Bionanocomposites based on layered double hydroxides as drug delivery systems

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ABSTRACT

The present work introduces new biohybrid materials involving layered double hydroxides (LDH) and biopolymers to produce bionanocomposites, able to act as effective drug delivery systems (DDS). Ibuprofen (IBU) and 5-aminosalicylic acid (5-ASA) have been chosen as model drugs, being intercalated in a Mg–Al LDH matrix. On the one side, the LDH-IBU intercalation compound prepared by ion-exchange reaction was blended with the biopolymers zein, a highly hydrophobic protein, and alginate, a polysaccharide widely applied for encapsulating drugs. On the other side, the LDH-5-ASA intercalation compound prepared by co-precipitation was assembled to the polysaccharides chitosan and pectin, which show mucoadhesive properties and resistance to acid pH values, respectively. Characterization of the intercalation compounds and the resulting bionanocomposites was carried out by means of different experimental techniques: X-ray diffraction, infrared spectroscopy, chemical and thermal analysis, as well as optical and scanning electron microscopies. Data on the swelling behavior and drug release under different pH conditions are also reported.

Keywords: bionanocomposites, layered double hydroxide, drug delivery systems, zein, alginate, pectin, chitosan, controlled release

1. INTRODUCTION

Bionanocomposites are nanostructured biohybrid materials in which a biopolymer is assembled to a nanosized inorganic solid.[1] They constitute a growing field of research not only as ecological materials, but also for other applications including biomedicine, such as in tissue engineering or drug delivery systems (DDS).[2] Layered double hydroxides (LDH) are versatile, biodegradable and biocompatible inorganic materials (Figure 1) whose anion-exchange ability results specially attractive for the preparation of a large variety of hybrid and biohybrid materials, for diverse applications with special emphasis in the biomedical field.[1,2] In this way, anionic drugs, pesticides or DNA biomolecules have been satisfactorily assembled to LDH,[3] typically Mg-Al and Zn-Al, and tested as controlled delivery systems (DDS) in which their sensitivity to pH is used to provoke the release of the intercalated species.[4,5] DDS with a core-shell structure in which the LDH-drug hybrid is coated with a protective polymeric matrix show a more controlled release of the drug. Due to the availability of a large variety of biocompatible polymers, including biopolymers, this is an emerging field of research of great interest in the development of new and more controlled DDS.[6] The formation of bionanocomposites by assembling of LDH to biopolymers, such as polysaccharides, and other biomolecules, such as phospholipids, profits from the versatility of methodologies for the LDHs synthesis in comparison to smectites and other clays.[7] Moreover, the possibility of using each component (biomolecule, LDH, drug and other species) as individual building-blocks which can be combined using sequential and alternative methodologies can result in nanostructured biohybrid materials for novel DDS applications.

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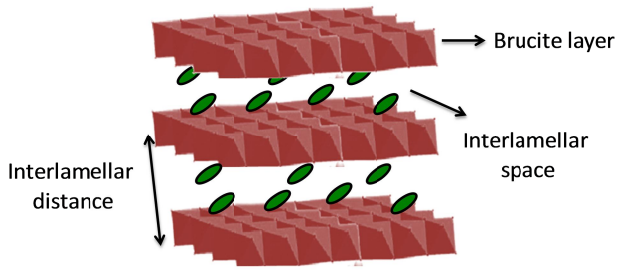

Figure 1. Schematic representation of the crystal structure of the layered double hydroxide

Our alternative consists in the use of bionanocomposites in which LDH-drug hybrid systems are either combined to biopolymers of different hydrophilic character or to biopolymers provided of special functionalities that may favor the specific action in a part of the gastro-intestinal tract. In this communication we will show two types of LDH-based bionanocomposites for DDS applications: i) a LDH intercalated with ibuprofen (IBU) (Figure 2a), as a model drug, assembled to mixtures of the polysaccharide alginate and the hydrophobic protein zein; ii) 5-aminosalicylic acid (5-ASA) (Figure 2b), anti-inflammatory drug for treatment of ulcerative colitis, intercalated in a LDH-chitosan bionanocomposite assembled to pectin that acts as a gastroresistant coating. In both approaches, the synergistic properties afforded by each component of the bionanocomposite result in DDS resistant to pH changes, making possible the drug release in a controlled manner or even acting only in the focus of the disease, minimizing side effects.

