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Physical - Chemical Issues of Cephalosporin Intercalated Nanoparticles for Life - Threatening Infections Treatment

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Abstract: *The fourth generation cephalosporin antibacterial agent, cefepime, was loaded into layered double hydroxides for enhancing antibiotic efficiency, reducing side effects, as well as achieving the sustained release property. The intercalation of antibiotic into the inter-gallery of ZnAl-layered double hydroxide (LDH) was carried out using ion exchange method, by this constituting a nano-sized organic-inorganic hybrid material for a controlled release novel formulation. Although cefepime is a broad spectrum antibiotic, it has various adverse effects and a significant degradation rate. Thus, the preparation and physico-chemical characterization of nanomaterials able to intercalate this drug is an important study for medical and pharmaceutical field. The antibiotic inclusion into LDHs nanostructure was confirmed by advanced characterization techniques and the release profile of cefepime was analysed with the respect to pH of the simulated media.*

Keywords*: nanomaterials, cephalosporin, epidemiology, neurosurgery, paediatrics surgery/pediatric disorders, pulmonology, obstetrics/gynaecology. drug release*

1. Introduction

In last few years, antibiotic administration mechanisms were intensively studied by word wide researchers, especially prolonged release of the bioactive molecules from delivery systems at a proper rhythm in order to enhance antibiotic efficiency. These carriers major goal is to maintain the antibiotic concentration in target area at the best levels for longer time and to limit eventual adverse effects [1-4].

Nowadays, nanohybrids based layered double hydroxides (LDHs) are widely used in medical field as drug delivery systems because these nanomaterials are able to protect active biomolecules from decomposition thus making them useful in sustained drug delivery. LDHs also called anionic clays gained attention due to their diverse properties and important usage in many fields especially medicine. These nanostructures consist of positive charged brucite-like sheets counterbalanced by exchangeable interlayers anions being expressed by general formula $[MII1-xMIIIx(OH)2]x+(An-)x/n\cdot mH2O$, where MII and MIII are di- and trivalent metal cations, An- are interlayer anions of

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valence n, x value represent the molar ratio MIII/(MII + MIII) and m represent the quantity of water (in moles). Due to their anion exchange property, these nano-sized architectures can form organic/bio-inorganic nanohybrids where LDHs represent the matrix for a wide variety of antibiotics and other drugs [5-15].

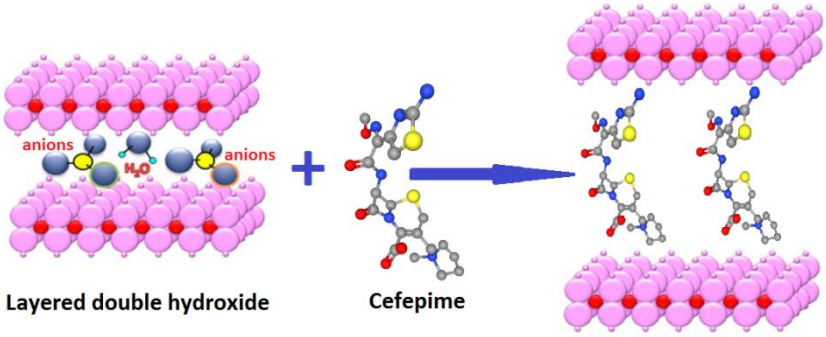

Figure 1. Schematic representation of nanohybrids

Cephalosporin, an important class of antibiotics is of the major importance in pharmaceutical medicine for diseases treatment caused by infectious agents by preventing or by limiting bacteria multiplication. Among these antibiotics, cefepime is a fourth generation cephalosporin with a broad spectrum of activity against dangerous pathogens [16-24]. One of the prime concerns in medical area is multi-drug resistant pathogens that cause severe infections.

Novel formulation based on antibiotic intercalated layered double hydroxides (Figure 1) brought worldwide attention given the serious healthcare conditions. These nanohybrids based on cefepime intercalated layered double hydroxides are used in various medical specialties such as cardiology, pulmonology, paediatrics, paediatrics surgery, neurosurgery, obstetrics/gynecology and epidemiology for the prophylaxis and treatment of several serious infections caused by different bacteria. Due to all these reasons, cefepime was loaded into interlayer space, structurally and morphologically characterized along with antibiotic release behavior in order to study the ability of layered double hydroxides for antibiotic formulation of the administration [25-33].

2. Materials and methods

ZnAl-LDHs synthesis

Preparation of pristine ZnAl-LDHs was accomplished using a 200 ml of solution containing metal salts and a solution containing 1M NaOH added drop wise to a 200 ml deionized water under vigorous stirring and nitrogen gas flow for maintaining the pH to 9.0±0.5 until the end of the procedure. The obtained suspension was aged for 24 h at room temperature, then centrifugated at 6000 rpm for 20 minutes, washed with deionized water and stored as a suspension to avoid aggregation of the crystals, thus obtaining small-sized particles.

