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STUDIES OF THE INTERCALATION AND "*IN VITRO*" LIBERATION OF AMINO ACIDS IN MAGNESIUM ALUMINIUM LAYERED DOUBLE HYDROXIDES

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Layered double hydroxides (LDHs) can be structurally described as the stacking of positively charged layers with hydrated anions intercalated in the interlamellar domain. LDHs are a class of materials represented by the general formula: $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]A^{m-}_{x/m} \cdot nH_2O$ *where:* M^{2+} *is a bivalent cation;* M^{3+} *is a trivalent cation; A is an anion with* m^- *charge.*

*We have studied the intercalation and "*in vitro*" liberation of aspartic and glutamic amino acids in Mg-Al-LDHs. LDHs intercalated with this type of anions could combine both the compound and the LDH properties. The materials were prepared by the coprecipitation method under constant pH, followed by a hydrothermal treatment for 18 hours, and subsequent characterisation of intercalated materials using PXRD, ATG/DTA and FT-IR. Studies of "*in vitro*" liberation were made by determination of amino acid concentrations in solution through UV-VIS (nynhydrin methods). Moreover, simultaneous pH monitoring was conducted during the experiment.*

Keywords: layered Double Hydroxides; anionic Clays; hydrotalcite; amino Acids; "*in vitro*" liberation; organic Compounds

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INTRODUCTION

Layered double hydroxides (LDHs), are a class of materials with a great number of applications due to their structural, chemical, electronic, optical, magnetic and medical properties. These compounds can be used as catalysts and catalyst supports, polymer stabilisers, adsorbents, anion exchangers and in medical applications.

The structure of the LDHs can be described considering the brucite-like structure, $M^{II}(OH)_2$, where Mg(II) cations are in the centre of an octahedron, sharing edges with hydroxyl groups in the vertices resulting in a planar structure. When part of the divalent cations are isomorphously replaced by trivalent cations, positively charged layers are formed. In order to neutralise the positive charge, anions must be intercalated between the layers, resulting in the hydrotalcite-like structure.

LDHs may be represented by the general formula: $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]A^{m-}_{x/m} \cdot nH_2O$, where: M^{2+} represents a bivalent cation; M^{3+} represents a trivalent cation; A^{m-} is an anion with charge m−. The layer specific charge is directly related to the exchange ratio [x in the general formula represents LDHs: $x = M^{3+}/(M^{2+} + M^{3+})$] of the trivalent cation [1,2].

A wide variety of LDHs can be obtained by the variation of cations M^{2+} and M^{3+} and the intercalated anion A^{m-}. LDHs can encapsulate organic anions into their interlayer region, with potential use in medicine as drug supports or hosts [3,4]. LDHs intercalated with organic anions could combine both the drug and the LDH properties resulting in another way of drug administration. Furthermore, once encapsulated, the drug can be released at a pH dependent rate which occurs due to destruction of the layer by acid attack. The antacid evolution and anti-pepsin activity, as well as liberation of metal ions in LDHs has been studied. Results show that LDHs reduces free hydrochloric acid concentration and present prolonged buffering action in optimum pH range [5–7].

The aim of this work was to study the intercalation and "*in vitro*" liberation of aspartic and glutamic amino acids intercalated in magnesium aluminium layered double hydroxides (MgAl-Asp-LDH and MgAl-Glu-LDH) and to study the evolution of the pH and Mg(II) concentration during the experiments. Furthermore, the buffer potential evaluation of LDHs was evaluated.

EXPERIMENTAL

All reagents used are of analytical grade and were supplied by Merck. All water used was distilled and further purified using a Millipore MilliQ® system.

Synthesis of the LDHs

MgAl-Asp-LDH and MgAl-Glu-LDH were prepared using the constant pH coprecipitation technique: a solution containing 0.250 mol of $Mg(NO_3)_2{\cdot}6H_2O$ and 0.125 mol of $Al(NO_3)_3{\cdot}9H_2O$ in 260 cm^3 water was added in a solution containing 0.0333/m (where m is the anion total charge of aspartate or glutamate) mol of the desired anion in 1050 cm^3 of water. During the addition a 2 mol dm^{-3} NaOH solution was added in order to keep a constant pH of 10.0 ($\pm$ 0.2 units). The solid product was separated and washed by centrifugation. A portion of the material was dried under vacuum in the presence of activated silica gel at room temperature and the other portion was submitted to hydrothermal treatment.

