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# Intercalation of hydrophilic and hydrophobic antibiotics in layered double hydroxides

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**Abstract**

Four pharmaceutically active molecules, each representing a different class of antibiotic, were intercalated in layered double hydroxides. Two of them, gramicidin and amphotericin B, are hydrophobic, surface active drugs that were incorporated in artificial membranes formed in the interlayer of the inorganic host. The other two, ampicillin and nalidixic acid, are water soluble, commonly used antibiotics that were directly intercalated by using simple ion exchange reactions. The synthetic nanohybrid materials were characterized by various methods, as X-ray diffraction, infrared spectroscopy and ultraviolet–visible spectroscopy that verified the successful intercalation of the antibiotics and provided information regarding the interlayer structure of the nanohybrids. The reversible interaction of the antibiotic molecules with the inorganic host leads to release of the active drugs under the appropriate conditions. The release studies showed that the synthetic nanohybrids can successfully serve as controlled release systems for different kinds of antibiotics.


*Keywords:* Layered double hydroxide; Gramicidin; Amphotericin B; Ampicillin; Nalidixic acid; Drug delivery

## 1. Introduction

Systemic drug administration results in distribution of the drug throughout the patient's body through blood circulation. This can lead to elevated drug concentrations in undesired parts of the body that cause severe side effects. Additionally, there are many cases where conventional drug administration methods do not provide satisfactory pharmacokinetic profiles because the drug concentration rapidly falls below desired levels. Drug delivery and controlled release systems are more sophisticated drug administration systems designed to overcome such problems (Kidane and Bhatt, 2005). These systems utilize carriers that slowly release their contents in order to maintain drug concentrations at the desired levels for a longer period of time. Moreover, drug carriers can be surface modified in order to target specific cells or tissues and thus reduce the risk of toxic side effects (Brannon-Peppas and Blanchette, 2004; Petrak, 2005). Drug carriers are usually polymers or various types of lipid vesicles, like liposomes, that form micro- or nano-particles (Brannon-Peppas, 1995; Zimmer and Kreuter, 1995; Labhasetwar et al., 1997; Müller et al., 2000). Recently, biocompatible inorganic materials, like layered double hydroxides, are being used in drug delivery and controlled release systems. These materials are more stable and less toxic than conventional drug carriers.

Layered double hydroxides (LDHs), commonly known as hydrotalcites or anionic clays, are a family of natural and synthetic materials represented by the general formula $[M_{(1-x)}^{II}M_x^{III}(OH)_2][A^{n-}]_{x/n}\cdot zH_2O$ where $M^{II}$ and $M^{III}$ are a divalent and trivalent metal, respectively, and $A^{n-}$ is the interlayer anion (Cavani et al., 1991). These materials form successive positively charged metal hydroxide layers and negatively charged anion layers. The metal hydroxide layers have a structure similar to brucite and are 4.8 Å thick, while the thickness of the intermediate layers depends on the size of the anion. Among the properties of LDHs, anion-exchange provides a simple method to replace the interlayer anion and thus synthesize a variety of different layered materials (Meyn et al., 1990). These materials have been used as anion-exchangers and catalysts (Cavani et al., 1991; Pinnavaia et al., 1995), but recently medical applications of LDH-biomolecule nanohybrids have gained attention.

