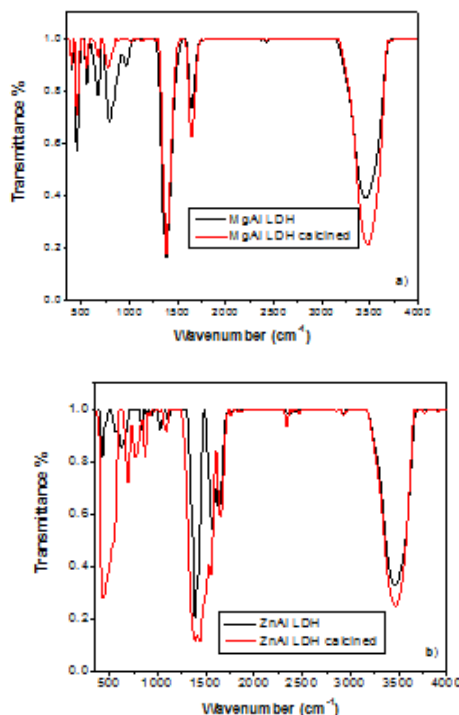


Fig. 2. FTIR spectra of a) calcined and uncalcined MgAlLDHs; b) calcined and uncalcined ZnAlLDHs

Metal-oxygen (M-O) bonds can be observed at 620 cm^{-1} and 430 cm^{-1} . For calcined ZnAlLDHs sample peaks at around 550 cm^{-1} show that M-O bonds are more prominent.

Scanning electron microscopy images was used to analyze the surface morphology of LDHs and Tob/LDHs samples [41-43].

Morphological features of the obtained samples reveals that LDHs and Tob/LDHs nanostructures are crystalline having approximately hexagonal shape.

It can also be seen interconnected particles with uniform size distribution typically for layered double hydroxides clays morphology [41-43].

As shown in figures 3, the nanoparticles size of MgAlLDH samples are about 30-40 nm and the sizes of ZnAlLDH nanoparticles, synthesized in the same conditions are larger varying from 150 to 250 nm. The nanoparticle size and distribution can strongly influence their antibacterial efficiency.

From figure 4, it can be observed that morphology characteristics alter when LDHs matrix is incorporated with antibiotic molecules. Furthermore agglomerated nanoparticles with irregular size distribution and an imprecisely defined organization of them are noticed.

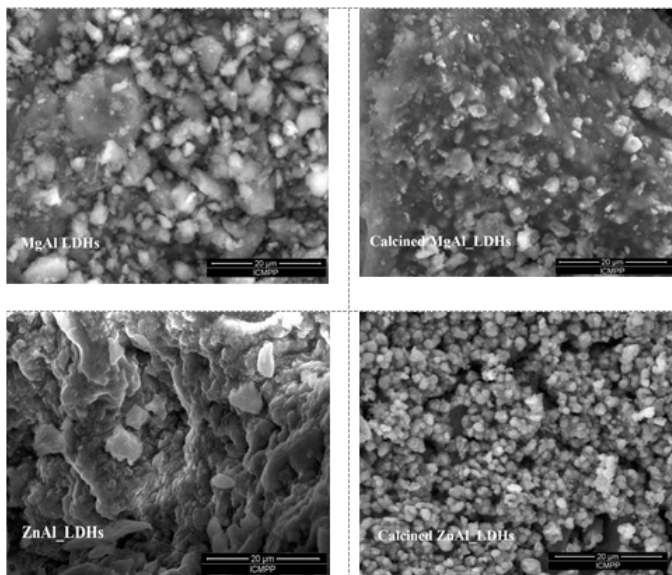


Fig. 3. SEM images for as synthesized and calcined samples: MgAl_LDHs and ZnAl_LDHs

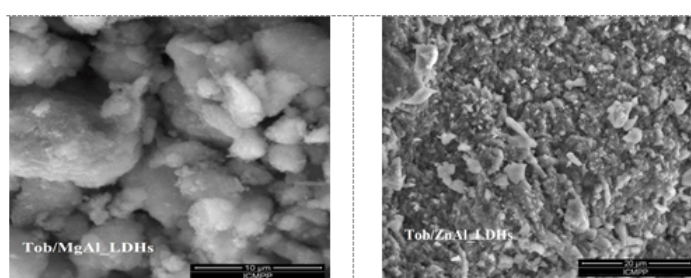


Fig. 4. Scanning electron microscopy images for Tob/MgAl_LDHs and Tob/ZnAl_LDHs

Conclusions

Synthesis and morphological features of hybrid nanomaterials type tobramycin-LDHs was studied. Layered double hydroxides have been used as inorganic matrix for drug intercalation for many different pharmaceutically active compounds and, as shown in this study, they can also serve as carriers for several types of antibiotics. The drug delivery properties of LDHs along with the possibility of targeting through surface make these nanohybrids very promising antimicrobial agents. Additionally, the methods presented here can be applied for the formation of nanohybrid materials using almost any biomolecule, ionic or non-ionic, water soluble or hydrophobic.

Self-assembled nanostructures type LDHs intercalated with drug molecules can be successfully used as drug delivery systems. However, their toxicity is not fully revealed and further researches must be done. It is also intended to have these advanced nanomaterials more efficient than simple antibiotic molecule for microorganisms targeting, controlled size and agglomeration degree of them limiting thus size effects and enhancing their efficiency.

Taking into account that these nanomaterials are cost effective and easy to synthesize, nanoparticles type LDHs-antibiotic can be obtained as drug nanocarriers having uses for the controlled release of active principle. Furthermore, magnetic nanoparticles based on LDHs-drug matrices improve the release process and the availability of antibiotic molecules resulting in an efficient and precise delivery.

This research reveals that MgAlLDHs and ZnAlLDHs nanoparticles have great physicochemical properties as ideal nanovectors for drug delivery.

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