Antibiotic intercalation

Cefepime was loaded into LDH structure by anion exchange method which consists of mixing antibiotic aqueous solution and the as obtained LDH suspension. The synthesized product was collected after centrifugation, washed with deionized water, filtered under vacuum and finally dried at 40°C in air flow. The obtained samples were symbolized as LDH and C-LDH.

The structural and textural properties were confirmed by powder X-ray diffraction (XRD), Fourier-transform infrared spectroscopic (FTIR) analyses and Scanning electron microscopy (SEM). Moreover, thermo gravimetric analysis (TGA) was carried out with a heating rate of 5°C/min from room temperature up to 1.000°C under nitrogen and the release profile was investigated by using UV–VIS spectrophotometry.

3. Results and discussions

Structural feature of novel formulation cefepime-layered double hydroxide (C-LDH) was confirmed by XRD patterns as shown in figure 2. The pristine LDH revealed a (003) crystalline peak at 11.5 2θ degree that matches to a basal spacing of 7.7Å being assigned to nitrate anions between the layers. After incorporation of cefepime into the interlayer space of LDH, all specific peaks of LDHs are noticed in XRD pattern of C-LDHs nanohybrid.

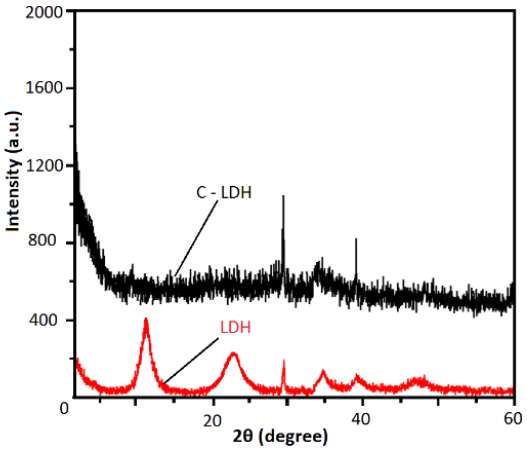

Figure 2. XRD patterns of LDH and C-LDH nanohybrid

It can be observed that (003) crystalline peak of LDH is modified to 3.8 2θ degree in nanohybrid structure indicating the enlargement in the basal spacing of LDH from 7.7Å to 22.7Å, thus intercalation of cefepime into LDH.

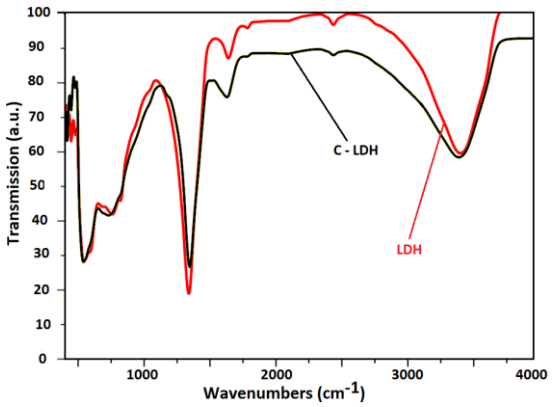

Figure 3. FTIR spectra of pristine LDH and C-LDH nanohybrid

FTIR spectra of LDH and C-LDH samples are presented in figure 3. Simple LDH spectrum reveals a broad absorption band at 3400 cm^{-1} typically for LDHs nanomaterials which can be attributed to the hydroxide groups stretching vibration from interlayer space and intercalated water molecules. The absorption band around 3600 cm^{-1} is assigned to hydrogen bonds formed between water molecules present into interlayer gallery and the –OH group of LDH sheets. LDH sample present a strong peak around 1340 cm^{-1} due to nitrate stretching vibration and a weak band at 1640 cm^{-1} attributed to water molecules stretching vibration. The absorption spectrum between 400 and 800 cm^{-1} correspond to metal oxide and metal hydroxyl bonds in the layers confirming the LDH structure.

C-LDH nanohybrid spectrum shows an absorption band around 3390 cm^{-1} characteristic to –OH group and the appearance of absorption bands bellow 1000 cm-1 reveals that the layered structure of

LDH was not altered during antibiotic intercalation action. Moreover, absorption bands appeared at around 1630 and 1450 cm^{-1} are related to both carboxylate symmetric and asymmetric stretching vibration. Taking all these issues into account, cefepime was included and stabilized in the interlayer gallery of LDH.

