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# Layered double hydroxides: application in the inhibition of coliforms

## *Hidróxidos dobles estratificados: aplicación en la inhibición de coliformes*

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**Abstract. -** *In this work, the preparation of different organic/inorganic hybrid materials and their evaluation as bactericides against Escherichia coli (E. coli) and Salmonella typhi (S. typhi) was studied. The main objective of the present investigation was to synthesize and characterize biocompatible hybrid materials that immobilize molecules with antibacterial activity in inorganic lamellar double hydroxides based inorganic lamellar matrices and to evaluate their antibacterial activity against Escherichia coli (E. coli) and Salmonella typhi (S. typhi). The hybrid materials consist of the association of an inorganic lamellar double hydroxide, or hydrotalcite-type compounds, with organic molecules with antibacterial activity, hosted in solids. Lamellar double hydroxides (LDH) are synthetic structures formed by positively charged metal hydroxide films that are stabilized with interlamellar anions. Different hybrid materials have been studied from hydrotalcite-type compounds, such as MgAl, ZnAl and MgFeAl, containing organic species of sodium cephalexin and nalidixic and pipemidic acids. The intercalation of the different anions was performed by one of the different existing methods: coprecipitation of the hydrotalcite-type compounds in the presence of the molecule of interest, and by the memory effect. The characterization of the materials was carried out by X-ray diffraction, IR and solid nuclear magnetic resonance spectroscopy, specifically analyzing the $^{27}Al$ and $^{13}C$ nuclei, and thermogravimetric analysis. The evaluation of the antibacterial activity of these materials was evaluated on cultures of Escherichia coli (E. coli) and Salmonella typhi (S. typhi) strains. The antibacterial activity of the tested hybrid systems is not always a direct function of the amount of antibiotic intercalated. It was obtained that the LDH ZnAl-NADmem presents a controlled release, since when the material was exposed three times against Escherichia coli (E. coli) bacteria, it continued eliminating bacteria, presenting a bacteriostatic effect in the third exposure, since it did not eliminate bacteria.*

**Keywords:** inhibition, hybrid materials, antibacterial.

**Resumen. -** *En este trabajo se estudió la preparación de diferentes materiales híbridos orgánicos / inorgánicos y su evaluación como bactericidas frente a Escherichia coli (E. coli) y Salmonella typhi (S. typhi). El objetivo principal de la presente investigación fue sintetizar y caracterizar materiales híbridos biocompatibles que inmovilizan moléculas con actividad antibacteriana en matrices lamelares inorgánicas basadas en dobles hidróxidos lamelares inorgánicos y evaluar su actividad antibacteriana frente a Escherichia coli (E. coli) y Salmonella typhi (S. typhi). Los materiales híbridos consisten en la asociación de un doble hidróxido laminar inorgánico, o compuestos tipo hidrotalcita, con moléculas orgánicas con actividad antibacteriana, alojadas en sólidos. Los hidróxidos dobles lamelares (LDH) son estructuras sintéticas formadas por películas de hidróxido metálico con carga positiva que se estabilizan con aniones interlaminares. Se han estudiado diferentes materiales híbridos a partir de compuestos tipo hidrotalcita, como MgAl, ZnAl y MgFeAl, que contienen especies orgánicas de cefalexina sódica y ácidos nalidíxico y pipemídico. La intercalación de los diferentes aniones se realizó mediante uno de los diferentes métodos existentes: la coprecipitación de los compuestos tipo hidrotalcita en presencia de la molécula de interés y por el efecto memoria. La caracterización de los materiales se realizó mediante difracción de rayos X, espectroscopia de IR y resonancia magnética nuclear sólida, analizando específicamente los núcleos $^{27}Al$ y $^{13}C$, y análisis termogravimétrico. La evaluación de la actividad antibacteriana de estos materiales se evaluó en cultivos de cepas de Escherichia coli (E. coli) y Salmonella typhi (S. typhi). La actividad antibacteriana de los sistemas híbridos probados no siempre es una función directa de la cantidad de antibiótico intercalado. Se obtuvo que el LDH ZnAl-NADmem presenta una liberación controlada, ya que cuando el material fue expuesto tres veces contra la bacteria Escherichia coli (E. coli), continuó eliminando bacterias, presentando un efecto bacteriostático en la tercera exposición, ya que no eliminar las bacterias.*

**Palabras clave:** inhibición, materiales híbridos, antibacteriano.



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## 1. Introduction

There are many disadvantages associated with the use of certain drugs. Drugs are distributed in the body according to their physical properties, such as solubility, partition coefficient and charge. Consequently, drugs can reach a wide variety of sites where they may be outside their therapeutic range, may be inactive, or their action may be undesirable or harmful, and therefore have negative side effects. There are currently two methods to enhance drug action [1]:

Controlled release, which attempts to eliminate or reduce side effects by producing a therapeutic concentration of the drug that is stable in the organism. It attempts to achieve zero-order release kinetics and there are usually no changes in the concentration of the drug in the body (compared to intermittent concentration changes in conventional dosing), and site-directed release, which tries to ensure that the drug is released at the required site, while keeping the drug inactive elsewhere in the body.