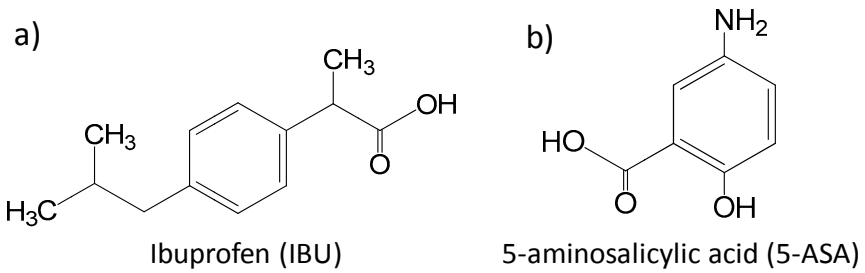

Figure 2. Molecular structure of the (a) IBU and (b) 5-ASA drugs used in this work.

2. METHODOLOGY

1.1 Starting Materials

Zein (Z) from maize, sodium alginate (A), chitosan (CHT) and pectin (PCT) biopolymers as well as IBU and 5-ASA drugs were purchased from Sigma-Aldrich. Aqueous solutions simulating the gastrointestinal media were prepared from chemicals of analytical reagent grade.

1.2 Materials preparation

2.2.1 Synthesis of MgAl/Cl LDH

A mixture of $MgCl_2 \cdot 6H_2O$ (17.5 mmol) and $AlCl_3 \cdot 6H_2O$ (8.74 mmol) was dissolved in 250 mL of decarbonated bidistilled water. This aqueous solution was added dropwise with a peristaltic pump to 100 mL deionized water kept under a nitrogen flow for removing CO_2. Simultaneously, a water solution of 1 M NaOH was also added to the aqueous system through an automatic dispenser (Metrohm Dosimat 765), in order to keep a constant pH of 10 during the synthesis. The resulting suspension was stirred for 24 h under a nitrogen flow. The solid product was isolated by

centrifugation, washed three times with bidistilled water, and dried overnight at 60 ºC. The $[Mg_{0.67}Al_{0.33}(OH)_2]Cl_{0.33}{\cdot}nH_2O]$ LDH was denoted as MgAl/Cl LDH.

2.2.2 Synthesis of LDH-IBU

Intercalation of IBU into the MgAl/Cl LDH was carried out by an ion exchange reaction. Briefly, 1 g of IBU was dissolved in 21 mL of a 50% (v/v) ethanol/water solution and then the pH adjusted to 10.0 ± 0.1 by addition of 1M NaOH. This solution was slowly added to a suspension containing 0.5 g of a freshly prepared LDH in 21 mL of distilled water. The pH of the resulting system was resettled back to 10 and then maintained under magnetic stirring at 60 °C under a nitrogen flow for 7 days. Afterwards, the solid product was isolated by centrifugation, washed thoroughly with distilled water and dried overnight at 60 ºC. The resulting hybrid material was denoted as LDH-IBU.

2.2.3 Synthesis of LDH-5ASA

The LDH-5ASA intercalation compound was prepared by co-precipitation of the MgAl LDH in the presence of the drug. In the present case, the mixture of 18 mmol and 9 mmol of Mg and Al salts, respectively, was dissolved on 400 mL of de-carbonated bi-distilled water and this aqueous solution was added to 100 mL of 50% (v/v) ethanol-water solution containing 0.008 mol 5-ASA at pH 9. The resulting suspension was kept under nitrogen and stirred for 48 h at 60 °C. The solid product was centrifuged, washed, and dried overnight at 60 °C. The resulting hybrid material was denoted as LDH-5ASA.

