

*Dedicated to the memory of
Professor Cristofor I. Simionescu (1920–2007)*

NEW HYBRID COMPOUNDS CONTAINING INTERCALATED CIPROFLOXACIN INTO LAYERED DOUBLE HYDROXIDES: SYNTHESIS AND CHARACTERIZATION

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This work presents the synthesis, physical and chemical characterization of layered double hydroxides containing Zn and Al (ZnAlLDH) intercalated with ciprofloxacin (CFX) (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid). The products were prepared by two different techniques: coprecipitation and ion exchange. The hybrid materials were studied by X-ray diffraction (XRD), FTIR spectroscopy and thermogravimetry. The results of the XRD analysis confirm that all the products present the crystalline structure characteristic for hydrotalcite-like compounds. The FTIR and TG-DTG analyses confirm the intercalation of the organic compound within the hydrotalcite matrix.

INTRODUCTION

Ciprofloxacin (1-cyclopropyl-6-fluoro-4-oxo-7-(1-piperazinyl)-1,4-dihydroquinoline-3-carboxylic acid) is a synthetic antibacterial fluoroquinolone and it is given to patients orally in the form of its hydrochloride salt. Ciprofloxacin is a potent and broad-spectrum antibiotic with high antibacterial activity against most gram-negative bacteria and gram-positive cocci. It has a superior ability to penetrate most tissues compared to other antibiotics¹⁻⁴ and neutrophils⁵ and it is bactericidal in low pH environment.⁶

The major side effect of CFX treatment is the gastro-intestinal irritation, which is a common disadvantage observed for most antibiotics. Intercalating the antibiotics into hybrid drug-layered double hydroxide materials can eliminate this problem.

Layered double hydroxides (LDHs), a class of materials known as anionic clays or hydrotalcite-like compounds are described by the general formula: $[M_{1-x}^{2+}M_x^{3+}(\text{OH})_2]^{x+}(\text{X}^{n-})_{x/n} \cdot x\text{mH}_2\text{O}$

They are widely used in pharmaceutical formulations due to their properties in protecting

the drugs against light, oxygen and water vapor effects. They are also applied as carriers in controlled or targeted release compounds. Hybrid drug-inorganic matrix materials were synthesized by various methods using layered double hydroxides reversibly intercalated with different active ingredients such as dichlofenac, gemfibrozil, ibuprofen, naproxen, fenbufen, indometacin and salicylic acid.⁷⁻¹⁴ This paper reports the incorporation of ciprofloxacin into ZnAlLDH by two synthetic methods: ion exchange and coprecipitation. The goal of this work is to intercalate the organic molecules of the drug into the interlayer space of the LDH structure in order to improve its solubility in acid conditions and to eliminate the gastro-intestinal side effects.

The characterization of the synthesized materials was achieved by using powder X-ray diffraction, FTIR spectroscopy and thermal analysis.

EXPERIMENTAL

Chemical reagents for this work ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, NaOH and ciprofloxacin) were purchased

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from Sigma-Aldrich and used without further purification. XRD analysis was performed on a Bruckner AXS D8 X-ray diffractometer using a Cu-K α radiation ($\lambda = 0.154$ nm) at 40 KV at 35 mA between 4° and 70° (2θ) with a secondary graphite monochromator. FTIR spectra were recorded using a Bomem MB 104 spectrophotometer. The differential thermal analysis was performed on a Mettler Toledo TGA-SDTA851e derivatograph under nitrogen atmosphere with flow rate of 20ml/min, using a heating rate of 10K/min and sample weight in the range of 4–6 mg. The parameters were kept constant for all the samples in order to obtain comparable data. The molecular modelling studies were performed using the Hyperchem software package.

Preparation of ZnAILDH precursor: NaOH 1M solution was added, under vigorous stirring and nitrogen blanket, to 100 mL aqueous solution containing 0.2 mol $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 0.1 mol $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ with the aid of an automatic titrator TitraLab TIM 854, maintaining the pH constant at 7.5. The white precipitate which was formed was allowed to age for 24h at 40°C , under stirring. It was then separated by centrifugation, washed extensively with deionized water and dried at 40°C under vacuum.

Synthesis of ZnAILDH compounds with intercalated ciprofloxacin (ZnAILDH_CFXc) using the coprecipitation method: 150 mL aqueous solution of 0.2 mol $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 0.1mol $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ and 100ml aqueous solution containing 0.01 mol CFX were added to the reaction vessel using a peristaltic pump. The pH of the mixture was maintained at the constant value of 7.5 during the whole process by continuously adding 1M NaOH using an automated titrator TitraLab TIM 854. The product was aged for 36h. The obtained precipitate was separated by centrifugation, washed with deionized water and dried in vacuum at 40°C .

