

Stabilization of Cefotaxime in Hydrotalcite - Like Anionic Clay Matrix and its Controlled Release

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The incorporation of cefotaxime in the brucite (Mg(OH)₂)-like inorganic network gives rise to a hybrid pharmaceutical formulation that contains both the properties of inorganic clay and the drug in a single material. The structural properties of the drug - clay assembly are described by using the results of X-ray diffraction, FTIR and TGA-DTA analyses. The TEM micrographs give information about the micromorphology features of the hybrid structures pointing out to the exfoliation of the clay layers. The drug content of the new designed pharmaceutical formulation is a function of the characteristics of the synthesis method. The confinement of the drug inside the clay matrix is stable up to 130 °C while its sustained release is a slow process. The results point out to an improved stability of the drug in the hybrid formulation as well as the possibilities to tailor its release behaviour as a function of the synthesis procedure.

Keywords: drug delivery, cefotaxime, drug-clay assembly, controlled release

A significant demand exists nowadays for the advanced delivery of the drugs very sensitive to ambient environments [1]. The incorporation of the drug molecules in a layered inorganic matrix is able to isolate the organic molecules from the environment thereby can improve the drugs long-term stability and storage [2, 3]. Moreover, the intercalation of drugs into biocompatible inorganic materials provides a useful and convenient route to prepare hybrids that contain properties of both the inorganic host structure and the organic guest, in a single material. Among a variety of biocompatible inorganic matrices, layered double hydroxides (LDHs) (also known as hydrotalcite-like anionic clays) are cheap materials with a well organized nanotexture. Represented by the general formula: $[Me^{II}_x Me^{III}_y (OH)_z]^{x+} [A^{m-}_{x/m} \cdot nH_2O]^{x-}$ (Me=metal) these materials are built up of sheets of edge - sharing metal octahedra, where by comparison to brucite $M(OH)_2$, part of Me^{II} is replaced by Me^{III} metal cations; the excess of the positive charge is counterbalanced by exchangeable anions A^{m-} , located as water molecules, in the interlayer space [4]. The large variety of compositions that can be developed by altering the nature of the divalent and trivalent cations (Me^{II} , Me^{III}), the interlayer anions (A^{m-}) and the stoichiometric coefficient (x) afford the incorporation of many biologically important molecules (including genes or drugs [4-7]). The rich intercalation chemistry of these anionic clays, their high affinity to carbonate ion and liability to acid dissolution is able to offer desirable discharge of the loaded drugs as well as compatibility with diverse biosystems [8]; further, the possibility to tailor the magnetic properties of LDHs opens interesting perspectives to use them for targeted drug delivery [9]. Cefotaxime is a third generation cephalosporin antibiotic; despite its high biological activity cefotaxime is less stable than the other cephalosporin derivatives in aqueous solutions due to its photodegradation process [10]. The incorporation of cefotaxime in the biocompatible, inorganic layered structure of LDHs opens new perspectives to improve its stability. In these view this work aims to report the first synthesis and characterization of a new hybrid

nanostructure type cefotaxime - LDHs. Two different methods were used for the hybrids synthesis. Preliminary studies regarding the release of the drug from the clay matrix are also reported.

Experimental part

Samples synthesis

All chemicals were commercially purchased and used without further purification. The parent MgAlLDH was prepared by the pH controlled co-precipitation of the corresponding metal nitrate salts, followed by an ageing step of the synthesis medium, at 45°C for 24 h. Typically, an aqueous solution of 0.075 mol/l $Mg(NO_3)_2 \cdot 6H_2O$ and 0.025 mol/l $Al(NO_3)_3 \cdot 9H_2O$ was added dropwise to 100 mL of deionized water at 65 °C under vigorous magnetic stirring. The pH of the mixture was kept at a constant value equal to 9 ± 0.1 by simultaneous addition of an aqueous solution containing 0.1 mol / l Na_2CO_3 and 0.5 mol / l NaOH.