2.2.4 Preparation of the alginate-zein bionanocomposite beads

For the preparation of alginate-zein beads incorporating IBU, 1 g of alginate was dissolved in 80 ml of water previously heated to 60 °C. Zein (1 g), and either 60 mg of IBU or the necessary amount of LDH-IBU intercalation compound containing 60 mg of IBU, were incorporated to 20 mL of 80% (v/v) ethanol/water. After homogenization, this mixture was gradually added to the alginate solution, forming a single batch that is kept in constant agitation overnight and the resulting gel was introduced in a burette and then slowly poured as small droplets into a solution of 5% (w/v) $CaCl_2$ solution. The resulting beads were filtered and washed with water, and dried in an oven at 40 ºC. The beads prepared from alginate-zein incorporating IBU alone were denominated AZ-IBU and those containing LDH-IBU as AZ/LDH-IBU.

2.2.5 Preparation of the chitosan-pectin bionanocomposite beads

For the bionanocomposite beads based on the chitosan and pectin biopolymers that incorporate 5-ASA drug, an aqueous solution of 1% (v/v) HCl containing 1% (w/v) chitosan was magnetically stirred with 0.2 g of pure 5-ASA or with the appropriate amount of LDH-5ASA bio-hybrid to achieve 0.2 g of 5-ASA until homogenization. The resulting gel was dripped into a solution of 2M NaOH. The resulting beads were isolated by filtration and washed with water. Subsequently, the chitosan beads loaded with 5-ASA or LDH-5ASA were immersed in a 1% (w/v) pectin aqueous solution, filtered and cross-linked in 10% (w/v) $CaCl_2$ aqueous solution. The resulting CHT/5ASA/PCT or CHT/LDH-5ASA/PCT bionanocomposite beads were filtered washed with water, frozen at -20 °C and lyophilized in a freeze-drier.

2.2.6 Characterization

Several physical-chemical techniques were employed in the characterization of the obtained materials, such as powder X-ray diffraction (XRD) (BRUKER D8-ADVANCE), Fourier transformed IR (FTIR) (BRUKER IFS 66v/S and NICOLET 20SXC), CHN elemental chemical analysis (LECO CHNS-932), simultaneous thermogravimetric (TG) and differential thermal (DTA) analyses (SEIKO SSC/5200). The surface morphology of samples was observed by FE-SEM (FEI Nova NanoSEM 230) and semi-quantitative analysis of elements was performed in certain samples with an EDAX Genesis XM2i detector coupled to the microscope.

The estimation of the drug content in the bionanocomposite beads was determined by UV-vis spectrophotometry. In the case of alginate-zein beads, 0.2 g of beads were immersed and dispersed in phosphate buffer at pH 6.8 for 24 h. Then, the solution was filtered, and the IBU content was calculated from the absorbance at λ = 262 nm measured in a Shimadzu UV1201 spectrophotometer. For the chitosan-pectin system, a certain amount of beads was immersed in phosphate buffer at pH 6.8 for 12 h and, then, the pH was adjusted to 1.2 by addition of 1 M HCl and kept in this solution for other 12 h more. The solution was filtered, and the 5-ASA content was calculated from the absorbance λ= 303 nm by UV spectrophotometry. The drug percentage loading and encapsulation efficiency of both systems were calculated using (1) and (2) equations.

$$\% \text{ drug loading} = \frac{\text{amount of drug in beads}}{\text{amount of beads}} \text{ x } 100 \qquad (1)$$

$$\% \text{ encapsulation efficiency} = \frac{\text{drug loading}}{\text{theoretical loading}} x\,100 \qquad (2)$$

The swelling behavior of the bionanocomposite beads was studied by placing a certain amount of them in a Petri dish containing 5 mL of phosphate buffer at pH 6.8, and visualized with an optical microscope (Motic, B3 Professional series). The drug release of the bionanocomposite beads was conducted as follows: a certain amount of beads were suspended in chosen release medium at 37 ºC under stirring. At determined intervals of time, an aliquot of the solution was removed, and the amount of IBU or 5-ASA released from the bionanocomposite beads was evaluated by UV spectrophotometry (λ = 262 nm and 303 nm for IBU and 5-ASA, respectively). The sequential pH changes that occur during the in vivo process were simulated by keeping the beads for 2h at pH 1.2 that simulates the gastric fluid, then for 2h at pH 6 simulating the first zone of intestinal fluid, and finally for 4h at pH 7.4, mimicking the second zone of intestinal fluid.[8] All the experiments were carried out in triplicate.

