KINETICS OF RELEASE OF CHLORAMPHENICOL INTERCALATED IN HYDROTALCITE

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Abstract: This paper reports the kinetic study of the release of chloramphenicol (CFN) intercalated in hydrotalcite. The release of CFN anion from the lamella of an organic-inorganic nanohybrid material, MgAl LDH_CFN nanocomposite, was found to be pH dependent. The release of CFN from the lamella of hydrotalcites at pH 2.0 was controlled by first-order kinetics. Microbiological tests with *Staphylococcus Epidermidis* demonstrated an antimicrobial activity for a period of 48 h.

Keywords: *hydrotalcite, layered double hydroxides (LDH), chloramphenicol hemisuccinate, controlled release, antimicrobial activity*

INTRODUCTION

Chloramphenicol (CFN) is a synthetic antibiotic with a large spectrum against both gram positive and gram negative germs. CFN hemisuccinate is water-soluble and therefore can be administered intramuscularly and intravenously. It is quickly absorbed in the body, where it undergoes a hydrolysis process (especially in the liver) which releases active chloramphenicol. It is eliminated mostly through the kidneys in its biological active form. A great disadvantage of this antibiotic is given by its high toxicity. The main toxic effect of CFN is its attack on the bone marrow, which can take

two forms. The first is reversible and dosage-dependent and it occurs when the plasmatic CFN concentration rises above 25-35 μg/mL. It consists in morphological changes of the marrow. The second, aplastic anemia (a severe toxic condition) is irreversible and fatal and occurs rarely (1 in 20,000 patients), when the dosage is well above 35 μg/mL of plasmatic CFN. For the purpose of improving the administration form and for reducing the toxicity of this powerful antibiotic we previously bound CFN covalently to xanthan (using the dicyclohexylcarbodiimide method) and ionic of chitosan [1-3] and evaluated the kinetic and pharmacologic characteristics of the new compounds [3-5]. In the present work we evaluate in vitro and in vivo behavior of CFN hemisuccinate intercalated in hydrotalcite. Previous reports regarding the characterization and pharmacological behavior of hydrotalcites with intercalated drugs like indomethacine, ibuprofen, diclophenac, gemfybrozil, salicylic acid or naproxen were found in the literature [6-9].

EXPERIMENTAL

Materials and method

For this study we used pharmaceutical grade CFN hemisuccinate. The intercalation of the CFN hemisuccinate was done by direct synthesis using a co-precipitation method [10]. The samples which were studied have the following molar ratios: MgAl LDH_CFN1 (Mg:Al = 2:1) and MgAl LDH_CFN2 (Mg:Al = 8:1). They have also been characterized by DRX, FTIR, thermogravimetry and elemental analysis [1]. The results confirm the intercalation of CFN in hydrotalcites. The nitrogen content (%) in the CFN intercalated products was determined by elemental analysis. These results were used to calculate the amount of drug bound to the support. In Table 1 the data regarding the samples used in this study and their content of intercalated CFN are presented.

Table 1. *Content and type of the studied sample*

Sample	Chloramphenicol (%)
MgAl LDH_CFN1 (s.d 2:1)	7.3
MgAl LDH_CFN2 (s.d 8:1)	21.0

Buffering effect and CFN release study

The buffering effect of MgAL LDH and MgAl LDH_CFN was studied by adding 100 mg of the materials, separately into 100 mL aqueous solutions at various pH values, under continuous stirring at 37^{o}C. The eluents used were: HCl acid solution (pH 2.0) and KH_2PO_4 / Na_2HPO_4 buffers (pH 5.5, 7.1 and 8.0 respectively). The sample, appearing as a fine powder, was introduced into the eluent and stirred continuously for 48 h. The pH values were monitored with an instrument connected to a personal computer. The pH values were recorded every minute. For quantitative determination of CFN release, 2 mL aliquots were extracted from solution and their absorbance was measured. Afterwards, 2 mL eluent was re-introduced into the release medium. The amount of released CFN was determined by measuring the solution absorbency.

