

## **Supplementary Material**

### **Anti-inflammatory and Analgesic evaluation of a phytochemical intercalated into Layered Double Hydroxide**

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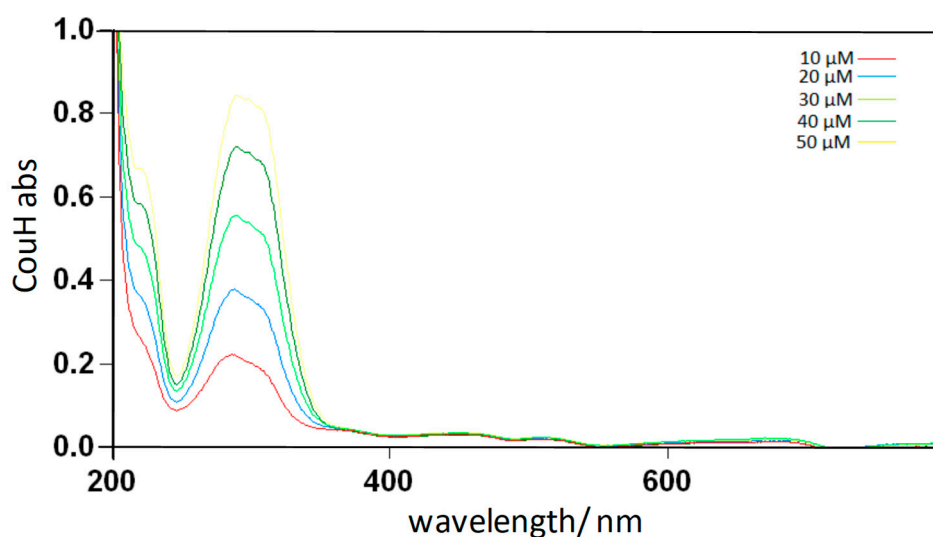
## 1. Materials and Methods

### 1.1. Reagents

*p*-coumaric acid ( $C_9H_8O_3$ , Sigma), aluminum chloride hexahydrate ( $AlCl_3 \cdot 6H_2O$ , Aldrich), zinc chloride ( $ZnCl_2$ , Aldrich), sodium hydroxide (NaOH, Merck), caffeine (Sigma-Aldrich), naloxone (Sigma-Aldrich), penicillin (Nutricell), neutral red (Sigma-Aldrich), phosphate buffer saline (PBS), Turk's solution, and Dulbecco's Modified Eagle Medium (DMEM, Nutricell) were used without further purification.

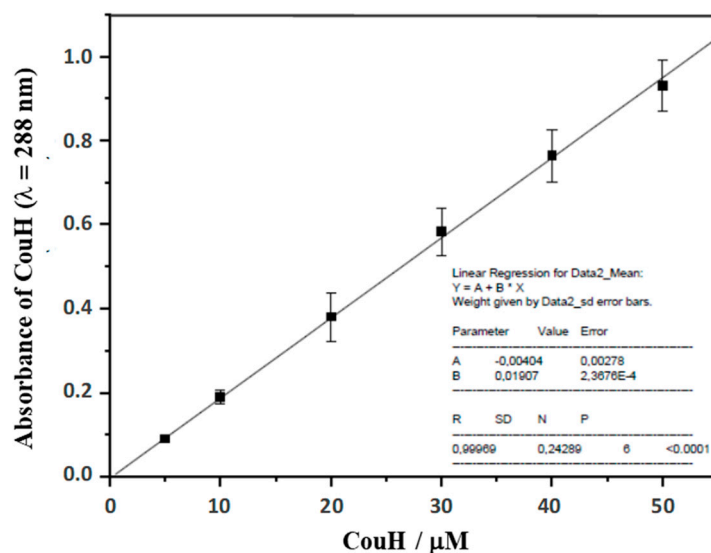
### 1.2. Spectrophotometric determination of *p*-coumaric acid concentration

The wavelength of maximum coumaric acid absorption in sodium chloride aqueous solution (NaCl 0.9 %), the UV-VIS spectra of CouH solution concentration from 10 to 50  $\mu\text{mol L}^{-1}$  were recorded in the 200 to 500 nm range, using a cuvette of 1 cm of optical pathway (Figure S1). The spectra were recorded in a spectrophotometer UV-VIS (Varian Cary 50 / Bio-Tek ELx 800) and values were analyzed by Graph Pad Software, Inc., USA and the Beer's Law was applied.



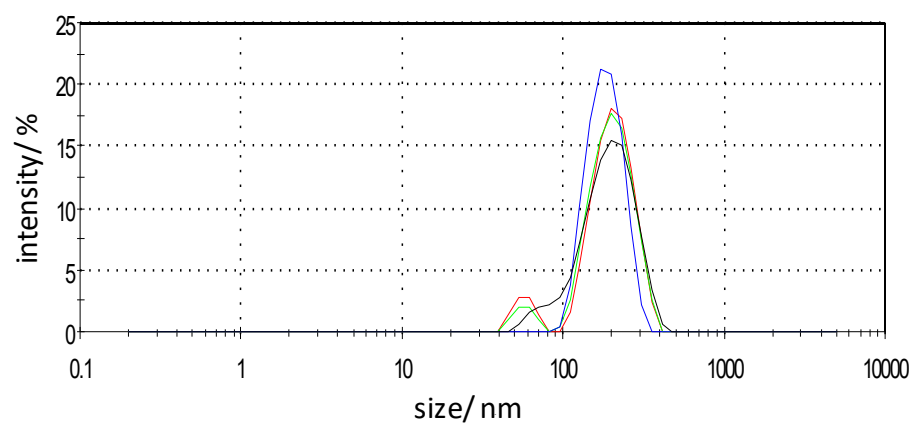
**Figure S1.** UV-VIS spectra of coumaric acid in different concentrations in sodium chloride aqueous solution (0.9 % NaCl).

The calibration curve obtained at 288 nm, Figure S2, provided the molar absorptivity of coumaric acid,  $1907 \text{ L mol}^{-1} \text{ cm}^{-1}$  with correlation coefficient ( $R^2$ ) of 0.99969 ( $y = -0.00404 + 0.01907x$ ) and it was used to determine the concentration of coumarate delivery in time, as well, to calculate the delivery constant.



**Figure S2.** Calibration curve of coumaric acid obtained by UV-VIS spectrophotometry at different concentrations ( $n=6$ ).

Zeta potential and particle size were measured with Zetasizer NanoZS, Malvern Instruments, Worcestershire, UK. The mean hydrodynamic diameter of the suspension of LDH-Cou obtained by Dynamic Light Scattering (DLS) is 226 nm (Pdl: 0.419). It can be noticed the slight particle size variation (Figure S3), obtaining a mono-modal system with a similar particle size population. Moreover, LDH-Cou shows low tendency towards agglomeration since its zeta potential is + 39 mV, which indicates a good stability of the aqueous dispersion of the material.



**Figure S3.** Particle size distribution of LDH-Cou sample.