Hydrothermal Treatment

Material obtained by coprecipitation was crystallised with a hydrothermal treatment, using a Parr 4842 reactor with mechanical stirring. The medium used was a solution containing 0.0333/m mol of the anion (aspartate or glutamate) in 140 cm^3 water. The pressure was adjusted to 3 bar with nitrogen, the temperature was set at 70°C and the ageing time was 18 hours. After the hydrothermal treatment the obtained materials were separated, washed and dried as described above.

Characterisation Techniques

The powder X-ray diffraction patterns (PXRD) were registered using a Siemens D5005 equipment with a graphite monochromator selecting the Cu Kα radiation ($\lambda = 1.5406$ Å). Steps of 0.02° s^{-1} were used in the continuous method in a 2θ range 2–70°. Fourier transform infrared (FT-IR) spectra were acquired over the range 4450-450 cm^{-1} with 60 scans per sample in a Nicolet 5ZDX instrument, using pressed KBr pellets containing 2% of the sample. Simultaneous thermal gravimetric analysis and differential thermal analysis (TGA-DTA) were performed with a Simultaneous DTA-TGA apparatus (TA Instruments SDT 2960). The conditions of analysis were: synthetic atmospheric air with flux of 100 cm^3 min^{-1}, heating rate of 10°C min^{-1}, from room temperature to 1000°C. The morphology of the powder LDHs was analysed by scanning electron microscopy (SEM) in a Zeiss DSM 960 – Digital Scanning Microscope. Surface area values and mean diameter of pores were analysed using nitrogen absorption in a Micromerits ASAP 2000. The C, N and H content was determined using a Perkin-Elmer 2400 CHN instrument.

Studies of "*in vitro*" Liberation

The MgAl-Asp-LDH and MgAl-Glu-LDH prepared by the coprecipitation method and crystallised by the hydrothermal treatment were submitted to "*in vitro*" liberation studies using a SOTAX AT7 apparatus. The tests were carried out in triplicate, at a constant temperature of 37°C, with stirring at 50 rpm in a propeller stirring device over 42 hours.

Samples containing 1.00 g of powdered LDHs were added in 700 cm^3 of a 0.01 mol dm^{-3} HCl solution. Aliquots of 5 cm^3 were removed at regular time intervals and the volume removed was replaced by the addition of 5 cm^3 of the same HCl solution. The quantity of amino acids liberated into the solution was analysed by UV-VIS using ninhydrin methods. As previously described, 0.4 cm^3 of a solution containing ninhydrin, hydrindantin, 2-methoxyetanol and sodium buffer acetate was added to 0.4 cm^3 of the aliquots containing amino acids [9,10]. The resulting solution was placed in a water bath at 65°C in closed flasks for 15 min. The same solution was later cooled to room temperature and a solution containing 50% ethanol was added. The absorbance of the resulting solutions was determined in a HP 8453 with wavelength of 570 nm [9,10]. The concentration of magnesium in the aliquots was determined by atomic absorption. After 42 hours of "*in vitro*" liberation test the remaining solid product was analysed by PXRD, FT-IR and elemental analysis.

Buffer Potential Evaluation

In order to evaluate the buffer effect of the MgAl-Asp-LDH and MgAl-Glu-LDH, aliquots of 0.5 cm^3 of HCl solution (1 mol dm^{-3}) were added to a suspension containing 200 mg of the LDH in 20 cm^3 of water. The pH value was registered using a Analyser 300 pH electrode, and a further aliquot of the HCl solution was added to the suspension. This procedure was repeated until the entire solid was dissolved.

RESULTS AND DISCUSSION

Characterisation of the Aspartate and Glutamate Containing LDHs

The PXRD patterns and FT-IR spectra of the obtained materials are shown in Figure 1. The PXRD for MgAl-Asp-LDHs showed a basal spacing of 12.1 Å and for MgAl-Glu-LDHs the basal spacing found is 12.5 Å [11]. The hydrothermal treatment improved structural organisation of the both materials.