3. RESULTS

1.3 Characterization of intercalation compounds

The intercalation of IBU and 5-ASA between the layers of MgAl/Cl LDH, was determined by XRD. Figure 3 shows the corresponding diffractograms of the pristine LDH and their intercalation compounds. XRD reflections can be indexed to a hexagonal lattice with rhombohedral symmetry, commonly used for description of LDH structures.[9] Thus, the observed first diffraction peak can be then ascribed to the (003) reflection, which gives a basal spacing (d_{003}) of about 0.77 nm characteristic of the MgAl/Cl LDH (Figure 3a).[10] Intercalation of both drugs into LDH is corroborated by the displacement of the reflection (003) towards lower 2θ values in the LDH-IBU (Figure 3b) and LDH-5ASA (Figure 3c), showing basal spacing values of 2.25 nm and 2.08 nm, respectively. Assuming that 0.48 nm corresponds to the brucite layer thickness, the interlayer spacing increases are 1.77 nm and 1.60 nm for LDH-IBU and LDH-5ASA, respectively. In both cases, these last values suggest the arrangement of the intercalated molecules most likely in a bilayer configuration. CHN chemical analysis of hybrids confirm the incorporation of around 226 mEq of IBU and 190 mEq of 5-ASA per 100 g LDH, respectively. Taking into account that the anion-exchange capacity of LDH is around 330 mEq/100 g, these values indicate that there are chloride ions remaining in the interlayer region.

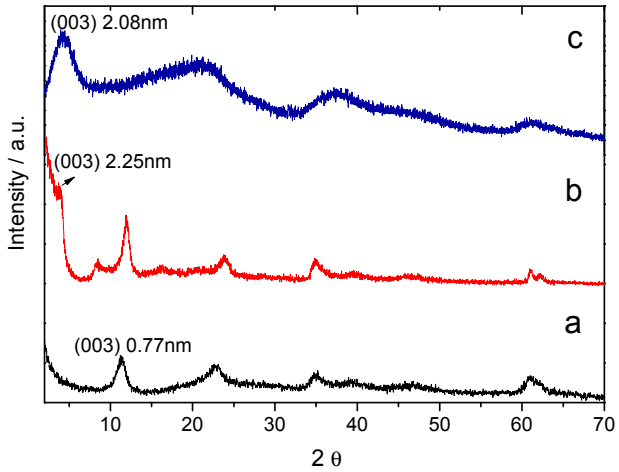

Figure 3. XRD patterns of (a) pristine MgAl/Cl LDH, and (b) LDH-IBU and (c) LDH-5ASA intercalation compounds prepared from ion-exchange and co-precipitation methods, respectively.

1.4 Characterization of bionanocomposites beads

The new DDS were prepared by incorporation of the LDH-IBU or LDH-5ASA intercalation compounds into an alginate-zein or chitosan/pectin matrix, respectively. For comparison, DDS in which the drug is directly associated to the biopolymers were also prepared. The amounts of IBU or 5-ASA incorporated as pure drug and as LDH-drug in the different bionanocomposites, as well as the encapsulation efficiency of the biopolymers matrix are listed in Table 1. For the systems zein-alginate, it is observed that the amount of IBU incorporated, as pure drug or intercalated in the LDH, increases with zein content in the blend. This increase in encapsulation efficiency is related to the organophilic character of zein, forming more stable beads when are based on the bionanocomposite systems. Good encapsulation efficiency is also found for the systems based on chitosan and pectin biopolymers that incorporate LDH-5ASA bio-hybrid in comparison to those systems loaded with pure 5-ASA drug. This behavior may be associated with the barrier effect afforded by the inorganic solid in which is incorporated the drug.

Table 1. Encapsulation efficiency and amount of IBU and 5-ASA loaded, either as pure drug or as LDH-drug hybrid compound, in different DDS beads.

