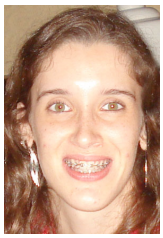
P-103	**Layered Double Hydroxide as a carrier of an anti-inflammatory drug.**	

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INTRODUCTION AND OBJECTIVES

The entrapment of drugs into layered inorganic nanoparticles can endorse the sustained release and minimize the usually side effects produced by them. Besides, inorganic framework can protect these molecules against degradation processes promoted by light, heat, molecular oxygen etc. and extend their shelf life. Layered Double Hydroxides (LDHs), also known as hydrotalcite like-compounds, are good candidate to carry pharmaceutical substances (Cunha 2010) for oral applications since they are common excipients or active principles in antacid products (Gordijo 2005).

In this work, the Mg,Al-LDH carrier containing the anionic form of mefenamic acid (Mef), a non-steroidal anti-inflammatory drug (abbreviated LDH-Mef) was characterized by chemical analysis, powder X-ray diffraction (PXRD), vibrational spectroscopy (FT-Raman) and thermal analysis (TGA-DTG).

The pharmacological potential of LDH-Mef compared to mefenamic acid and the LDH-Cl (with chloride ions intercalated) was evaluated by hemolytic and anti-inflammatory activity assays.

MATERIALS *AND METHODS*

Synthesis of LDHs materials

The LDHs materials (LDH-Mef and LDH-Cl) were synthesized by co-precipitation method (Constantino 1995). The hybrid LDH-Mef was obtained using the molar ratio $Mg^{2+}/Al^{3+} = 2$ and the molar ratio Mefenamate/$Al^{3+} = 1$.

Anti-inflammatory effect: writhing test

Male Swiss mice (25-30 g, n=6/group approved by Institutional Animal Care and Use Committee/UNICAMP) were treated by oral route with NaCl 0.9% solution (control), Mef and LDH-Mef (50, 100, 200 mg/kg), before the intraperitoneal injection of a solution 0.6 % of acetic acid (0.1 mL/10 g). The anti-inflammatory activity was evaluated by the number of writhings during 20 minutes (Koster 1959).

Cytotoxicity assays: evaluation of hemolytic effect

Human red blood cells (approved by Ethics on Human Research Committee, UNICAMP, 0.15 % hematocrit) were treated with LDH-Mef and LDH-Cl (1 to 90 mM, at 37 ºC, 15 min). After supernatant separation, the amount of hemoglobin released (412 nm) was expressed as the percent of hemolysis, as follows: hemolysis %= (Aa – Ac_1/ Ac_2 - Ac_1) x 100, where Aa is the absorbance of sample, $A_{PBSbuffer}$ is the absorbance of c_1, A_{Water} is the absorbance of c_2 (Malheiros 2004).

RESULTS AND DISCUSSION

The PXRD pattern (Figure 1) of LDH-Mef shows the displacement for low angle (2θ) of the peak (003) related to the basal spacing, evidencing the intercalation of the organic species. The d_{003} value (2.06 nm) suggests a bylayer arrangement of the guest anions in the interlayer space. The chemical composition of LDH-Mef, $[Mg_2Al(OH)_6](C_{15}H_{14}NO_2)_{0.55}Cl_{0.45}\cdot 2H_2O$, was proposed according to elemental analysis (CHN) data, metals contents and the percentage of H_2O (obtained from TGA curve). The amount of the organic species present in 100 g of LDH-Mef is 33 g.

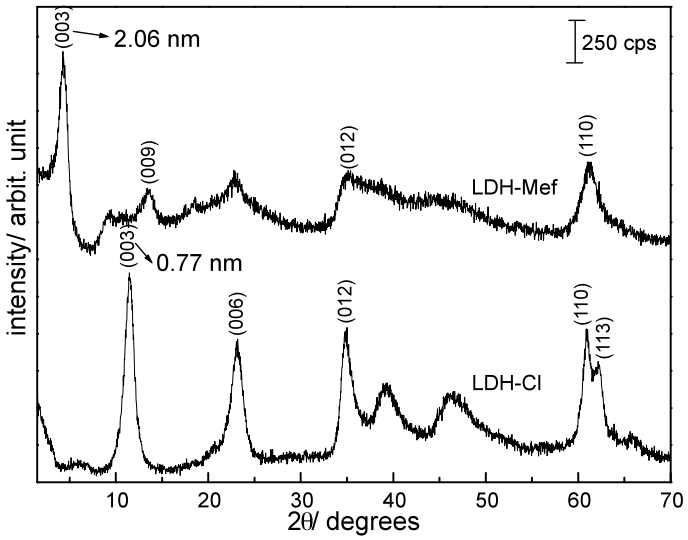

Figure 1: PXRD patterns of LDH-Mef and LDH-Cl.

Compared to the mefenamate sodium salt, the FT-Raman spectrum of hybrid material shows the characteristic bands of deprotoned mefenamic acid, *i.e.*, the presence of the mefenamate anion: ν_{as} COO^- at 1582 cm^{-1} and ν_s COO^- at 1383 cm^{-1}(Figure 2).