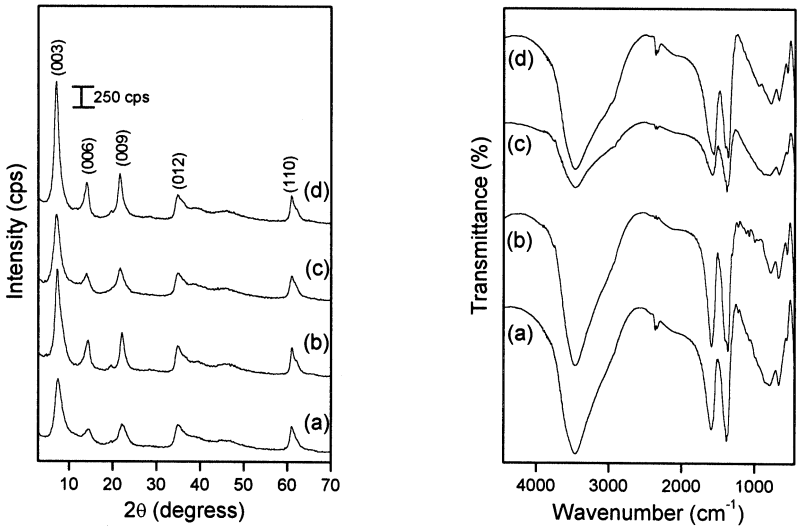

FIGURE 1 Left – PXRD patterns; and Right – FT-IR spectra for (a) MgAl-Asp-LDH without hydrothermal treatment, (b) MgAl-Asp-LDH with hydrothermal treatment, (c) MgAl-Glu-LDH without hydrothermal treatment, (d) MgAl-Glu-LDH with hydrothermal treatment.

The FT-IR spectra for all LDHs were very similar. The presence of the bands at 3410 cm^{-1} are correlated with OH^- stretching. The presence of the amino acids is confirmed by two bands related to the carboxylate group at 1590 and 1400 cm^{-1} characteristic of the RCO_2^- asymmetric and symmetric stretches [11,12].

The TGA-DTA curves are shown in Figure 2. Adsorbed and interlayer water was lost during heating from room temperature to 300°C. The decomposition of the hydroxyl groups of the layers and intercalated amino acids occurred in the range 300–700°C, with constant loss of mass around 30–40%. Above 700°C no further mass loss is observed in any case, indicating that the material was converted to the mixed oxide (MgO and $MgAl_2O_4$). DTA analysis showed an exothermic process due to the decomposition of the amino acids.

Figure 3 shows the SEM images of the powder LDHs. The materials prepared without hydrothermal treatment present a spherical shape with increased number of pores. On the other hand, the materials with hydrothermal treatment present smoother surfaces due to stacking of the particles in the planar form. These results are in accordance with the specific surface areas shown in Table 1. The materials prepared without

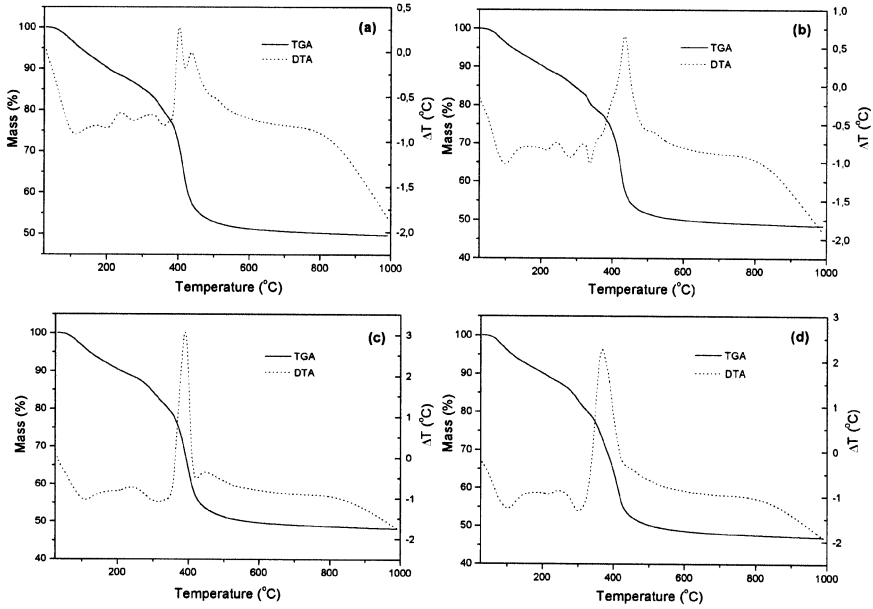

FIGURE 2 TGA-DTA curves (a) MgAl-Asp-LDH without hydrothermal treatment, (b) MgAl-Asp-LDH with hydrothermal treatment, (c) MgAl-Glu-LDH without hydrothermal treatment, (d) MgAl-Glu-LDH with hydrothermal treatment.

hydrothermal treatment presented an increased surface area compared to material prepared by the hydrothermal treatment.

