

## Fluorouracil - layered double hydroxides nanocomposites obtained through LDH-LDO-LDH structural conversion

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### Introduction

In the last several decades, but mainly in the last 10+ years, nanocarrier formulation of anticancer drugs has strongly stimulated the research, becoming a key topic in cancer nanotechnology [1]. Layered double hydroxides (LDH) with two dimensional structure are among the nanocarriers holding promise for oncologic applications due to their low toxicity, particular structure and morphology, relatively high specific surface areas, anion exchange capacity, positive Zeta potential, to name only a few of their attracting physico-chemical properties [2]. Their unique structure, consisting of compact layers embedding inorganic cations separated by a space accommodating anions and water, make them a generous platform for developing an extremely large number of nanodrugs for cancer, but not limited to (Figure 1).

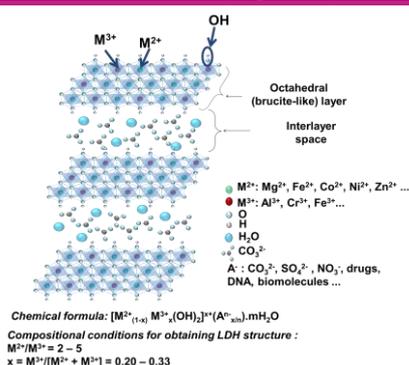


Figure 1. Schematic structure of LDH

Essentially, the layers contain cations ( $M^{2+}$  and  $M^{3+}$ ), each of them being surrounded by six OH groups forming octahedra linked together by the edges [3]. The trivalent cations in the layers bring a positive charge, which is beneficial for both hosting organic anionic molecules, such as drugs, in the space between the layers and for interaction with the negatively charged phospholipids in the cell membrane. Such materials can be conveniently synthesized by several methods [4]. However, the appropriate method has to be selected depending on the desired properties. The most employed

methods to synthesis organic-inorganic LDH hybrids, are coprecipitation at low and high supersaturation, anion exchange and reconstruction. *Coprecipitation* implies the addition of two solutions, one containing the metallic precursors and the other one the precipitation agent to a solution containing the drug at controlled pH, temperature, stirring, time, and aging conditions. The *anion exchange* supposes putting into contact a pre-synthesized LDH with a solution of the drug to be encapsulated. In this case, the inorganic anion between the layers is replaced by the drug. Finally, the late approach, *reconstruction* implies three main stages, that is, (i) synthesis of LDH, (ii) thermal degradation through calcination at high temperatures (usually 400 - 450 °C for 4 - 6 h) when the layered double oxides (LDO) are formed, followed by (iii) rehydration of LDH to recover their initial structure. In this case, the drug to be incorporated is dissolved in the solution used for rehydration of LDO and incorporated during this step. This unique LDH-LDO-LDH structural conversion is based on the so called “*memory effect*” of these exciting materials. While the first two methods do not allow to reach the maximum loading degree due to the presence of the inorganic anions ( $Cl^-$ ,  $NO_3^-$  and or  $CO_3^{2-}$ ), the reconstruction occurring in the absence of those inorganic anions has all the premises to incorporate the highest amount of drug that the gallery space *in tandem* with the amount of  $M^{3+}$  cation can accommodate.

### Originality

In this work, we studied the ability of a  $MgOAl_2O_3$ -LDO obtained from the corresponding  $MgA$ -LDH to accommodate fluorouracil (FU). FU is a heterocyclic aromatic organic compound with a structure similar to that of the pyrimidine molecule and is one of the most used anticancer drug for a large pallet of malign tumors [5]. Herein, we were particularly interested in seeing how the rehydration time impacts both (i) the degree of crystallinity and thus the recover of the initial order of the materials and (ii) the amount of FU sealed off between the reorganized layers. Also, how the drug molecule is placed between the octahedral layers, as an indication of the amount of drug that can be loaded in the LDH, was also in our area of interest.

### Experimental

**Synthesis Step 1:** The starting material,  $MgAl$ -LDH with  $Mg/Al$  molar ratio of 3/1 was synthesized by coprecipitation under low supersaturation method at 25 °C. A solution containing the metal nitrates (1 M) and an alkaline solution ( $[NaOH] = 2$  M) were simultaneously added in an automated double jacked reactor from Syrris, which allowed to carefully control the temperature, pH and stirring. The suspension was vigorously stirred and maintained at the desired pH (~10) by adjusting the flow rates of the solutions. The final suspension was aged under stirring at 90 °C for 14 h. The white precipitate was filtered, washed with deionized water and then dried at 80 °C for 24 h. The sample was denoted as  $NO_3$ -LDH. **Step 2:** To obtain  $MgOAl_2O_3$ -LDO, the obtained  $MgAl$ -LDH was calcined at 400 °C with a