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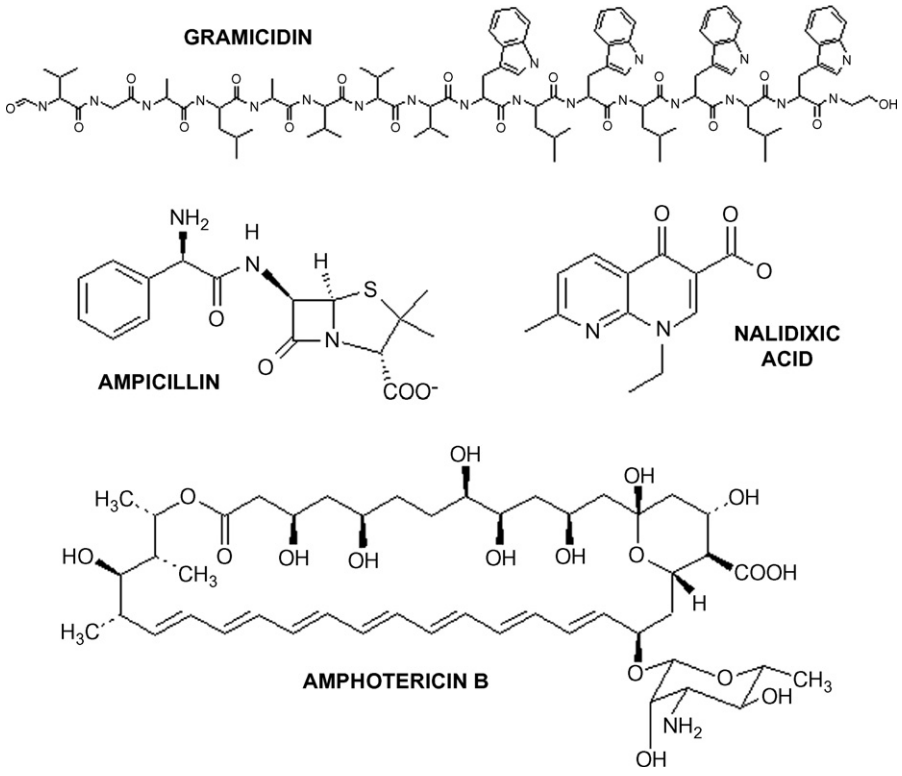


Fig. 1. Chemical structures of the four antibiotics that were used.

LDH became an attractive material for drug delivery and controlled release applications when Choy et al. intercalated DNA in Mg–Al LDH (Choy et al., 1999) and showed that the LDH–DNA nanohybrids can deliver the DNA into cells (Choy et al., 2000). Furthermore, it was shown that these nanohybrids can be injected intravenously in rats without toxic side effects (Kwak et al., 2004). Even the gene of the green fluorescent protein was intercalated in LDHs and was then delivered and expressed in various cell lines (Tyner et al., 2004a). Additionally, the possibility of surface modification of the inorganic layers, leading to targeted drug delivery to specific cells or organs, makes LDH a very attractive drug carrier. Anti-inflammatory drugs like ibuprofen (Ambrogi et al., 2001; Khan et al., 2001) and naproxen (Khan et al., 2001; Wei et al., 2004) and anti-cancer drugs like camptothecin (Tyner et al., 2004b) and folate derivatives (Choy et al., 2004) have been intercalated in LDH. In this study, four different antibiotics, gramicidin, amphotericin B, ampicillin and nalidixic acid (Fig. 1), were intercalated in layered double hydroxides.

Gramicidin is a polypeptide ionophore antibiotic produced by the bacterium *Bacillus brevis* (Wallace, 1998) that is active against Gram-positive bacteria. It is a hydrophobic peptide consisting of 15 amino acids that forms dimeric channels across biological membranes. These channels are permeable to monovalent cations but are blocked by divalent cations. Amphotericin B is a polyene antibiotic that is used for treatment of fungal infections in immunodepressed patients (Schreier et al., 2000). It forms pores in fungal membranes by complexing with ergosterol. However, due to severe side effects, amphotericin B is usually administered in lipid complexes (Espuelas et al., 1997; Hargreaves et al., 2006) and was one of the first drugs that were commercially available in liposomal form (AmBisome®).

Ampicillin belongs to the family of penicillins or β-lactam antibiotics that are widely used against bacterial infections. It inhibits the synthesis of peptidoglycan in bacterial cell walls (Tipper and Strominger, 1965) and thus is more active against Gram-positive bacteria. Some drug delivery and controlled release systems for ampicillin have been developed using polymethacrylate (Fernández Degiorgi et al., 1995) or hydroxyapatite (Queiroz et al., 2001) as carriers. A similar antibiotic, phenoxymethylpenicillin has been intercalated in LDH and the activity of the intercalated material against *Staphylococcus aureaus* was demonstrated (Li et al., 2006). Quinolone antibiotics, such as nalidixic acid, are inhibitors of bacterial DNA gyrases (Shen et al., 1989; Kampranis and Maxwell, 1998). They are more active against Gram-negative than Gram-positive bacteria and find clinical application mostly in the treatment of urinary tract infections. Nalidixic acid (Clerc and Barenholz, 1995) and enoxacin (Fang et al., 2001) have been loaded into liposomes in order to produce drug delivery systems.