Studies of "*in vitro*" Liberation

Figure 4 shows the effect of pH on the free amino acids concentration as a function of time.

The initial pH value is close to the pKa value of aspartic and glutamic amino acids, 1.88 and 2.19, respectively. At low pH *(Region I)* the amino acids are protonated, therefore electrostatic repulsion between the layers and the amino acids occurs which rapidly drives the intercalated amino acids into the solution. The destruction of the layered material due to acid attack increases the pH of the solution. In this pH range *(Region II)* which is close to the isoelectric point, 2.77 for aspartic and 3.22 for glutamic, the amino acids become uncharged and the rate of the amino acid liberation is reduced [13]. The maximum rate of amino acids liberation in solution occur in a period of 3 hours. After this time the solution pH is around 4.0 *(Region III)* and next to the pKx values of the aspartic and glutamic acids, 3.65 and

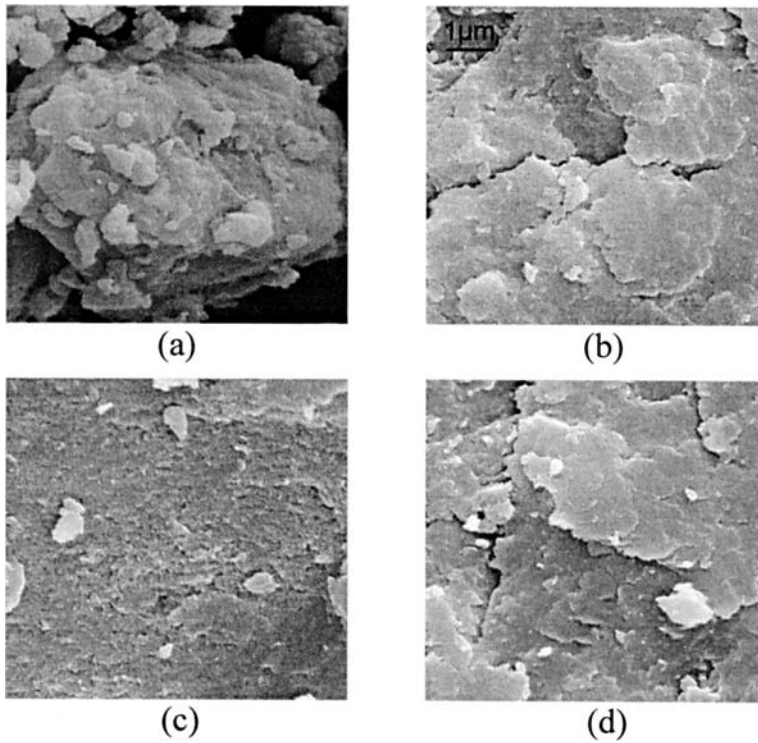

FIGURE 3 SEM images (10.000X): (a) MgAl-Asp-LDH without hydrothermal treatment, (b) MgAl-Asp-LDH with hydrothermal treatment, (c) MgAl-Glu-LDH without hydrothermal treatment, (d) MgAl-Glu-LDH with hydrothermal treatment.

TABLE 1 Values of Specific Surface Areas and Mean Diameter of Pores for the Prepared Materials

Samples	Hydrothermal treatment	Specific surface areas (m^2/g)	Mean diameter of pores (Å)
MgAl-Asp-LDHs	Before	1,029	346,74
	After	0,816	206,36
MgAl-Glu-LDHs	Before	0,722	259,98
	After	0,525	209,37