Today there is an incessant demand for advances in the field of controlled release of biologically or chemically active and environmentally sensitive molecules. The incorporation or immobilization of biologically active molecules into lamellar inorganic matrices allows their isolation from the environment while improving their stability and long-term storage. Thus, the stabilization of active molecules in biocompatible inorganic materials constitutes an interesting route for the preparation of hybrid materials that possess both the advantages of the properties of the inorganic host material and those of the organic host, in the same material. Subsequent release of the active species, if desired, is carried out by simple processes of bipolar or anion exchange interactions with ions present in the medium with which the hybrid material is contacted.

Apart from the problem of storage and stability of the active species we can find one more problem linked to the release process of the active species. An inefficient release system can result in high concentrations of the drug where it is not needed causing possible side effects; or in a rapid drop of the drug concentration below the desired levels. These problems can be solved by designing new systems for the administration and controlled release of active ingredients. These systems should provide kinetic profiles in which the concentration of the molecule remains at the appropriate concentration levels and for an adequate period of time [2].

Technologies to minimize the presence of polluting chemical species in effluents or watercourses have been studied and developed for many decades. Among the many existing possibilities, the immobilization of pollutants, i.e., their passage from the liquid phase to a solid phase, is widely used. Transport between these phases can be driven by various physicochemical phenomena. The processes involved can be as varied as adsorption, dissolution-reprecipitation, co-precipitation, occlusion and ion exchange.

The strategies used to date have been based mainly on adsorption or precipitation/arrestration. However, given that the choice of substrates to be used depends not only on the type of pollutant, but also on its chemical, structural and textural characteristics, which offer a universe of possibilities, these systems are still under study. In addition to the various immobilization alternatives, both anions and cations can be fixed by ion exchange reactions with suitable substrates. However, this process has not been massively applied on a real or plant scale due to the high cost of exchange resins of synthetic origin. As a counterpart, the use of inorganic exchangers seems promising. Most of the available information deals with cation exchange in natural clays. To a large



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extent, this is due to their implications on the mobility and bioavailability of cations (contaminants, or not) in soils and water reservoir beds} Complementing the behavior of clays, LDH-type structures allow the possibility of exchanging the anions occupying their interlaminar spaces. LDHs are easily synthesized, in a wide range of compositions, and at low cost. If the LDH components are properly chosen, we will have an inorganic anion exchanger [3];

Although the ability of LDHs to exchange anions has been known for decades, few works have focused on their study, and of all of them, only a few discuss the application of these processes in a real problem [4]. It is interesting then, to evaluate the factors that control the immobilization of ecotoxic anions in synthetic Mg-Al LDHs, and to explore the possible application of these solids as retention agents in effluent treatments or remediation processes. The use of layered double hydroxides (LDHs) as anion immobilization agents also requires knowledge of their stability under the operating conditions in which they are planned to be used. Interestingly, the thermodynamic and kinetic stability of these phases is practically unknown. Although it is known that these phases can be prepared by coprecipitation, which implies that, under certain conditions, they are more insoluble than their component hydroxides, their solubility products are still an unknown.

There is only one paper on this subject [5]. Nothing is known about their dissolution kinetics either. Some authors only mention that they have observed the leaching of the most soluble cation [6]. Others suggest that this process is slower than the dissolution of pure hydroxide [7]. Still others simply ignore this possibility, and subject LDH to conditions where dissolution may be total [8].

In the case of nanopharmaceutics, the efficacy of active ingredients depends on their intrinsic physical and chemical properties, in addition to their ability to be properly administered into the body. In this sense, we are currently seeking to overcome the limitations of therapeutics and to minimize toxic side effects in order to carry out good drug delivery. Thus, work has been done, for example, on the development of mechanisms to increase the half-life of the drug in plasma, increase the stability of the active ingredient or maximize its therapeutic activity.

The ideal objective of systems for the administration and controlled release of biologically active molecules contemplates two important aspects: spatial localization and temporal or controlled release of the active molecule. Spatial localization is related to the fact that the molecule can reach a specific organ or tissue. Controlled release refers to the control of the rate of release of the active species at the site where it is required. These two aspects cannot always be achieved and, therefore, in many cases, advances in research are still needed to propose new systems for the administration and controlled release of active molecules. For this, both the vehicle and the route of administration, as well as the target (organ or tissue) must always be taken into consideration in order to propose a strategy that allows increasing therapeutic efficiency and, in many cases, decreasing the effects.

In recent research (2012) Martinez D.R. & Carbajal G.G. on the subject of LDHs, were dedicated to review the chemical and structural characteristics of these compounds, the methods of synthesis, their intercalation or functionalization products and reaffirm the various areas in which they can be applied.

In 2019 Aristizabal D. managed to optimize the conditions to synthesize nano LDH with the



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appropriate properties to be used as drug nanocarriers and describe the interaction of nanocarriers with biological fluids, starting with the incorporation of a protein, up to more complex fluids, such as fetal bovine serum.

## 2. Methodology

The method of preparation of the LDH ZnAl is described, as well as the preparation of the hybrid materials from the LDH and the organic anion of nalidixic acid. The techniques used for the characterization of the different materials are also described.