Synthesis of ZnAILDH compounds with intercalated ciprofloxacin (ZnAILDH_CFXsi) using the ion exchange method: 150 mL aqueous solution with 0.01 mol dissolved

CFX were added to 250 mL aqueous dispersion containing 1g ZnAILDH under vigorous stirring and nitrogen blanket. During this process, the pH of the mixture was maintained constant at 7.5 by adding 1M NaOH. The reaction was allowed to proceed for 24h at room temperature. The precipitated was separated by centrifugation, washed with deionized water and dried at 37°C .

RESULTS AND DISCUSSION

XRD analysis

The analysis of the XRD patterns reveals the fact that each synthesis resulted in layered double hydroxide structures containing well-crystallized phases. The basal reflections are characteristic to successive layer ordering and can be used for finding the basal distance c' , which represents the thickness of one brucite layer and one interlayer spacing.

In Fig. 1 the reflection peak of the (d_{003}) plane recorded at low 2θ diffraction angle values is 8.74 \AA for the ZnAILDH precursor. After the CFX intercalation, the powder XRD patterns of the products maintain the characteristic features of layered double hydroxides. Moreover, the (d_{003}) parameter increases to 20.61 \AA for the ZnAILDH_CFXc sample and to 20.37 \AA respectively for the ZnAILDH_CFXsi sample, due to the presence of the CFX molecules in the interlayer space.

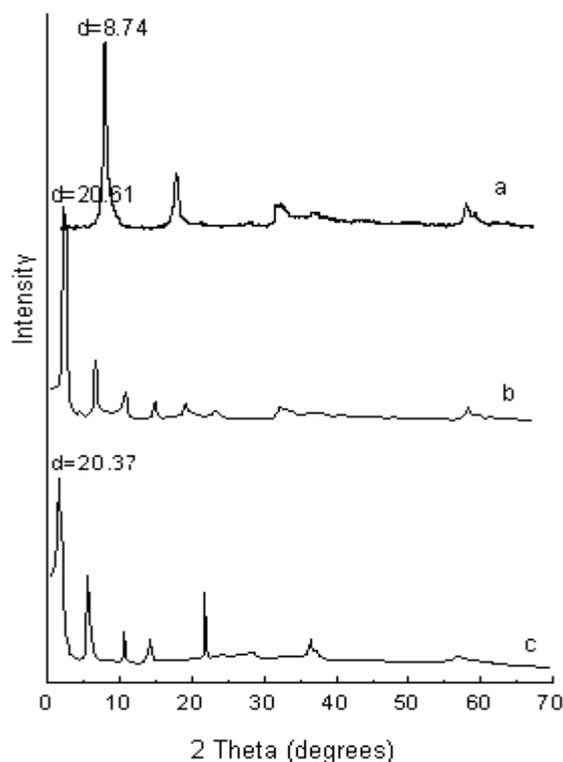


Fig. 1 – XRD diffractograms of a) ZnAILDH, b) ZnAILDH_CFXc, c) ZnAILDH_CFXsi.

The reflection parameters for all the synthesized compounds can be described as belonging to a hexagonal structural unit with rhombohedral symmetry R3m, characteristic to layered double hydroxides. The lattice parameters shown in Table

1 are calculated from the positions of the maxima due to reflections by planes (003) and (001) and from the interlayer distances of the precursor and the samples with intercalated CFX.

Table 1

Lattice parameters and interlayer distances

Compound	$d_{003}(\text{Å})$	$c=3 d_{003}(\text{Å})$	$a=2 d_{110}(\text{Å})$	Interlayer distance (Å)
ZnAILDH	8.74	26.10	3.04	3.94
ZnAILDH_CFXc	20.61	61.63	3.05	15.81
ZnAILDH_CFXsi	20.37	61.11	3.05	15.57

Structural modelling

The molecular dimension of CFX compound, calculated using the chemical bond lengths and atomic angles (using Hyperchem software), is 4.21 Å along the y-axis and 11.83 Å along the x-axis. According to the XRD data, the intercalation of the CFX molecule leads to an increase of the interlayer

distance of 15.81 Å for the ZnAILDH_CFXc and 15.57 Å for the ZnAILDH_CFXsi sample respectively.

The proposed orientation of the CFX anions is presented in Fig. 2. The model takes into account the length of the hydrogen bonds established between the anions and the LDH hydroxyl groups.