Synthesis of cefotaxime-LDHs hybrid material by using the direct coprecipitation method

CefLDH1: 200 mL of an aqueous solution of $Mg(NO_3)_2 \cdot 6H_2O$ (0.05 mol)/ $Al(NO_3)_3 \cdot 9H_2O$ (0.025mol) and 150 mL of an aqueous solution containing 1 g of cefotaxime sodium (Fluka product) were added together over a period of 90 min under bubbling constant flow of nitrogen in the reaction medium and stirring at 37°C. The pH of the synthesis medium was kept to a constant value equal to 8.9 by using a solution of NaOH, 0.5M.

Synthesis of Cefotaxime-LDHs hybrid material by using the ion exchange method

CefLDH2: 1 g of freshly calcined MgAlLDH was introduced in an aqueous solution of $NaNO_3$ at 40°C for 7 h. After washing and drying the obtained sample was added in 150 mL of aqueous solution containing 1 g of cefotaxime sodium for 1 h at 37°C under nitrogen. All the synthesis procedures of the hybrid materials have done in the absence of light.

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Release of cefotaxime from the clay layered matrices into the media (phosphate buffer solution) was performed by adding about 0.1g of LDHs into 300 mL of the buffer solutions at 37 ± 0.5 °C and a pH value equal to 6.7 ± 0.2 . The amount of the drug released into the solution was measured as a function of time using an UV-visible spectrophotometer (Jasco V 550), at 752 nm, as detailed described in Ref. 11. The tests were made in duplicate and the results were recorded as an average.

Characterization methods

XRD: X-ray powder diffraction patterns were recorded on a Philips PW 1840 diffractometer using monochromatic $\text{CuK}\alpha$ radiation ($\lambda = 0.154$ nm), operating at 40 kV and 30 mA over a 2θ range from 2 to 70 degree. FTIR spectra were recorded on a FT-IR Perkin Elmer (Spectrum 100) spectrometer under the following experimental conditions: 200 scans in the mid-IR range ($500 - 4000$ cm^{-1}) using KBr (ratio 4 / 96 wt %) pellets, and a resolution of 4.0 cm^{-1} (all the samples were quantitatively weight out). Thermal analysis was carried out on a Diamond TG-DTG Perkin Elmer analysis system (under air flow, 150 ml/min, with a temperature ramp rate of 10 degree /min). TEM analysis was performed on a Hitachi H-900 instrument operating at 200 kV; the samples were prepared by dispersing them in ethanol.

Results and discussion

XRD analysis

Figure 1 illustrates the XRD patterns of the cefotaxime-anionic clay hybrid samples synthesized by using either coprecipitation or ion exchanged method; for comparison the XRD pattern of as-synthesized MgAlLDH clay is also shown.

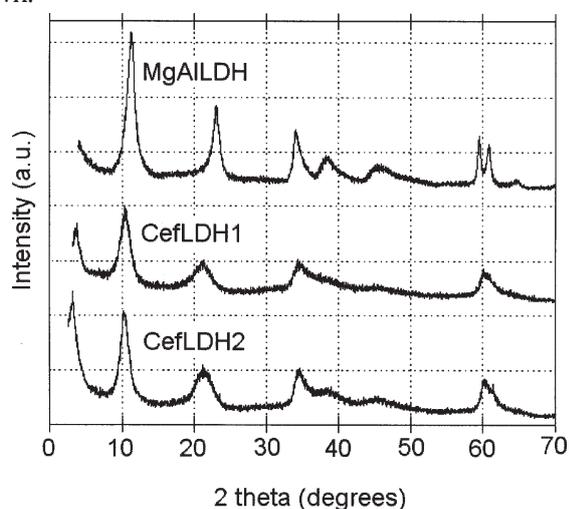


Fig. 1. Powder XRD patterns for MgAlLDH and the cefotaxime-anionic clay hybrid samples