### *2.1 Homogeneous coprecipitation method with urea*

The ZnAl-$NO_3$ solid was synthesized by the urea hydrolysis method, traditionally, due to the products of urea hydrolysis (A. Inayat et al., 2011), this method leads to the formation of LDH with carbonate anions in the interlamellar region. This result is independent of the type of metal salts used in the syntheses (chlorides or nitrates). Thus, $Zn^{+2}$ and $Al^{+3}$ cations precipitate in the form of LDH due to the controlled hydrolysis of urea at 90°C from a solution of Zn and Al nitrates.

During the synthesis, the pH of the solution is gradually increased as the hydrolysis of urea proceeds, while achieving homogeneous local concentrations resulting in the formation of solids of higher crystallinity, larger crystal size (on the order of µm) and homogeneous crystal size distribution, compared to solids synthesized by the coprecipitation method under high or low supersaturation conditions. To avoid the intercalation of $CO_2$, in the form of $CO_3^{-2}$, from the hydrolysis of urea, an excess of $NH_4NO_3$ was added to provide $NO_3^-$ ions in solution.

For the synthesis of the solid ZnAl-$NO_3$, 0.335 mole of Zn $(NO_3)_2 \cdot 6H_2O$ and 0.165 mole of Al $(NO_3)_3 \cdot 9H_2O$ were dissolved in 500 ml of $CO_2$-free deionized water at room temperature. Subsequently, 1.65 mole urea and 1 mole $NH_4NO_3$ were added and the resulting solution was placed in a 500 ml three-hole ball flask equipped with a reflux system. The system was purged by bubbling argon gas for 1 h and the temperature was increased to 90°C using a thermostated bath with sand.

After 10 h at this temperature, the white precipitate obtained was centrifuged for 15 min, washed several times with hot $CO_2$-free deionized water. Finally, the solid was dried at 120°C for 12 h in an oven. The prepared solid has a $Zn^{+2}$ / $Al^{+3}$= 2 ratio.

### *2.2 Synthesis of Hybrid Materials*

An intercalation reaction in LDH can be carried out by several pathways, or by only one depending on the LDH/anion system studied. In this work, a strategy similar to that reported in the literature was approached in order to perform the intercalation reactions for each LDH/anion system (U. Costantino et al., 2008).

### *2.3 Collation by Memory Effect*

This is an indirect intercalation method in which the mixed oxide obtained after heat treatment of the corresponding LDH is brought into contact with a solution containing the anion of interest. After a certain time, the LDH will regain its original lamellar structure and the anions contained in the solution will reside in the interlamellar region. This property is very useful when you want to intercalate an anion different



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from the original one, especially in the case of large organic anions.

First, 0.5 g of the LDH was subjected to a heat treatment at 500°C (5°/min) for 5 h in a flow of $N_2$. The mixed oxide obtained was put in contact with 30 ml of a solution, previously bubbled with argon, containing the anion of interest (4.8 mmol) and adjusted the pH to a value of 9 with NaOH (0.1 M). The obtained suspension was left in agitation for 7 days. After this time, the solid was separated from the solution by centrifugation and washed with $CO_2^-$free deionized $H_2O$ to finally dry it at 50 °C for 48 h. In this way the solids ZnAl-PIPmem, MgAl-PIPmem, MgAlFe-PIPmem, ZnAl-NADmem, MgAl-NADmem and MgAlFe-NADmem were obtained.

### *2.4 Experimental characterization techniques*

The methodology of the techniques was followed as reported by (Alejandra Santana Cruz, 2014).

*X-Ray Diffraction*

X-ray diffractograms (XRD) of the powder samples were obtained on a Philips X'PERT PRO diffractometer; the samples were analyzed in the range 3.4-80 (2θ) and with wavelength CuKα1 =1.5418 Å. Voltage-amperage of 45 kV and 40 mA were used respectively.

*Fourier transform infrared spectroscopy.*

Infrared spectroscopy is used for the identification and study of the functional groups of the molecules that make up the material to be analyzed. Measurements are performed with a Perkin-Elmer FTIR Spectrum Two™ spectrometer, with which powdered solids, rigid solids, plastics, elastic materials and liquids can be analyzed. The advantage of this equipment is that it is not necessary to prepare the sample for measurement; the material to be analyzed is deposited directly on the lens.

The infrared spectra were obtained using a NICOLET MAGNA IR 750 spectrophotometer. The analyzed region was 4000-400 $cm^{-1}$. The methodology used to obtain the spectra was by forming a pellet by mixing the sample with KBr in a sample:KBr ratio of 1:100 by weight in a manual press. The IR spectra were obtained in the Transmittance mode.

*Thermogravimetric Analysis (TGA)*

Thermogravimetry is part of a set of thermal analyses that have been developed to identify and measure the physical and chemical changes that materials undergo when exposed to controlled temperature variations *(Conesa Ferrer, 2000*).

Specifically, thermogravimetric analyses have been used to study the primary reactions in the decomposition of solid and liquid materials. With thermogravimetry, desorption, adsorption and decomposition reactions are analyzed in an inert gas environment or in the presence of oxygen *(Fraga Grueiro, 2001).*

The thermograms were obtained from the thermogravimetric analysis equipment SDT Q 60. A mass of between 5 and 20 mg of the samples was used and analyzed in a temperature range of 25 to 850 °C at a rate of 10 °C/min, in nitrogen atmosphere.