Sample	Drug load (%)*	Encapsulation efficiency (%)*
AZ/IBU	2.73 ± 0.17	45.5 ± 0.21
A/LDH-IBU	0.58 ± 0.71	9.74 ± 0.50
AZ/LDH-IBU	3.14 ± 0.02	52.4 ± 0.42
CHT/5ASA/PCT	2.49 ± 0.05	12.5 ± 0.22
CHT/LDH-5ASA	4.86 ± 0.17	24.4 ± 1.10
CHT/LDH-5ASA/PCT	6.99 ± 0.06	34.9 ± 0.30

* Data are mean ± S.D., n = 3

Swelling behavior of beads is a very important property regarding the controlled drug delivery performance of the DDS. Alginate-zein systems were evaluated by optical microscopy following the evolution of swelling of A/IBU and AZ/IBU beads in contact with a pH 6.8 buffer solution (Figure 4). The alginate bead (A/IBU; Figure 4a) swells rapidly and after 40 minutes it becomes almost completely disintegrated. This result is due to the removal of calcium ions that maintain the network of cross-linked alginate chains by phosphate ions,[11] favoring a higher uptake of water molecules. In contrast, the sequence of swelling of the bead that contains zein (AZ/IBU; Figure 4b) clearly shows a swelling effect that provokes an increase in the bead size but, at same time, the integrity of the bead is preserved. This observation indicates that the role of zein in the system favors the stability of the microspheres due to its hydrophobic character, limiting the incorporation of water and the easy uptake of calcium ions by the phosphate anions of the buffer solution.

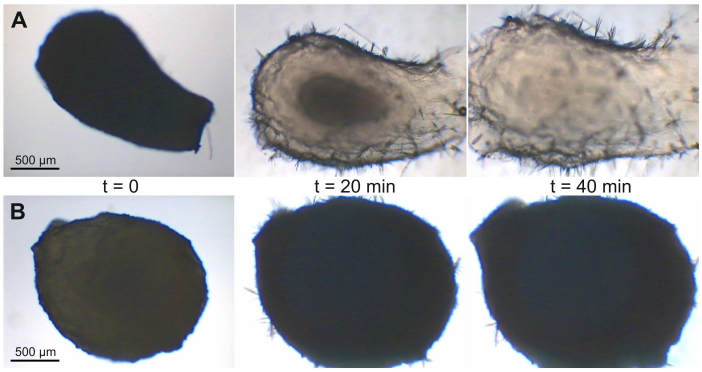

Figure 4. Swelling images of (A) pure alginate and (B) alginate-zein beads loaded with IBU, at different time of exposure in pH 6.8 phosphate buffer solution.

The mucoadhesion properties of chitosan were evaluated by checking its interaction with mucin, in an assay that simulates *in vivo* conditions taking place in the intestinal tissue. Table 2 presents the initial concentration of mucin and the percentage of mucin adsorption by chitosan. It is observed that chitosan shows a great capacity for mucin adsorption (ranging between 67 and 83%) in a wide range of mucin concentrations. This result points out to the existence of strong interaction between the negatively charged mucin and positively charged chitosan, suggesting the possibility of using this system in the targeted delivery of drugs at the intestinal colon tract.

Table 2. Mucin adsorption by chitosan from different initial mucin.concentrations.

Mucin concentration (mg/mL)	**Mucin adsorption (%)**
0.5	82.6 ± 0.8
0.375	81.8 ± 0.6
0.25	70.8 ± 0.6
0.125	66.7 ± 2.2

Figure 5 shows the release profiles of IBU and 5-ASA from alginate-zein and chitosan-pectin beads, respectively, under pH conditions that simulate the sequence in the gastrointestinal tract. The LDH-drug systems without biopolymer protection are very sensitive to acid environments and a complete release of drug in the stomach media (pH 1.2) is achieved in short time.[8] The beads based on alginate-zein biopolymers (Figure 5a) show a progressive release of IBU, being this more controlled when zein content increases. The systems based only on alginate are very sensitive to pH changes and quickly release the drug loaded in conditions that simulate the intestinal fluid (pH 6.8-7.4), where a

complete liberation of the encapsulated drug can be reached. Moreover, AZ/LDH-IBU system shows a slower IBU release, not only because the hydrophobicity introduced by zein, but also because the encapsulated drug probably requires additional time to be released from the MgAl LDH. Hence, AZ/LDH-IBU shows a maximum release value of around 60%. It is remarkable the possibility to prepare DDS that can deliver controlled doses of drug, which can remain constant along their passage through the intestinal tract.