The diffraction peaks, typical to the layered double hydroxides structure [12], with sharp and symmetric basal reflections of (003), (006) and (009) planes and broad, less intense and asymmetric reflections for the nonbasal (012), (015) and (018) planes, can be clearly identified for all the samples. For the drug - clay hybrids the (110) diffraction peaks become broader and lowered in intensity, possibly indicating that the ordered arrangement of the layered sheets decreased after the drug incorporation. The XRD reflections were indexed by using a hexagonal cell with rhombohedral symmetry ($R - 3m$), commonly used as a description of the LDH structure [13]. The basal spacing (d_{003}) of the pristine MgAlLDH is 7.7\AA while the cefotaxime - LDHs materials show expanded structures;

the basal spacing value increases to 12.7\AA for CefLDH1 and reaches 14.3\AA for CefLDH2; this can be a consequence of different orientation of the incorporated drug between the layers of the clay [14]; this result points out that the microstructural features of the new hybrid materials is dependent on the synthesis method. The reflections arising from the nonintercalated LDHs precursor can also be observed in the diffraction patterns of the hybrid samples, suggesting that the intercalation process of the drug in the layered clay is not complete. The results of the elemental analysis (by ICP), table 1, reveal that the carbon content increases by nearly 6 % for the CefLDH2 sample in comparison to the CefLDH1 sample; this result indicates that content of the drug of the biocompatible inorganic matrix can be tailored as a function of the synthesis procedures.

Table 1
THE BASAL SPACING AND ELEMENTAL ANALYSIS OF THE SYNTHESIZED SAMPLES

Sample	d_{003} (\AA)	Elemental analysis (%)
MgAlLDH	7.7	n.d.*
CefLDH1	12.7	Mg : 17.1 Al : 8.34 C : 21.71
CefLDH2	14.3	Mg : 17.7 Al : 7.90 C : 27.2

(*) n.d. = not determined

FTIR spectroscopy

The FTIR spectra of CefLDH1 and CefLDH2 are shown in figure 2; the spectrum of the parent MgAlLDH anionic clay is also included.

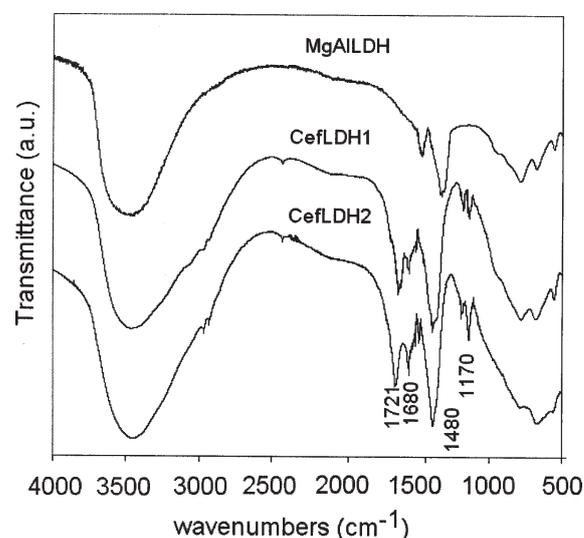


Fig. 2. FTIR spectra for MgAlLDH and the cefotaxime-anionic clay hybrid samples

For the parent clay the sharp absorption band observed at 1381 cm^{-1} indicates the presence of the nitrate anions in the interlayer while the broad absorption band around 3500 cm^{-1} is attributed to the O-H vibration mode of hydroxyl group and water molecules; a lower intensity absorption band corresponding to water deformation is also observed at ca. 1640 cm^{-1} [15]. For the cefotaxime-LDHs samples the band assigned to the interlayer nitrate anion is vanished and the FTIR spectrum becomes somehow different, indicating that the organic compound alters the structural features of the clay interlayer; the absorption band around 1170 cm^{-1} can be assigned to ν -CN vibration band while the bands at 1680 cm^{-1} and 1480 cm^{-1} can be attributed to C=O and multisubstituted pyrimidine group of the drug [16]. The bands less than 850 cm^{-1} appear in all the samples and are due to the M-O and O-M-O vibrations of the hydroxylate [12]. Considered together, the FTIR and XRD results clearly show the formation of a new hybrid layered microstructure by incorporation of cefotaxime into the LDHs inorganic matrix.