Analysis of CFN

The amount of CFN released from MgAL LDH_CFN was evaluated using a spectrophotometric method. A calibration curve was first obtained from the study of six aqueous solutions of CFN, with concentrations in the range of 5 to 30 μg/mL and six solutions with concentrations between 20 and 70 μg/mL. All solutions were prepared by diluting a standard solution with the concentration of 0.1 g/dL. The absorbance was measured at 280 nm using a spectrophotometer with a 1 cm or 0.5 cm thick quartz cell, depending on the sample concentration. The calibration curves are plotted in Figures 1 and 2. In all cases the experimental points were found on the same straight line, with an error of less than 1%. The equations of these curves were obtained by the least - square method and are shown below: A = 0.0153 x C (μg/mL) in the range 5-30 μg/mL and A = 0.0174 x C (μg/mL), in the range 20-70 μg/mL, (C - concentration of the CFN solution, A - absorbance). They can be used for direct determination of drug concentration.

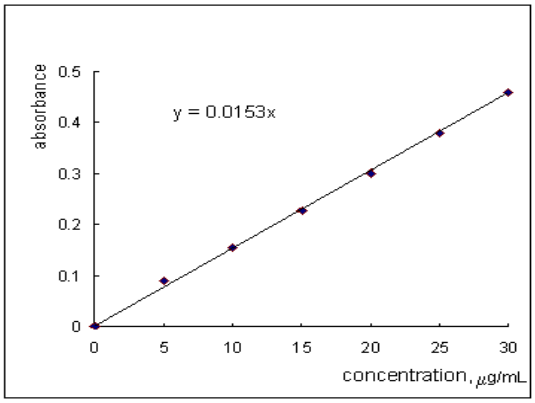

Figure 1. *Calibration curve for chloramphenicol in the range 5-30* μg/mL

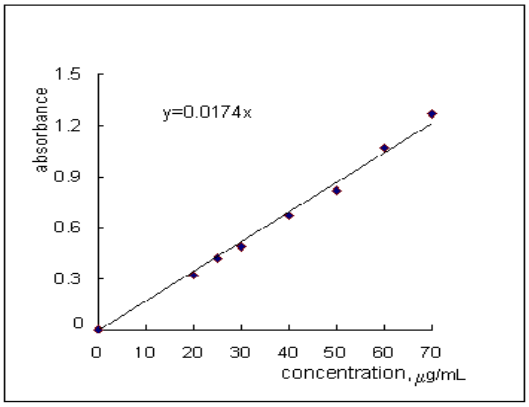

Figure 2. *Calibration curve for chloramphenicol in the range 20-70* μg/mL

RESULTS AND DISCUSSION

In the case of the MgAl LDH_CFN2 sample with a high content of intercalated CFN (21.0%), (Figure 3), in an acidic medium (pH 2.0) the pH of the solution rises due to the consumption of H^+ ions by the CFN hemisuccinate anions and the release of the HO^- ions from the compound. For the MgAl LDH_CFN1 sample, which contains 7.3% of intercalated CFN, the pH variation is insignificant due to the fact that the amount of CFN is less and the distance between the layers of the compound is smaller, therefore it does not allow the release of the HO^- ions (present in all the samples as shown by FTIR spectroscopy) [11]. For MgAl LDH the variation of the pH is relatively small, probably due to the absorption of the H^+ on the particles' surface. At a pH of 5.5 (Figure 4) the changes in the ion content of the releasing systems is insignificant for all the samples. The ionic strength of the environment does not allow the release of CFN or HO^-.