### ***2.5 Evaluation techniques for the inhibition of the growth of microorganisms.***

*Bacterial strains:*

*Purity tests*



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Gram staining was performed to verify the purity of the *E. coli* strains, observing their morphology under an optical microscope at 100x.
The corresponding smear was made on the strain to be analyzed with crystal violet and an iodine solution, all of them were stained with a purplish color. Subsequently, they were treated with a decolorizing solution (alcohol-ketone), the gram-positive bacteria retained the dye, due to the composition of their wall, while the gram-negative bacteria did not retain it, the dye being eliminated. Next, they were treated with a contrast dye (diluted fuchsin), the gram-negative bacteria were stained, which ensures that they are gram-negative bacteria.

*Turbidity standard for inoculum preparation*

To standardize inoculum density, a barium sulfate suspension was used as a turbidity standard (0.5 on the McFarland scale). Turbidity tests were performed on a Varian Cary 4000 UV-Vis spectrophotometer. The absorbance was measured at 625 nm and incubated under the same conditions until growth was achieved in the range between 0.7 and 0.8 optical density. The standards were stored at room temperature away from light.

*Preparation of inoculum*

For inoculum preparation, 3 isolated colonies of the same type of morphology of the strains maintained on tryptic-casein soy agar wedges were taken and grown in tubes containing 5 ml of tryptic-casein soy broth at 37 °C until standard turbidity was reached. This suspension contained approximately $1x10^8$ Colony Forming Unit (CFU)/ml of *E. coli*. The inoculum was reseeded every 12 h for 5 days to confirm the exponential phase of growth. These strains were stored at 4 °C in order to maintain viability.

In order to facilitate the identification of lamellar double hydroxides and hybrid materials, the following nomenclature was adopted for LDH: ZnAl-X, where X indicates the resident anion in the interlaminar region. In our specific case, X can be $NO_3^-$, $Cl^-$ or $CO_3^{2-}$ .

### 2.6 *Bacterial growth in the presence of hybrid materials.*

Bacterial growth in the presence of the biocidal materials was determined. For this purpose, the bactericidal capacity of the materials was evaluated in relation to time and average CMB. A standardized inoculum was challenged at fixed concentrations of antimicrobial in a broth. A 0.5 ml sample of the liquid systems was inoculated with bacteria (*E. coli*) in 10 ml of tryptic-casein soy broth contained in screw-capped test tubes. An amount of biocidal material (amount determined as average CMB) was added to each tube and incubated at 37°C with agitation at 30 rpm. Samples were taken at different times (0, 5, 15, 30, 60, 90 and 120 min). The sample taken was seeded in Petri dishes with 20 ml of MacConkey agar by the streak-plate technique.

As a control, one plate was inoculated with culture without bactericidal material, at the beginning and at the end. The plates were incubated inverted at 37 °C for 24 h in an aerobic atmosphere and colony counting was performed.

## 3. Results and Discussions

### 3.1 *Characterization of ZnAl-X materials*

Zn- and Al-based lamellar double hydroxides were synthesized with different anions by different methods to obtain the solids ZnAl-$CO_3$, ZnAl-Cl and ZnAl-$NO_3$. The X-ray diffractograms of these solids and the interplanar distances $d_{003}$ are shown in Figure 1. All the



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diffractograms show peaks associated to the characteristic planes of hydrotalcite, no other phases foreign to this mineral are observed; that is to say that pure LDH were obtained.