TGA-DTA analysis

The pattern of TGA-DTA curves of CefLDH1 and CefLDH2 are very similar, as a consequence we present in figure 3 (a) only the TGA-DTA profile of the CefLDH1 sample while the TGA-DTA profile of MgAlLDH is shown in figure 3 (b).

For the cefotaxime containing hybrid structure three events weight loss are observed. The first one ($30 - 130^\circ\text{C}$) is attributed to the loss of adsorbed water, while the second event ($130 - 360^\circ\text{C}$) is assigned to the desorption - decomposition processes of cefotaxime adsorbed on the external surface of the inorganic matrix. The third sharp

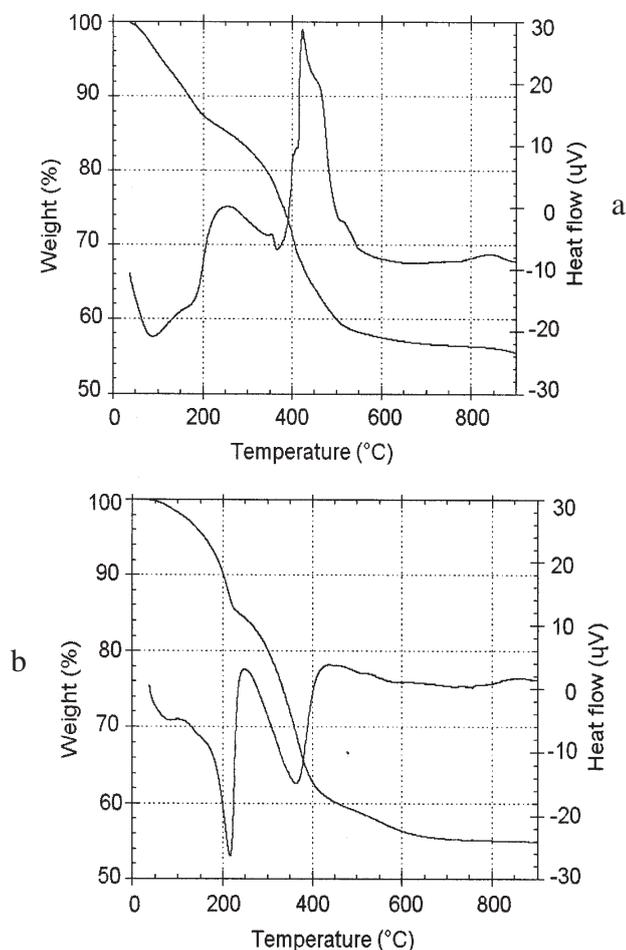


Fig. 3. TGA and DTA profiles for (a) CefLDH2 and (b) MgAlLDH

weight loss is assigned to the further decomposition [17] as well as combustion of the drug incorporated in the clay layered matrix. The characteristics of DTA patterns of CefLDH1 are defined by two exothermic peaks; only two strong endothermic peaks define the DTA pattern of MgAlLDH. The TGA-DTA results show that the confinement of cefotaxime inside the clay matrix is stable up to 130°C . The total weight loss is equal to nearly 45 % for CefLDH1 though the value increases to 52% for CefLDH2; this demonstrates that the synthesis method is important to establish the drug content of the hybrid materials.

TEM analysis

TEM images of CefLDH1 and CefLDH2 are presented in figure 4. The flat, plate-like LDH characteristic nanoparticles [18] with an average size around 80 nm and a high degree of agglomeration can be observed. The TEM results show also that some of the anionic clay layers are exfoliated and dispersed disorderly. In the TEM image of CefLDH2 the white arrow points to one area where these individual exfoliated layers can be clearly observed.