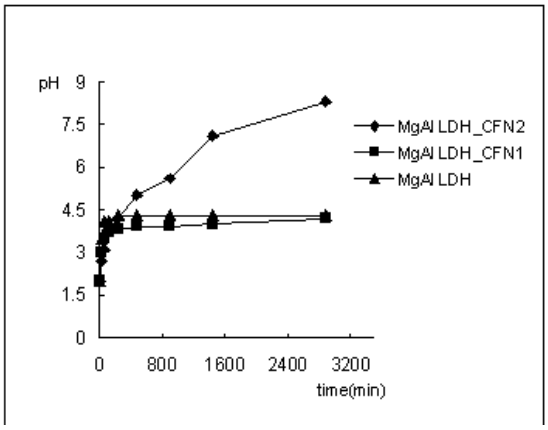

Figure 3. *pH profile of the release media, (pH = 2) for MgAl LDH_CFN2, MgAl LDH_CFN1 and MgAl LDH*

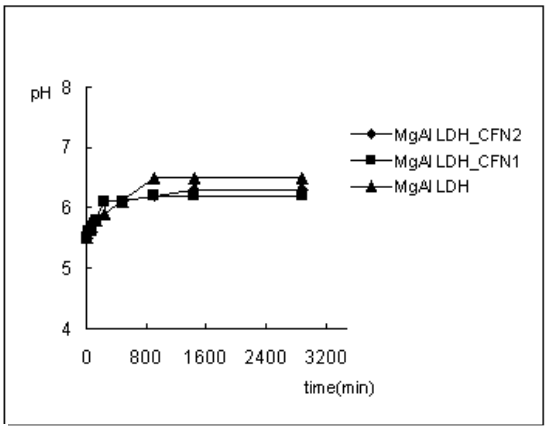

Figure 4. *pH profile of the release media, (pH = 5.5) for MgAl LDH_CFN2, MgAl LDH CFN1 and MgAl LDH*

At a pH of 7.1 and 8.0 (Figure 5 and Figure 6) the changes are significant for the sample with a high content of CFN, in which the distance between the layer is wider, allowing the release of HO^-; meanwhile, in the case of the low CFN content sample and the MgAl LDH, where the variation of the pH is significant.

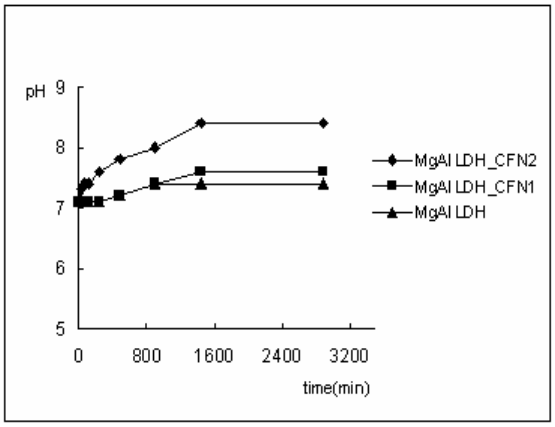

Figure 5. *pH profile of the release media, (pH = 7.1) for MgAl LDH_CFN2, MgAl LDH_CFN1 and MgAl LDH*

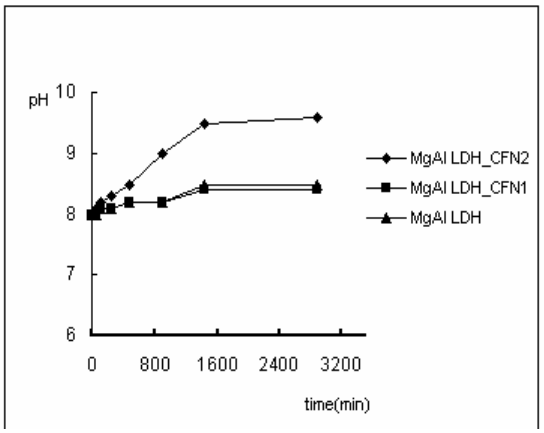

Figure 6. *pH profile of the release media, (pH = 8.0) for MgAl LDH_CFN2, MgAl LDH_CFN1 and MgAl LDH*