In the case of the chitosan-pectin bionanocomposite materials containing 5-ASA drug (Figure 5b) the behavior is strongly dependent on the composition of beads. In this way, beads based on 5-ASA loaded chitosan show a release close to 90% of the drug within the first 2 hours (i.e., when the system is at pH 1.2). This behavior is expected due to the high solubility of chitosan in acid media. Therefore, these DDS will not resist the acid pH of stomach in *in vivo* applications. On the contrary, these chitosan beads coated with pectin are protected towards degradation at stomach pH, achieving elevated release values when the pH changes the 1.2 to 6.8. These beads can be completely dissolved at pH 7.4, causing the total release of the encapsulated drug. Such release behaviors could not be suitable for DDS in colon diseases, since the drug release does not occur in a controlled manner in the intestinal tract. A more effective controlled release is achieved when 5-ASA drug is present as LDH-5ASA bio-hybrid and then incorporated in chitosan-pectin system. In this case, a controlled release at pH 6.8 is now observed with the complete drug delivery at pH 7.4, just in the intestinal colon tract conditions. From these results, it can be concluded that beads of chitosan/LDH-5ASA coated with pectin can be very promising DDS for controlled release of drugs for treatment of colon diseases.

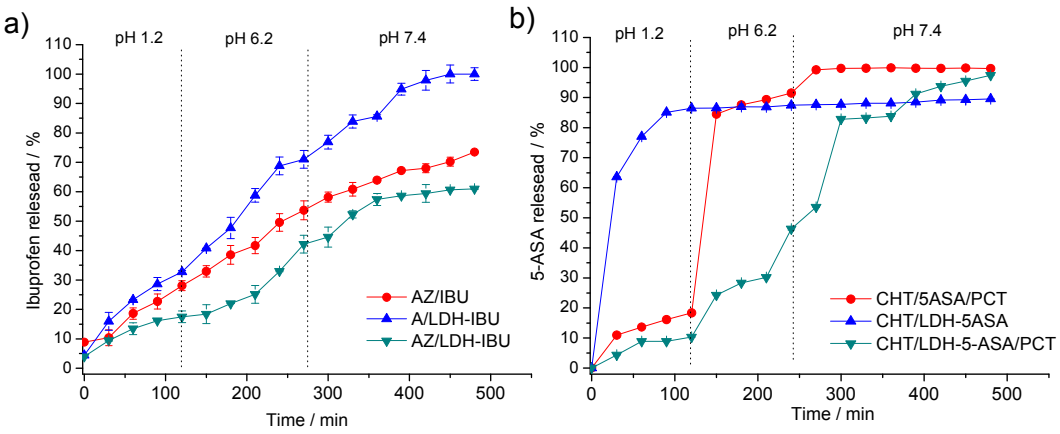

Figure 5. Profiles of IBU and 5-ASA release from (a) alginate-zein and (b) chitosan-pectin beads, in conditions that simulate the gastrointestinal tract passage (pH & time) at 37ºC.

4. CONCLUSIONS

We have reported two types of LDH-based bionanocomposites for drug delivery applications in which the drug is supported in a MgAl-LDH substrate and further assembled to biopolymers of different hydrophilic character (alginate-zein system) or to biopolymers provided with special functionalities (CHT-PCT system). The synergistic properties afforded by each component of the bionanocomposite result in DDS resistant to pH changes, making possible the drug release in a controlled manner or even acting only in the focus of the disease, minimizing side effects which may be especially useful for different *in situ* therapies for treatment of specific diseases

Both, AZ/LDH-IBU and CHT/LDH-5ASA/PCT based-systems are approaches for the preparation of DDS that can be applied to other oral drugs that also require a controlled release in the digestive tract. The possibility of adjusting the composition of the systems (biopolymers and LDH-supported drug) can determine different kinetics in the release that may favor the specific action in a part of the gastro-intestinal tract.

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