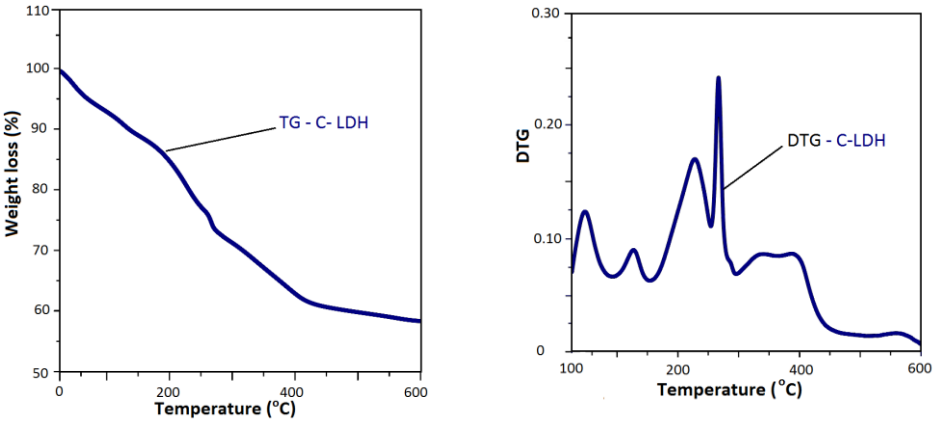

Figure 4. Thermo-gravimetric (TG) analysis curves and differential thermo-gravimetric (DTG) of nanohybrid C-LDH

From TG analysis it can be observed that are noticeable stages of weight loss in agreement with DTG results, as shown in figure 4. First two steps, from 25 to 160°C the loss of physically absorbed water and water molecules between layers happened are about 7.5 and 3.5% respectively. In addition, in DTG analysis are observed two peaks at 50°C and 130°C. in the interval 160-250°C occurred the third weight loss (almost 12% mass loss) corresponding to a peak in DTG curve at 230°C maybe due to the loss of –OH groups from LDH, of which condensation leads to formation of water vapour.

A sharp peak at 250°C and two peaks at 350°C and 400°C present in DTG diagram are attributed to the combustion of the intercalated cefepime.

Related to TG curve, mass loss took place in three consecutive steps (in 250-450°C interval) leading to the generating of NOx, CO2 and H2O. Cefepime ions from nanohybrid had an increased decomposition temperature than pure antibiotic. The final step at 550°C is caused by the formation of metal oxides and mixed metal oxides conducting to complete calcination of LDH as well as full burn of antibiotic.

Figure 5 present UV-VIS absorption spectra of pure antibiotic and antibiotic intercalated layered double hydroxide nanohybrid. Absorption spectrum of cefepime reveals two characteristic absorption band of the drug structure.

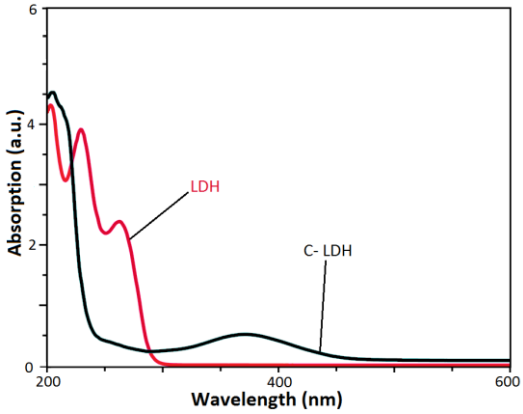

Figure 5. UV–VIS absorption of pristine cefepime and nanohybrid C-LDH

After loading of cefepime in LDH host, the absorption band could not be identified maybe due to the effect of LDH nanoparticles scattering.

The pristine LDH and C-LDH nanohybrid micrographs are presented in Figure 6. Simple layered double hydroxide reveals regular and uniform nanoparticles having a hexagonal plate-like feature while nanohybrid morphology shows irregular and non-uniform aggregates of compact with a microblock-like shape.

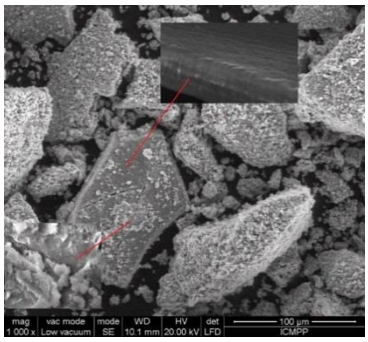

Figure 6. SEM micrographs of LDH and nanohybrid C-LDH

Release profile was performed in different solution media in the absence or presence of Cl- ions. Standard curve according to the absorbance of the drug solution concentration reveals the final quantification of the cefepime. Figure 7 shows release of the antibiotic into buffer solution depending on time referring to strong acidic, mild acidic, neutral and slightly basic.

Under strong acidic conditions simulating gastric fluid, LDH is dissolved fast and completely and the intercalated antibiotic is entirely released in its active molecule but not totally dissolved: 70% of cefepime is dissolved after the procedure is ended.