The release of intercalated CFN in a strong acid medium (pH 2.0) has been verified by quantitative determination of CFN in aqueous solutions by a spectrophotometric method. In the case of the samples in which the release occurred at a pH of 5.5, 7.1 and 8.0 there was no detection of CFN in the solutions after 48 hours of elution. Instead, for MgAl LDH_CFN1 the recorded data show a CFN release for a period of 250 minutes and for MgAl LDH_CFN2 the CFN release during 400 minutes (Figure 7).
The amount of CFN released from the samples was calculated as:

$$m_{CFN} = c_n \cdot V_e + \sum_{i=0}^{n-1} c_i \cdot V_i \qquad (1)$$

where: m_{CFN} is the amount of CFN released from the sample at a certain time (μg); c_d is the concentration of drug in the extracted sample (μg/mL); V_e is the volume of eluent from the system (100 mL); V_i is the volume of extracted sample, *i,* (2 mL); and c_i is the concentration of CFN in the extracted sample, *i*, (μg/mL) determined spectophotometrically.

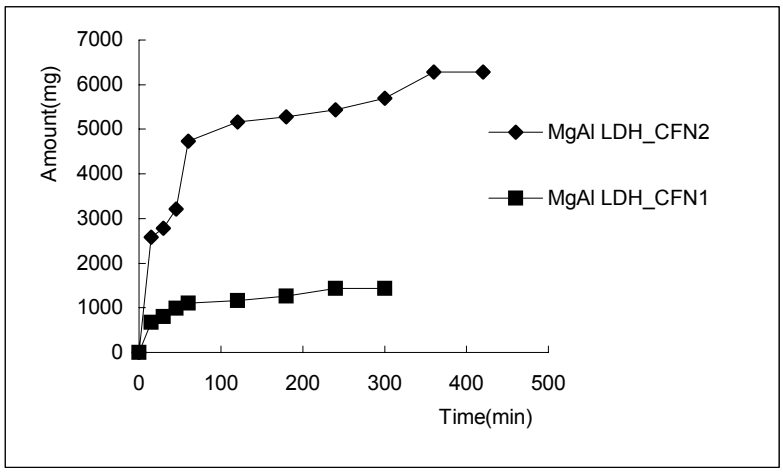

Figure 7. *Variation of the amount of CFN released, with time for MgAl LDH_CFN2 and MgAl LDH_CFN1 at pH 2.0*

The general aspect of the CFN release from the MgAl LDH_CFN2 and MgAl LDH_CFN1 is presented in Figure 8. The CFN release profiles suggested that the first-order kinetic equation could be used to describe the release behavior, equation 2.

$$\ln\left[1-\frac{m_{CFN}}{m_f}\right]=k\cdot t \qquad (2)$$

in which m_{CFN} is the amount of CFN released from the sample at a certain time (μg) and m_f is the final amount of CFN released (μg), t-time, k- constant of rate.