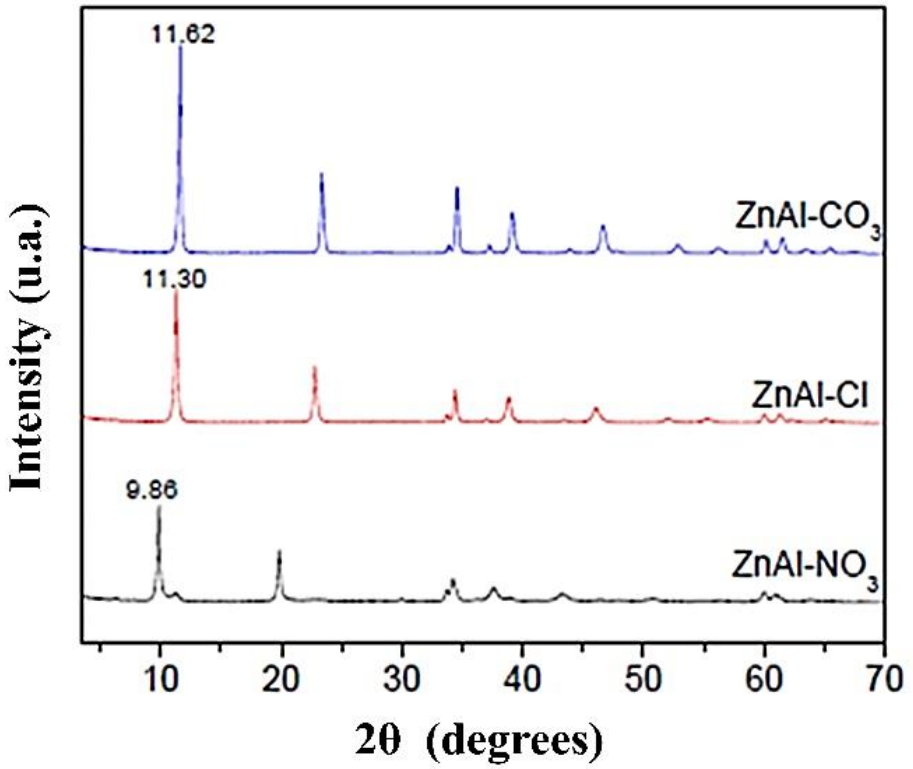


**Figure 1.** X-ray diffractogram of LDH ZnAl-X.

On the other hand, the infrared spectra shown in Figure 2 (for reasons of clarity the infrared spectrum for the ZnAl-Cl solid is not shown) are congruent with those reported in the literature. On the one hand, both ZnAl-$NO_3$ and ZnAl-$CO_3$ solids show a broad and very intense absorption band, centered around 3445 $cm^{-1}$, which is attributed to the vibrational frequency of the $\nu$OH stretching mode of the O-H groups forming the brucite-like films, so it can be stated that up to this point three different types of ZnAl-X solids are available and were used as starting materials for the subsequent preparation of hybrid materials.



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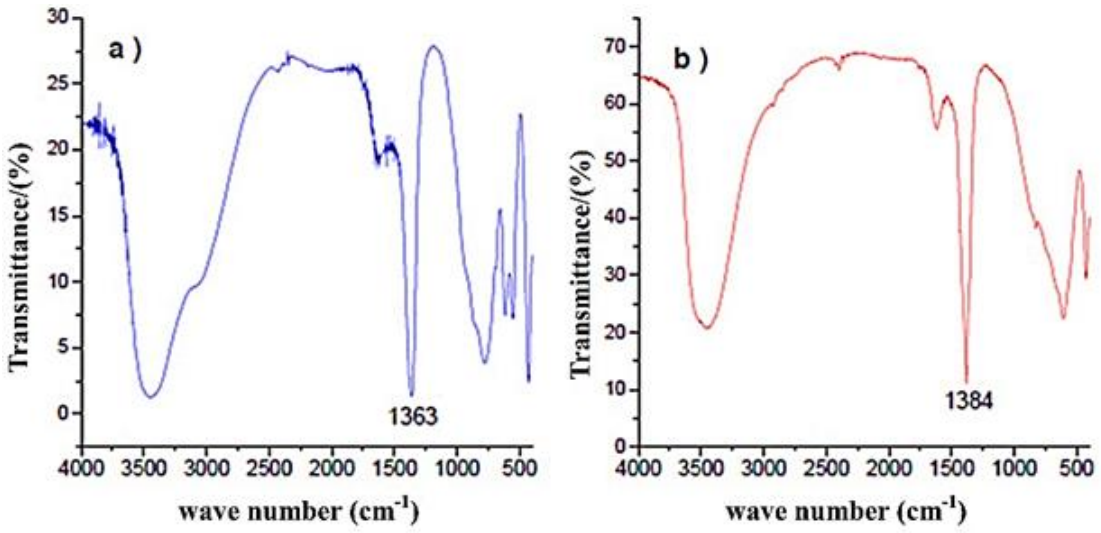