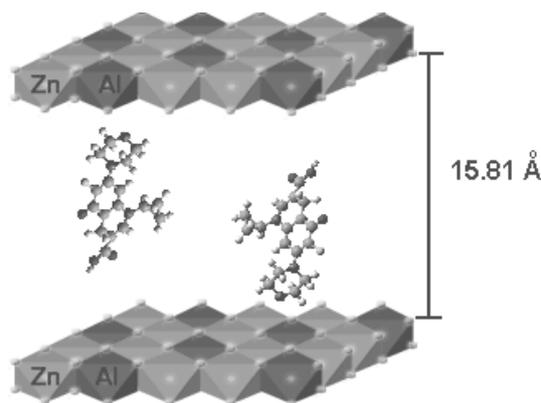


Fig. 2 – Proposed model for the orientation of the CFX anions in the LDH interlayer space for ZnAILDH_CFXc hybrid material.

FT-IR analysis

The FTIR spectrum for the ZnAILDH precursor (Fig. 3a) shows an absorption band centered at 3431 cm^{-1} , associated with the OH bonds due to the presence of hydroxyl groups in LDH and/or to the physically adsorbed water molecules. The strong band located at 1381 cm^{-1} associated with the ν_3 vibration of the nitrate group, the anion contained in ZnAILDH. The band at 1625 cm^{-1} is related to the $\nu_{\text{H-O-Hbent}}$ vibration. The bands in the low frequency region of the spectrum correspond to the vibration mode of the layer bonds such as Zn-OH at 611 cm^{-1} and Al-OH at 829 cm^{-1} , characteristic to this class of materials. The FTIR spectrum of ciprofloxacin is presented in Fig. 3d. The band at 1707 cm^{-1} is characteristic to the

carboxylic (C=O) bond. The absorption bands centered at 1280 and 1610 cm^{-1} are assigned to the vibration mode of the C-F bond and to the vibration of the phenyl group conjugated with -COOH. The bands characteristic to the vibration mode of the aromatic ring C-H bonds are located at 3083 and 2918 cm^{-1} .

Two absorption bands assigned to the asymmetrical [$\nu_{\text{as}}(\text{O}-\text{C}-\text{O})$] and symmetrical [$\nu_{\text{s}}(\text{O}-\text{C}-\text{O})$] vibration mode are centered in the range 1610–1550 cm^{-1} and 1400–1280 cm^{-1} respectively.

The intercalated products spectra show most of the organic anion vibration bands together with the absorption bands characteristic to the ZnAILDH, which confirms the presence of the drug in the LDH interlayer space.

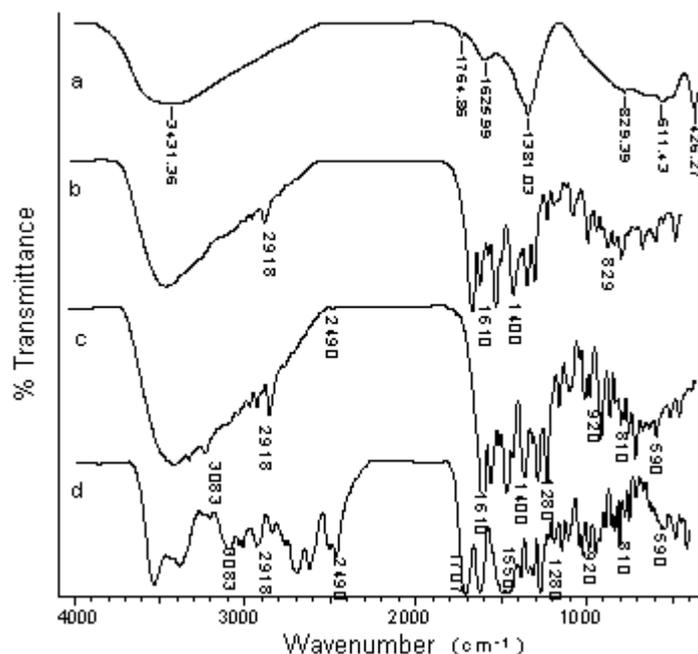


Fig. 3 – FTIR spectra for (a) ZnAILDH, (b) ZnAILDH_CFXsi, (c) ZnAILDH_CFXc, (d) CFX.

Thermogravimetric analysis

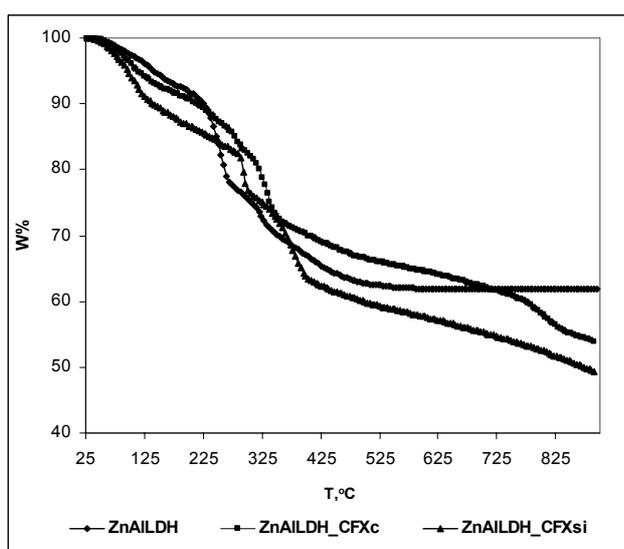
The thermogravimetric curves of the layered double hydroxides with intercalated ciprofloxacin and of the precursor are shown in Fig. 4.