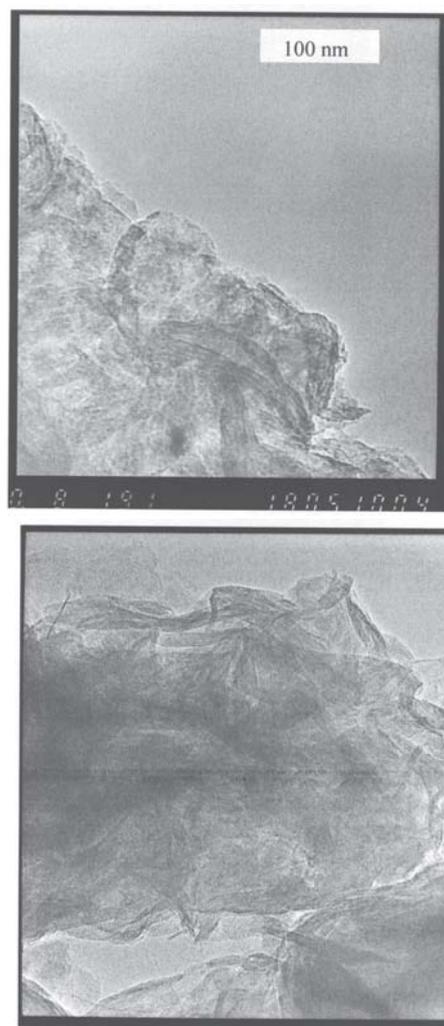


Fig. 4. The TEM images of (a) CefLDH2 and (b) CefLDH1

Controlled release of the drug in aqueous solutions

Figure 5 shows the release profile plots for the release process of cefotaxime from the layered anionic clay inorganic matrix in 300 mL of the buffer solutions at $37 \pm 0.5^\circ\text{C}$ at a pH value equal of 6.7 ± 0.2 .

For both samples it can be noted the rapid release during the first 45 min followed by a stable and more sustained release of the drug in the next 200 min. At equilibrium it

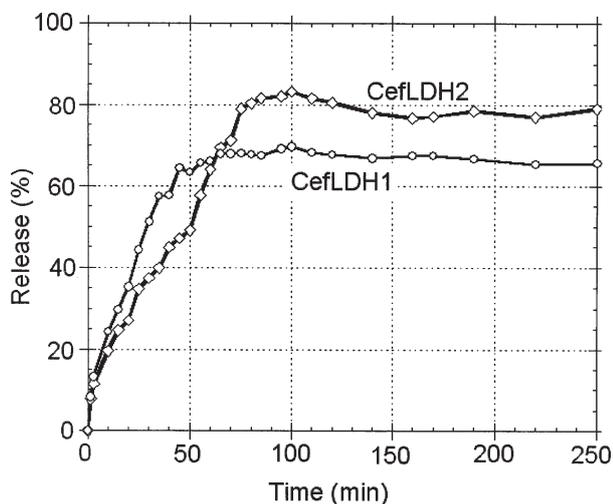


Fig. 5. Release properties of cefotaxime from MgAlLDH hosts

was estimated that almost 84% amount of cefotaxime could be released from CefLDH2 though only 68% amount of cefotaxime is released from CefLDH1. The difference in the release profile can be also a consequence of the different arrangement of the drug molecules inside the inorganic clay matrix. In addition the concentration of the used buffer solution could also affect the rate release of cefotaxime. Further studies are going to be done to study the influence of the characteristics of the hybrid materials on the stability of cefotaxime.

Conclusion

The direct coprecipitation method and the ion exchange method are used to obtain new hybrid nanostructures based on cefotaxime-hydroxalite-like anionic clays. XRD, FTIR and TG-DTA analysis demonstrate the incorporation of cefotaxime in the layered anionic clay matrix. The synthesis method is important not only for establishing the specific physico-chemical structural characteristics and the cefotaxime content of the hybrid matrices but also for defining the textural properties of the emerged hybrids. The cefotaxime antibiotic can be controlled released from the anionic clay matrix thus the new synthesized hybrid formulation can be used as a drug reservoir.

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