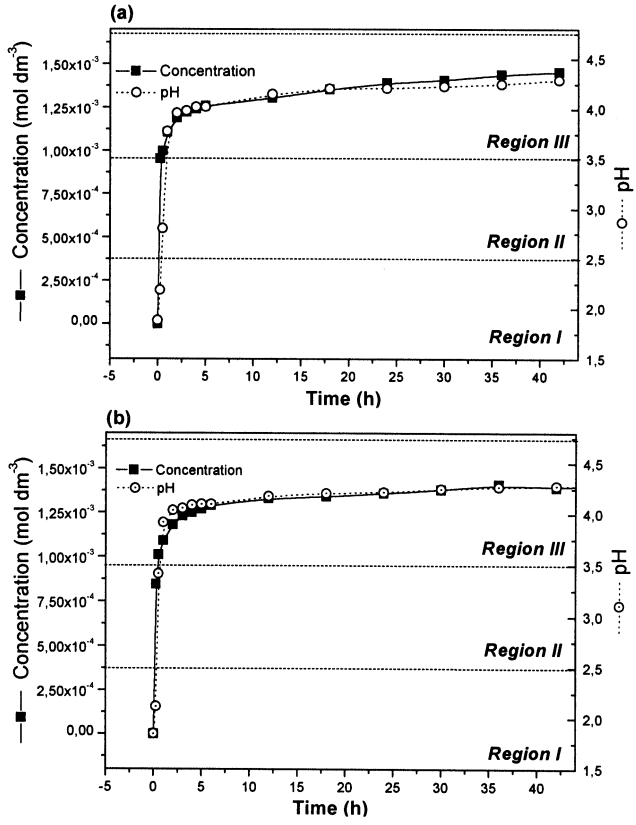

FIGURE 4 pH and concentration *versus* time in of "*in vitro*" liberation studies; (a) MgAl-Asp-LDH, (b) MgAl-Glu-LDH.

4.25, respectably. In this pH range the amino acids are found in the anionic form and their concentration in solution is almost constant at 1.4×10^{-3} mol dm^{-3}.

The regions I, II, and III defined in the interpretation of the liberation data arise from the pH ranges which result in the deprotonations of the groups shown in Figure 5.

The concentration of magnesium in the solution was determined by atomic absorption and the results are shown in Figure 6. The results show the same profile as those from the "*in vitro*" amino acids liberation studies. These results suggest that the destruction of the layered materials occurs in a gradual manner.

The resulting solid material was analysed by PXRD, FT-IR and elemental analysis which are shown in Figure 7.

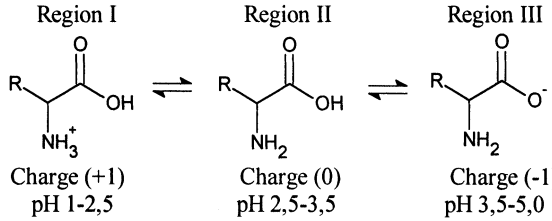

FIGURE 5 Amino acids forms in function of the pH in the "*in vitro*" liberation tests.

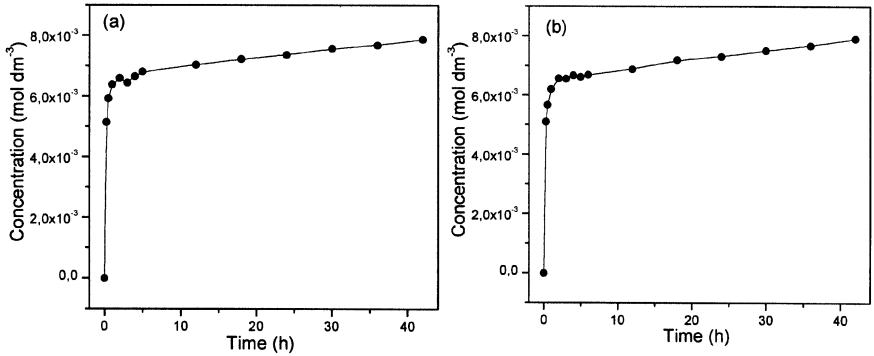

FIGURE 6 Concentration of magnesium in the "*in vitro*" liberation studies; (a) MgAl-Asp-LDH, (b) MgAl-Glu-LDH.