rate of 1 °C.min<sup>-1</sup> for 6 h under stagnant air in an oven. The sample was denoted as LDO. **Step 3: Reconstruction of MgAl-LDH from MgOAl<sub>2</sub>O<sub>3</sub>-LDO** while incorporating FU between the layers was performed by using an Al/FU molar ratio of 1.6. The rehydration was performed in a round bottom flask and using decarbonated water. The suspension (pH ~8) was gently stirred at 25 °C for 24 and 72 h. The samples are denoted as FU-LDH-24 and FU-LDH-72 for a treatment performed for 24 and 72 h, respectively. **Physico-chemical characterization** The samples were characterized by XRD, N<sub>2</sub> physisorption, and FTIR in order to assess the structure, texture, and interlayer composition. In order to evaluate the amount of FU in the sample (loading degree), both samples were dissolved in strongly acidic media (HCl + HNO<sub>3</sub>) aiming at returning the cations into solution while releasing the entire amount of FU previously enclosed in the solid. The concentration of was determined by the UV-Vis spectrometry based on the typical absorption of FU at 266 nm and using a calibration curve.

### Results and discussion

Figure 2 illustrates the XRD patterns of all samples, that is, original LDH, the calcined form and the organic-inorganic LDH hybrids reconstructed based on the “memory effect”.

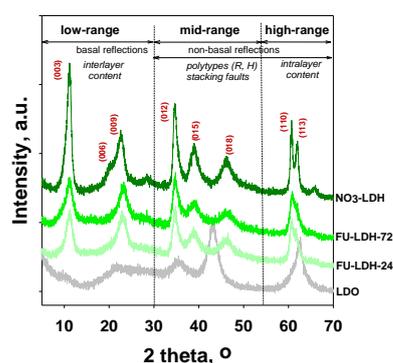


Figure 2. XRD patterns of samples.

The XRD pattern of NO<sub>3</sub>-LDH displays the typical reflections, that is, basal reflections ((003) and 006)) and non-basal reflection ((012), (015), (018), (110) and (113)) typical for a LDH material. Moreover, the shape of the peaks plus the well-shaped (110) and (113) reflections clearly show a high-quality crystalline material. The thermal treatment at 400 °C for 6 h was enough to completely break the high order of the materials, effect reinforced by the disappearance of the LDH reflection and appearance of the typical reflection of the mixed oxides in the pattern of LDO. The rehydration of the oxides-based sample in the presence of FU solution for 24 and 72 h led indeed to the reorganization of the samples in the 2D structural arrangement of LDH. Yet, the crystallinity is much lower as compared to the parent material, but even high enough according to the shape of the peaks. Interestingly, the position of the first basal reflection is not shifted at lower values of 2 theta, but it is placed at almost the same value as in the case of initial LDH for both samples. Because the

reconstruction took place in the presence of FU and not other anions were in the solution, it is hypothesized that the FU molecule was taken by the reconstructing layers and placed in a flat position instead of perpendicular against to the layers. Indeed, the FTIR spectra show clear evidence of the presence of FU inside the samples mainly based on the vibrations at 1654, 1246 and 807 cm<sup>-1</sup> belonging to  $\nu$ C<sup>4</sup>=O,  $\nu$ C-F and  $\delta$ N<sub>1</sub>-H, respectively. No clear indication on the amount of FU in the sample was provided by the FTIR spectra. The evaluation of the amount of FU by UV-Vis in the solution obtained upon acidic dissolution of solid samples provided a loading degree of 12.36 and 10.50% for FU-LDH-24 and FU-LDH-72, respectively. Comparing these values with that obtained for FU included in the same parent NO<sub>3</sub>-LDH by anion exchange, which was of only 3.52%, the improvement by reconstruction approach is obvious.

### Conclusion

To sum up, the LDH-LDO-LDH structural conversion led to materials of reasonable quality in terms of crystallinity irrespective of rehydration time when compared to the parent material obtained by coprecipitation. On the other hand, the results revealed that the amount of FU was slightly influenced by the time of treatment, longer time decreasing the loading degree by ~2%. However, irrespective of the amount incorporated, the FU occupied a parallel position against the brucite-like sheets in the interlayer space, which does not allow to additionally increasing the amount of FU as a perpendicular position would entail. Yet, when compared with the anionic exchange, it can be stated that indeed reconstruction proved to be a much more efficient approach in terms of FU amount and efficient exploitation of the interlayer gallery.

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