**Figure 2.** Infrared spectra of fresh LDH ZnAl-X. a) ZnAl-$CO_3$ and b) ZnAl-$NO_3$.

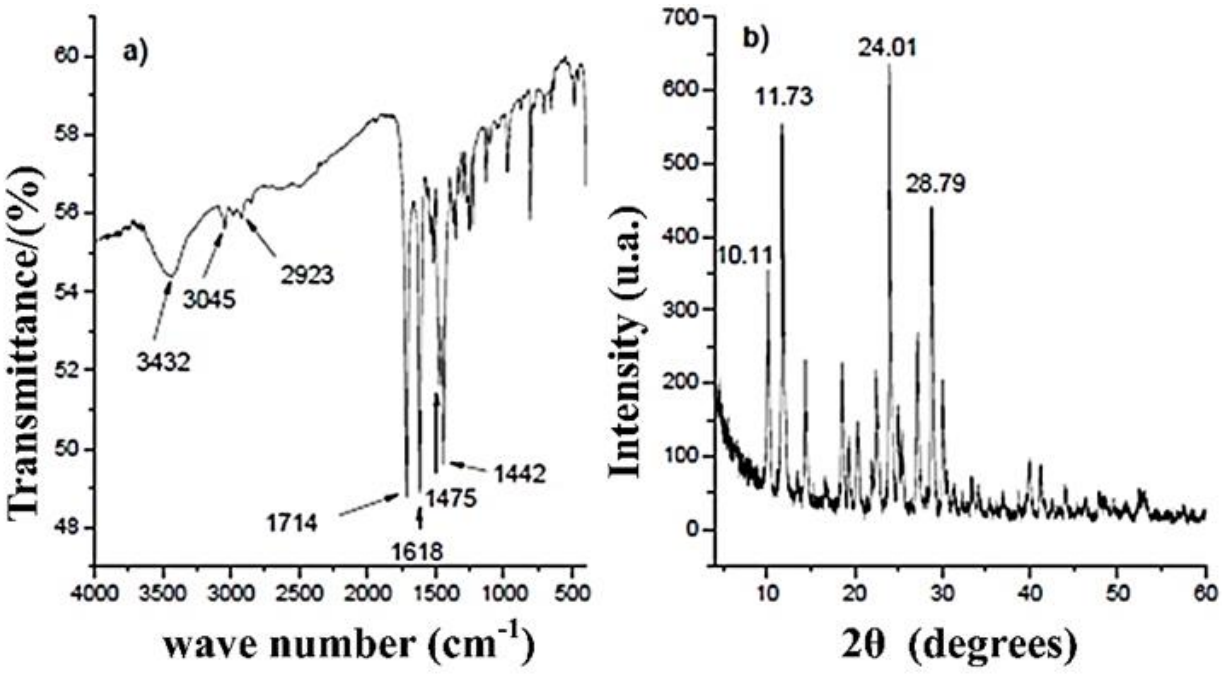


**Figure 3.** Characterization of the Nalidixic acid molecule. a) FTIR spectrum, b) XRD



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### *3.2 Characterization of hybrid materials*

In order to better understand the characterization of the hybrid materials, it is convenient here to present both the infrared spectrum and the X-ray diffraction pattern of the molecules that were studied; either of the starting acids or of the sodium salts generated from them. This is with the purpose of keeping them in mind as reference analysis results. Thus, Figure 3 shows the IR spectrum and the XRD pattern of nalidixic acid.

From the general formula of the LDH and the charges of the incoming and outgoing anions, the necessary amount of anions that can enter the interlaminar space is known.

Taking into account the stoichiometry of the exchange; for example, a $NO_3^-$ ion would be replaced by a $Cl^-$ ion and vice versa; however, a single $CO_3^{2-}$ anion would have to be replaced by two $NO_3^-$ ions or by two $Cl^-$ ions in order not to create a charge imbalance in the interlaminar region. Thus, the charges of both the anion initially present in the interlaminar region and the anion to be introduced must be taken into account to determine the minimum stoichiometric amount of the incoming anion necessary to achieve 100% replacement.

However, the intercalation process does not ensure that the anion will occupy all of that interlamellar space or achieve a 100% replacement rate. Because of the above, when only the minimum stoichiometric amount of incoming anion is placed in contact with an LDH, the exchange is likely to be only partial (Y. T. Kameda et al., 2006).

### 3.3 Nalidixic Acid CMBs and CMIs

In order to verify that Nalidixic Acid has a better bactericidal effect against *E. coli* with respect to other drugs, MIC and BMC tests were carried out on the most commonly used drugs for this purpose. Tables 1 and 2 show the MIC and BMC values, respectively, of the drugs as inhibitors of *E. coli*, using the method of dilutions and sowing on agar. Reproducibility was done in triplicate for each bacterial strain. The values reported are the average of the MICs and BMCs for each test, in broth and MH agar, respectively.

**Table 1.** CMIs of different antibiotics.

| | CMI (mg/mL) | | | | | |
|---|---|---|---|---|---|---|
| **Drug** | **0.11** | **0.17** | **0.22** | **0.27** | **0.33** | **0.38** |
| **Cloxacillin Sodium** | + | + | + | + | + | - |
| **Cephalexin Sodium** | + | + | + | + | - | - |
| **Sodium Ampicillin** | + | + | + | + | - | - |
| **Mg-Al Ampicillin** | + | + | + | + | - | - |
| **Nalidixic Acid** | + | + | + | - | - | - |
| **Pipemidic Acid** | + | + | + | - | - | - |



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**Table 2.** CMBs of different antibiotics.

| | CMB (mg/mL) | | | | | |
|---|---|---|---|---|---|---|
| **Drug** | **0.11** | **0.17** | **0.22** | **0.27** | **0.33** | **0.38** |
| **Cloxacillin Sodium** | + | + | + | - | - | - |
| **Cephalexin Sodium** | + | + | + | + | - | - |
| **Sodium Ampicillin** | + | + | + | + | - | - |
| **Mg-Al Ampicillin** | + | + | + | + | - | - |
| **Nalidixic Acid** | + | + | + | - | - | - |
| **Pipemidic Acid** | + | + | + | - | - | - |

Turbidity in the inoculated tubes allowed determining the MIC after 24 hours of exposure of the bacteria to the different materials. The quantification of bacterial colonies that grew on plates with MH agar was performed after 18 and 24 hours of incubation at 37$^{o}$C.

The exposed samples that did not show turbidity were sown in petri dishes with Müller-Hinton agar, showing that bacterial growth was null. This shows that the materials have a bactericidal effect on *E. coli*. It should be noted that those translucent tubes whose turbidity was very low (less than 50 NTU) or close to zero were considered as systems in which there was no growth. Those tubes with apparent turbidity and values greater than 50 NTU were considered systems with microbial growth.