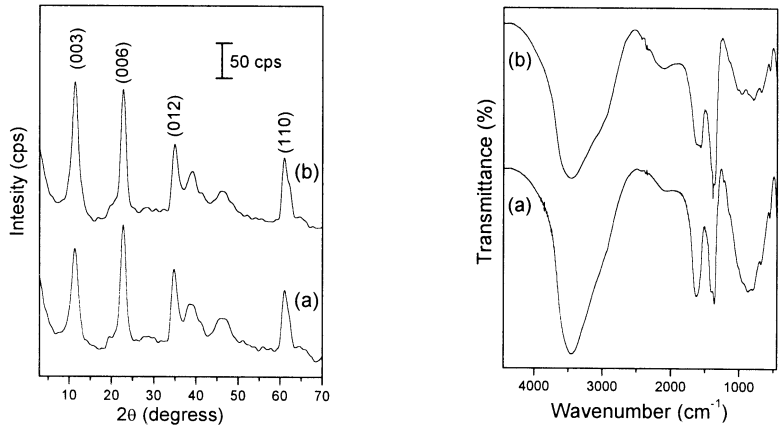

FIGURE 7 Left - PXRD patterns; and Right – FT-IR spectra for (a) MgAl-Asp-LDH, (b) MgAl-Glu-LDH; after 42 hours of "*in vitro*" liberation studies.

TABLE 2 Percentages of C, N and H Contents LDHs Before and After of the "*in vitro*" Liberation Tests

Sample	"*in vitro*" liberation tests	N (%)	C (%)	H (%)
MgAl-Asp-HDLs	Before	1.85	7.25	4.42
	After	1.31	5.01	4.16
MgAl-Glu-HDLs	Before	1.78	8.66	4.58
	After	1.07	4.79	4.20

The PXRD of MgAl-Asp-LDH and MgAl-Glu-LDH showed a basal spacing around 7.6 Å for both materials, which is in accordance with the basal spacing for chloride anions. In order to confirm this, a qualitative test with silver nitrate was performed and the formation of a white AgCl precipitate supported this hypothesis.

It may be noted that, FT-IR shows the presence of the amino acids by two bands related to the carboxylic group at around 1590 and 1400 cm^{-1}. These data suggest that amino acids remain associated with the material of over after acid treatment.

As show in Table 2 the materials presented a smaller percentages of C, N, and H after of the "*in vitro*" liberation tests. These results confirm the existence of the amino acids.

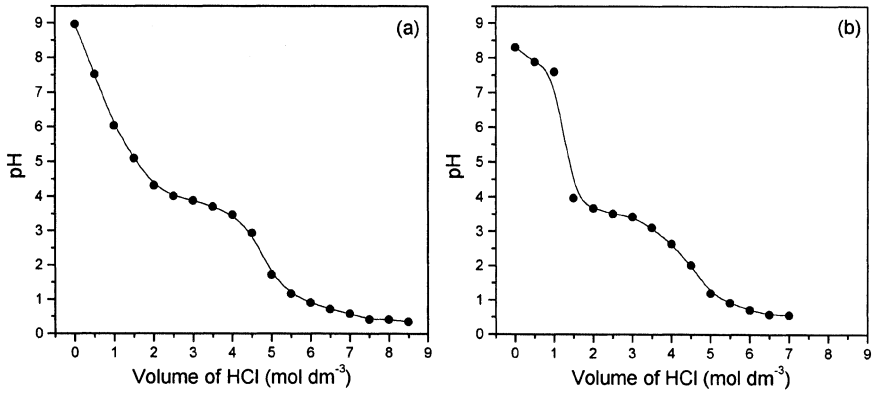

FIGURE 8 Titration of the (a) MgAl-Asp-LDH, (b) MgAl-Glu-LDH in buffer potential studies.

Buffer Potential Evaluation

The results for the titration of the LDHs prepared by coprecipitation are shown in Figure 8. It is possible to observe that the buffer effect of the LDHs occurred in the pH range 3–4, and is reduced on the complete destruction of the LDHs.

CONCLUSION

Based on the results obtained we conclude that aspartic and glutamic amino acids can be intercalated in MgAl-LDHs through the coprecipitation technique at constant pH. The hydrothermal treatment after coprecipitation improves the structural organisation of the both materials. Materials with hydrothermal treatment present smoother surfaces than the ones not submitted to hydrothermal treatment.

The liberation of the intercalated amino acids was dependent on the solution pH and consequently of the ionisation state of the amino acids. Two competitive processes occur, one with amino acids liberation and destruction of the layered material and another with interlayer change of the amino acids by chloride anions. The buffer effect of the LDHs in the pH range 3–4 seems to be ideal for the application as antacid.

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