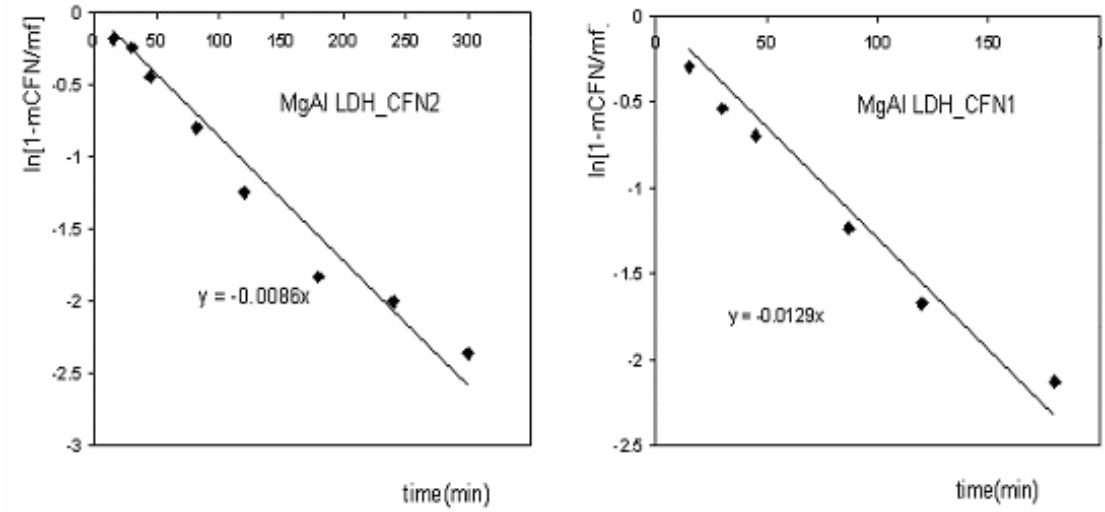

Figure 8. *Fitting of CFN release data into the aqueous solution with first-order kinetics at pH 2.0*

An attempt has been made to fit the data obtained from CFN released into the aqueous solution (pH = 2.0) to the first-order kinetic and the result obtained is shown in Figure 8. Antimicrobial activity of samples LDH, CFN, LDH-CFN1 and LDH-CFN2 was determined by their depositing on plates inoculated with *Staphylococcus Epidermidis* and measuring the diameter of the inhibition region in time, Figure 9.

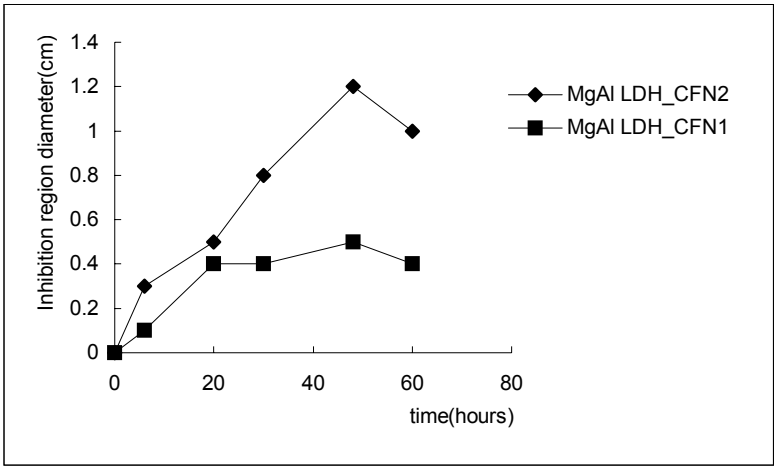

Figure 9. Variation of the diameter of the inhibition region with time

The plain hydrotalcite, MgAl LDH showed no sign of antimicrobial activity. For MgAl LDH_CFN1 and for MgAl LDH_CFN2 the diameter of the inhibition region increased up to 48 h and then decreased. The maximum values were 0.5 cm for MgAl LDH_CFN1 and respectively 1.2 cm for MgAl LDH_CFN2.

CONCLUSIONS

The release of CFN anion from the lamella of an organic-inorganic nanohybrid material, MgAl LDH_CFN nanocomposite can be controlled by adjusting the pH of the release medium in aqueous solution. The release rate of the anion was found to be dependent on the pH of the release medium. The CFN released from the samples was determined and quantitatively measured by a spectrophotometric method.
The release of the CFN intercalated in hydrotalcites takes place in a strong acid environment (pH = 2.0), similar to the pH of the gastric juice. At pH values of 5.1, 7.2 and 8.0, which can be found in the gastro-intestinal tract, CFN cannot be released.
It was found that the release of CFN anions from the lamella of MgAl LDH_CFN at pH 2.0 was controlled by first-order kinetics.
Microbiological tests of samples with CFN demonstrated an antimicrobial activity for a period of 48 h.

ACKNOWLEDGEMENTS

Funding for this research was provided by the Ministry of Research and Education, under the CNCSIS Grant 545.

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