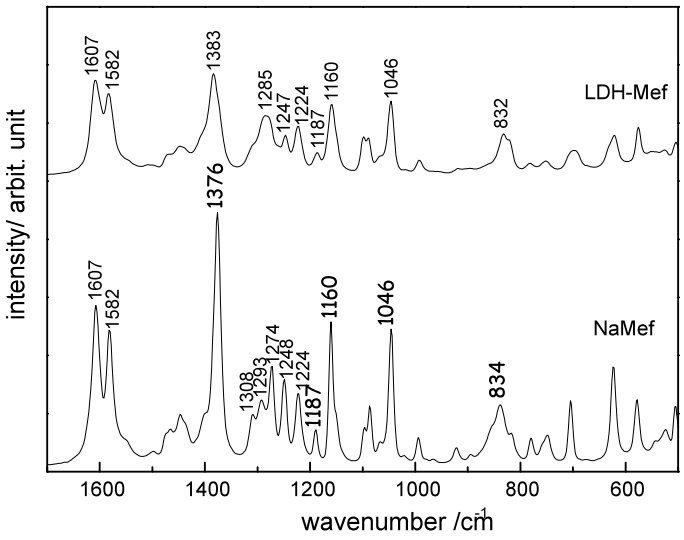

Figure 2: FT-Raman spectra of LDH-Mef and NaMef.

Thermal analysis data indicate that intercalated mefenamate thermal stability is slightly higher than the non-intercalated species. Sodium mefenamate decomposes at 260 °C while LDH-Mef sample is decomposed at about 275 °C.

In vitro cytotoxicity assays revealed that the concentration for the onset of hemolysis (C_{onset}) for Mef, LDH-Cl and LDH-Mef was 0.48 mM, 1.66 mM and 1.83 mM, respectively. The results indicated that the entrapment of Mef in LDH nanoparticles, impaired the hemolytic effects of Mef, as observed by the higher hemolytic concentrations registered for LDH-Mef association (Fig 3).

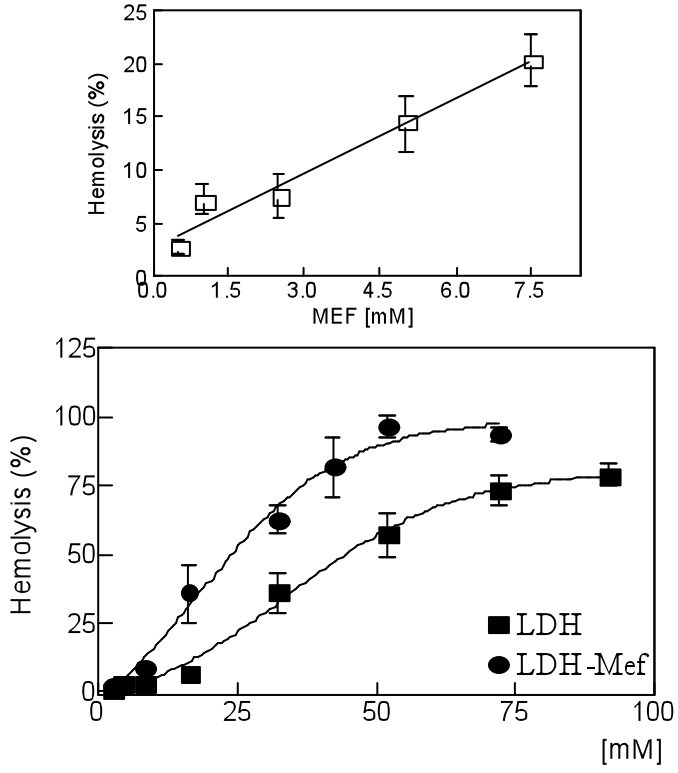

Figure 3: Hemolytic effects of Mef, LDH-Cl and LDH-Mef on human erythrocytes (Hematocrit 0.15%, pH 7.4, 37 °C, n = 4-6, mean ±SD).

Pharmacological evaluation (Figure 4) showed that the treatment with LDH-Mef nanoparticles reduced the number of writhings, enhancing the anti-inflammatory effects of Mef when compared to the non intercalated Mef, at three different evaluated doses ($p < 0.001$ for 50 or 100 mg/kg and $p < 0.01$ for 200 mg/kg).

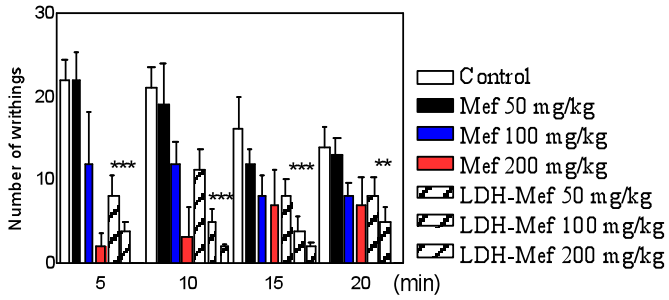

Figure 4: Anti-inflammatory effects of Mef, and LDH-Mef at 50, 100 and 200 mg/kg (n=6/group).

CONCLUSIONS

The results showed in this study demonstrate the entrapment of mefenamate anion in layered double hydroxides. In addition, in vitro cytotoxicity assays and pharmacological evaluation showed reduced hemolytic effects and increased anti-inflammatory activity, presenting it as an effective novel approach to enhance the pharmacological effects of mefenamic acid.

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