With this descriptive turbidimetric test method, some answers can be obtained about the behavior of bacteria in the presence of bactericidal agents, such as suppression in the level of growth in the stationary phase, decrease in the growth rate and lethality *(Davidson and Parish, 1989).*

Microbiological tests to evaluate inhibition times Before evaluating the bactericidal character of the materials, quality controls were performed on the working strains by microdilutions. The results of the quality controls (viability and purity) of *E. coli*, used in the bactericidal evaluation, met the acceptance criteria by obtaining viability values in the order of $1x10^{8}$, so that the purity results comply with the condition of cultures free of microbial contamination.

Table 3 and Figure 4 report the average values of *E. coli* colonies that survived in each of the three trials, for each of the exposure times to the different drugs evaluated as biocides. The microbial growth and colony count of the *E. coli* strains in the presence of the different bactericidal materials were calculated taking into account the number of initial colonies in the suspension of inoculated microorganisms and the colonies that grew or were eliminated during the exposure time.

The bactericidal effect of the materials was assessed by measuring cell viability at 0, 5, 30, 60, 90 and 120 minutes after exposure and incubation of the bacteria with the different materials. The tests were performed in triplicate on McConkey agar plates. These tests made it possible to define the moment at which the biocidal agent acts on the bacterial replication cycle, in such a way that each drug presents a different graph according to its mechanism of action.



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**Table 3.** Colonies of *E. coli* that survived against drugs.

| Amoxicillin Sodium($C_{16}H_{19}N_3O_5S$) | | | | | | MgAl -ampicillin($C_{16}H_{18}N_3NaO_4S$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time (min)** | **E1** | **E2** | **E** | **Average** | **Uncertainty** | **E1** | **E2** | **E3** | **Average** | **Uncertainty** |
| **0** | 170 | 170 | 174 | 171 | 1 | 172 | 171 | 170 | 171 | 1 |
| **5** | 153 | 150 | 155 | 152 | 2 | 71 | 70 | 71 | 70 | 0 |
| **15** | 117 | 120 | 118 | 118 | 1 | 40 | 42 | 42 | 41 | 1 |
| **30** | 86 | 88 | 88 | 87 | 1 | 31 | 30 | 33 | 31 | 1 |
| **60** | 68 | 71 | 67 | 68 | 1 | 18 | 19 | 21 | 19 | 1 |
| **90** | 44 | 48 | 45 | 45 | 1 | 11 | 12 | 14 | 12 | 1 |
| **120** | 16 | 15 | 14 | 15 | 1 | 0 | 1 | 2 | 1 | 1 |
| **Nalidixic Acid ($C_{12}H_{12}N_2O_3$)** | | | | | | **Pipemidic Acid($C_{14}H_{17}N_5O_3$)** | | | | |
| **Time (min)** | **E1** | **E2** | **E3** | **Average** | **Uncertainty** | **E1** | **E2** | **E3** | **Average** | **Uncertainty** |
| **0** | 171 | 170 | 168 | 169 | 1 | 169 | 170 | 170 | 169 | 0 |
| **5** | 109 | 110 | 111 | 110 | 1 | 82 | 80 | 81 | 81 | 1 |
| **15** | 63 | 62 | 61 | 62 | 1 | 52 | 51 | 51 | 51 | 1 |
| **30** | 11 | 10 | 12 | 11 | 1 | 5 | 6 | 5 | 5 | 1 |
| **60** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **90** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **120** | 0 | 0 | 0 | 0 | 0 | 169 | 170 | 170 | 169 | 0 |
| **Cephalexin Sodium($C_{16}H_{17}N_3O_4S$)** | | | | | | **Cloxacillin Sodium($C_{19}H_{18}N_3ClO_5S$)** | | | | |
| **Time (min)** | **E1** | **E2** | **E3** | **Average** | **Uncertainty** | **E1** | **E2** | **E3** | **Average** | **Uncertainty** |
| **0** | 172 | 171 | 175 | 172 | 1 | 172 | 170 | 172 | 171 | 1 |
| **5** | 124 | 121 | 131 | 125 | 4 | 121 | 120 | 123 | 121 | 1 |
| **15** | 91 | 83 | 85 | 86 | 3 | 90 | 85 | 87 | 87 | 2 |
| **30** | 54 | 52 | 53 | 53 | 1 | 43 | 42 | 45 | 43 | 1 |
| **60** | 33 | 30 | 31 | 31 | 1 | 27 | 29 | 32 | 29 | 2 |
| **90** | 27 | 26 | 28 | 27 | 1 | 15 | 17 | 18 | 16 | 1 |
| **120** | 8 | 7 | 8 | 7 | 0 | 7 | 8 | 6 | 7 | 1 |



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Figure 4 shows the results of the tests performed with different antibiotics as inhibitors of *E. coli*, showing that NAD and Pimemidic acid (PIP) were the ones that eliminated the bacteria in the shortest time; however, NAD is more economical than PIP, so it was decided to perform this work with NAD (Nalidixic acid).

The prolonged release of the biocide from biocidal LDHs must be attributed to dipolar interactions between the organic biocide and LDHs and occurs in other similar organic-LDH hybrid compounds [14,15].