The plot of the CFX could not be recorded due to the fact that the compound degrades rapidly once the decomposition temperature is reached.

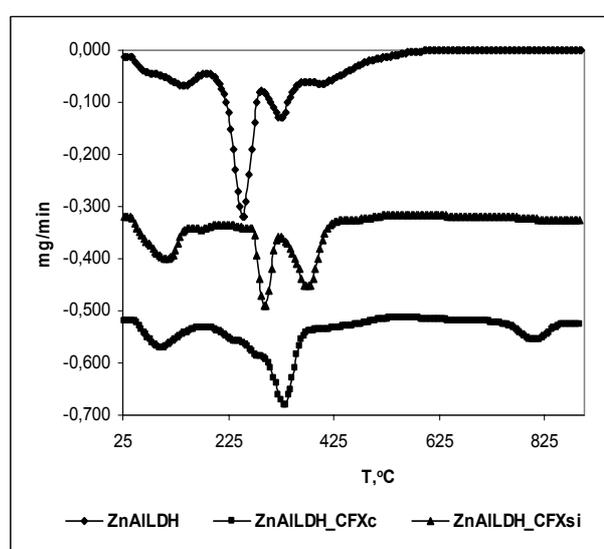
As shown in Fig. 4, the first stages in the weight loss process occur in the temperature range of 50 to 130-150°C and are attributed to the

removal of the physically and chemically bonded water from the interlayer space, which represents about 10 to 13% of the sample weight.

The next stages correspond to weight losses between 5 and 25%. The DTG curves presented in Fig. 4b reveal a complex degradation mechanism which takes place in multiple steps. The thermal degradation of ZnAILDH_CFXc exhibits four stages, while the other hybrid, ZnAILDH_CFXsi, degrades in a five stage process.



a) TG curves



b) DTG curves

Fig. 4 – TG and DTG curves for ZnAILDH_CFX.

The biggest weight loss is recorded in the 300–500°C temperature range: approximately 15% of the ZnAILDH_CFXc and 19% of the ZnAILDH_CFXsi sample respectively. This weight loss stage is associated with the layers dehydroxylation and the beginning of the ciprofloxacin decomposition. Taking into account the fact that the melting point of ciprofloxacin is 255°C, the results show an increase in the thermal stability of the drug upon intercalation.

The T_{peak} values corresponding to the temperatures of maximum degradation rate for each stage, were determined from the DTG curves.

The thermogravimetric parameters T_{onset} , T_{peak} and T_{endset} , in addition to the percentage weight losses are presented in Table 2. Different hydrotalcite degradation mechanisms take place at temperatures higher than 300°C depending on their synthesis method.

Table 2

Thermogravimetric parameters – ZnAILDH_CFX

SAMPLE	STAGE	T_{onset}	T_{peak}	T_{endset}	W%	Residue
ZnAILDH_CFXc	I	50	95	157	8.68	57.25
	II	157	331	349	19.41	
	III	349	650	772	14.66	
	IV	772	812	900	7.17	
ZnAILDH_CFXsi	I	50	111	132	10.02	49.66
	II	132	175	289	7.13	
	III	289	295	302	8.21	
	IV	302	379	407	11.39	
	V	407	650	900	13.59	

The amount of sample residue left after degradation at temperatures higher than 900°C is 10 to 20% lower for the layered double hydroxides containing ciprofloxacin than for the precursor, which is further proof of the presence of the organic compound intercalated within the LDH layers.

CONCLUSIONS

New layered double hydroxide derivatives have been synthesized and characterized. Both synthesis methods used here, coprecipitation and anion exchange, lead to formation of crystalline LDHs containing intercalated CFX. According to XRD, the main technique for discerning the intercalated material structure, the resulting basal spacing of ZnAILDH_CFX was expanded to 20.37–20.61 Å. A structural model has been proposed based on the optimized CFX geometry. The anions are therefore accommodated in the interlayer region as a monolayer with reactive groups interacting simultaneously with both upper and lower hydroxide layers. In the FTIR spectra, the intensity of the NO_3^- absorption peak decreased with increasing the amount of intercalated CFX. Thermal analysis of the ZnAILDH_CFX nanocomposites showed that CFX intercalated inside ZnAILDH is thermally more stable than its raw counterpart. The results show that ZnAILDH materials can be used as biocompatible inorganic host for drug reservoirs.

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