In blood, intestinal and lysosomal simulated fluid it can be noticed similar release behaviour for cefepime loaded layered double hydroxide. At *p*H 4.0, 6.8 and 7.4, antibiotic is released rapidly in percentage of 65, 50 and 59% respectively

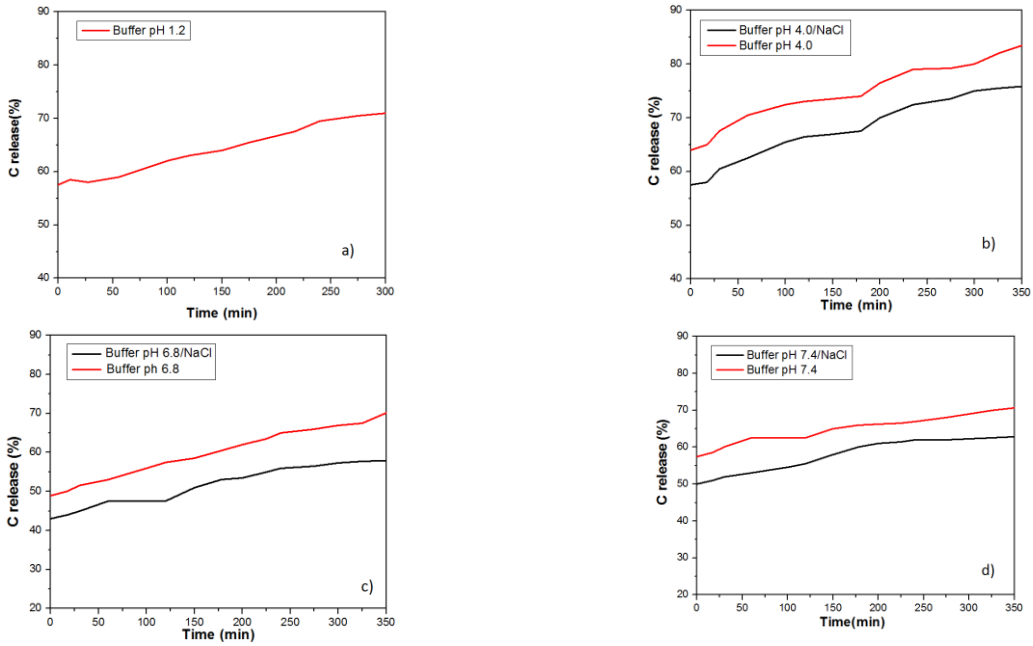

Figure 7. Release profiles of cefepime from the nanohybrid C-LDH at *p*H 1.2 (a), at *p*H 4.0 (b), at *p*H 6.8 (c) and at *p*H 7.4 (d)

After 6 h the release of cefepime is slow and sustained, almost 85% at *p*H 4.0, 70% at *p*H 6.8 and 72% at *p*H 7.4 possibly depending on the deliberate ion exchange between antibiotic from interlayer gallery and the anions present in different fluids of human body.

A general release mechanism is described in Figure 8. For drug delivery carriers, it can be distinguished, regarding drug release from the host, the penetration to the LDH followed by interaction of nanohybrid with the buffer solution ending with the diffusion of the drug into the release medium.

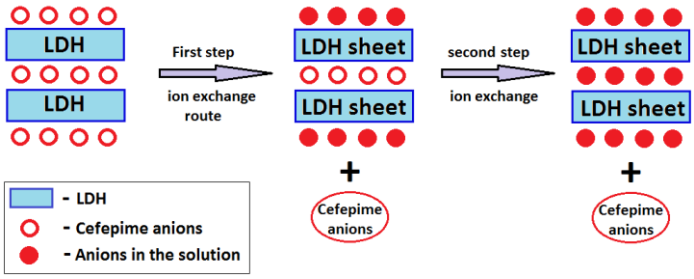

Figure 8. Schematic representation of drug release in buffer solution

Anion exchange process happens between drug anions included in the interlayer space of LDH and the anions existing in the release media.

4. Conclusions

The β-lactam antibiotic, cefepime, was successfully loaded and stabilized into LDHs nanostructure by anion exchange method. Advanced characterization techniques confirmed the presence of the drug molecules into inter-layered space by host-guest interactions. Investigation of antibiotic release at different pH values revealed that cefepime diffusion to the simulated media happened rapidly compared to ion exchange route. The obtained results give the perspective to research new formulations based on antibiotic intercalation into interlayer gallery of LDHs for further uses in serious infections treatment. Furthermore, LDHs is an exquisite biocompatible, inorganic matrix that may enhance the antibacterial efficiency of cefepime with sustained and prolonged release property and reduce side effects of the drug, respectively.

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