In similar studies by Santana Cruz et al. in 2018 refer (Although *S. typhi* is more resistant than *E. coli* to both nalidixic acid and pipemidic acid incorporated into LDHs, it is clear that LDH-biocide interactions are favorable for killing *S. typhi*) (Santana A.; Flores J.L.; Guerra R.; & Martínez M.J.et al., 2016). In results of Antibacterial activity of pipemidic acid-hybrid ions. MgFeAl-Cl allows the rapid development of colonies of *S. typhi*. In contrast, the MgFeAl-PIP hybrid material showed good activity to kill bacteria, as only 12% of colonies survived after 90 min of exposure. MgFeAl-PIP is less active in killing *S. typhi* than *E. coli* pathogens.

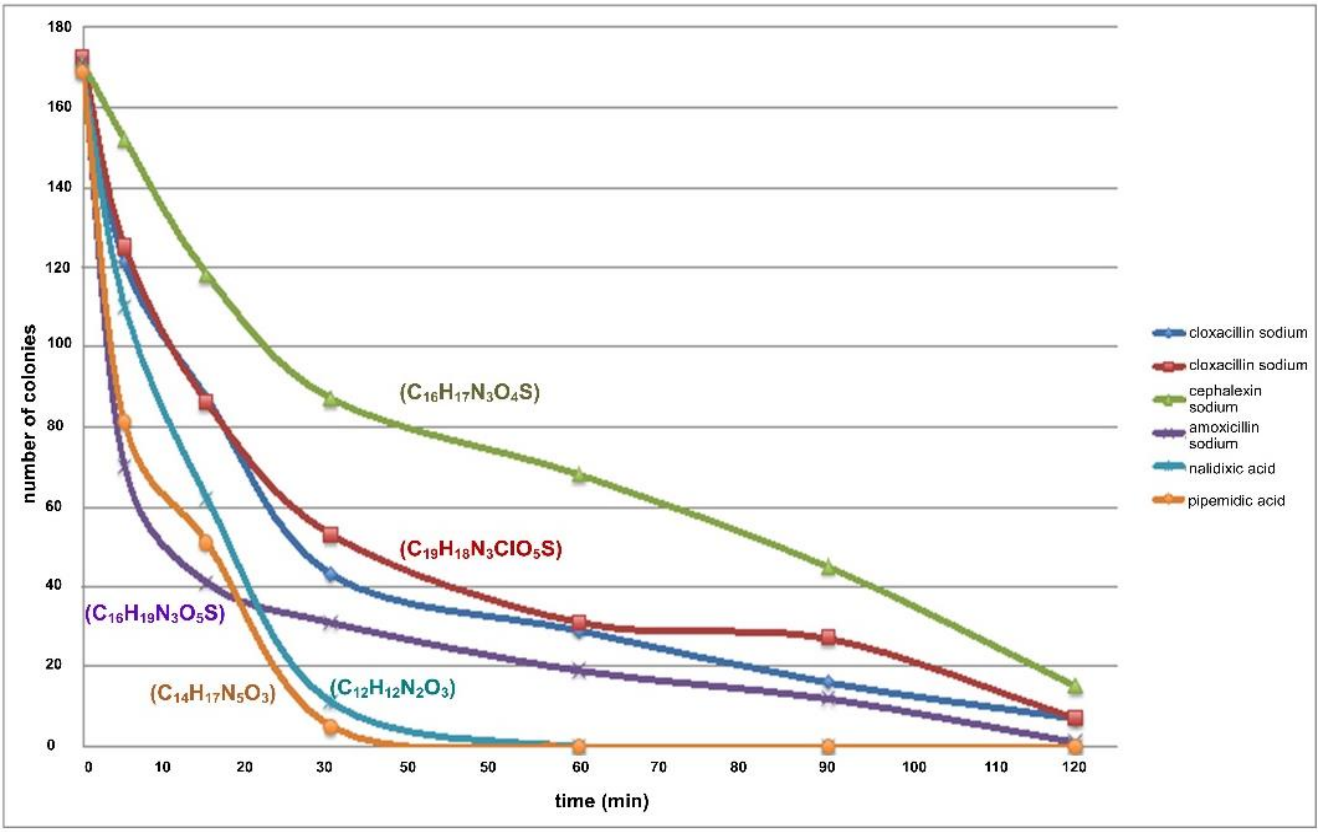


**Figure 4.** Colonies of *E. coli* that survived against drugs.



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## 4. Conclusions

According to the results obtained, the LDH ZnAl-NADmem presents a controlled release, since when the material was exposed three times to *E. coli* bacteria, it continued to eliminate bacteria, presenting a bacteriostatic effect in the third exposure, since it did not eliminate bacteria. The ZnAl-NADmem material obtained by memory effect, is a laminar and very crystalline material but with a very low degree of intercalation, which to some extent is an advantage because it does not require much antibiotic to present good antibacterial activity, this is due to a synergistic effect between the inorganic matrix and the antibiotic. LDH MgFeAl-NADmem also shows good antibacterial activity, however, its synthesis represents a higher cost with respect to ZnAl-NADmem. In general, on the hybrid systems analyzed, the antibacterial activity is not always a direct function of the amount of antibiotic intercalated. It seems that the activity depends precisely on the inorganic matrix-antibiotic system studied. From the above arises the idea that there is, in some systems, a synergistic effect between the inorganic matrix and the antibiotic that results in an efficient inhibition system.

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