In the present study two hydrophobic antibiotics, gramicidin and amphotericin B, and two hydrophilic antibiotics, ampicillin and nalidixic acid, where intercalated in layered double hydroxides. Surface active drugs like gramicidin and amphotericin B have been intercalated for the first time in LDHs by incorporating them in artificial membranes formed in the interlayer space of the inorganic host. Moreover, the LDH–cholate–gramicidin nanohybrid can be used as a model for membrane protein immobilization in LDHs as it is the first hydrophobic polypeptide

intercalated in this material. Ampicillin and nalidixic acid represent two major classes of antibiotics, penicillins and quinolones, which are widely used but have not previously been intercalated into LDHs. To examine the possibility of applying these LDH-antibiotic nanohybrids in drug delivery and controlled release systems, the release rates of the antibiotics were studied under different conditions.

## 2. Materials and methods

### 2.1. Reagents

Amphotericin B 80%, gramicidin D, nalidixic acid, sodium cholate hydrate 99%, 6-*O*-(*N*-heptylcarbamoyl)-methyl-α-D-glucopyranoside 90% (Hecameg) were purchased from Sigma. Ampicillin sodium salt 99% and 2,2,2-trifluoroethanol 99% were purchased from Fluka. 2-Amino-2-hydroxymethylpropane-1,3-diol (Tris) and dodecyl-β-D-maltoside were purchased from Biomol. All chemicals were used without further purification.

### 2.2. Synthesis of nitrate layered double hydroxides

Synthesis of layered double hydroxides was based on the protocol of (Miyata, 1975). About 200 ml of a solution containing 0.6 M $Mg(NO_3)_2$ and 0.3 M $Al(NO_3)_3$ and 200 ml of a solution containing 2 M NaOH were added dropwise to 200 ml of $H_2O$ with vigorous stirring and under nitrogen gas flow. The flow rate of the two solutions was adjusted so that the pH was equal to $10.0 \pm 0.5$ throughout the addition. All solutions were prepared using deionized water and were purged of $CO_2$ with nitrogen gas for 20 min. After the end of the addition the suspension of the synthetic LDH was aged for 1 day at room temperature to obtain small-sized particles (Kwak et al., 2004). The resulting LDH product was centrifuged at $8000 \times g$ for 15 min, washed twice with deionized and $CO_2$-free water and finally stored as a suspension to prevent aggregation of the crystals.

### 2.3. Intercalation of the antibiotics

All antibiotics were intercalated by simply mixing aqueous solutions of the antibiotic and the LDH. The solutions were prepared using deionized, $CO_2$-free water. After the reaction the nanohybrid products were collected by centrifugation at $12{,}000 \times g$ for 15 min, washed twice with water, filtered under vacuum and dried in air flow.

#### 2.3.1. LDH–cholate–gramicidin

Gramicidin D (15 mg, as a 10 mg/ml solution in 2,2,2-trifluoroethanol) was added in an aqueous solution of sodium cholate (0.65 g, 1.5 mmol) buffered at pH 9.0 with 10 mM Tris. Finally, the appropriate volume of the LDH suspension equal to 0.50 g of LDH-$NO_3$ was added (final reaction volume 50 ml) and the solution was stirred at room temperature overnight.

#### 2.3.2. LDH–cholate–amphotericin B and LDH–amphotericin B

Two amphotericin B nanohybrids were synthesized by using two different approaches: hydrophobic interaction with cholate micelles, as in the case of gramicidin, or direct ion exchange of the anionic form of the antibiotic. For the first nanohybrid, amphotericin B (100 mg, as a 25 mg/ml solution in DMSO) was added to an aqueous solution of sodium cholate (0.22 g, 0.5 mmol) buffered at pH 9.0 with 10 mM Tris. Then, 0.20 g of LDH-$NO_3$ suspension was added and the solution (final volume 50 ml) was stirred in the dark, at room temperature overnight.

To prepare the second nanohybrid, amphotericin B (125 mg, as a 25 mg/ml solution in DMSO) was added in an aqueous solution of the non-ionic detergent Hecameg (0.25 g, 0.75 mmol) buffered at pH 9.0 with 10 mM Tris. Then, 0.15 g of LDH-$NO_3$ suspension was added and the solution (final volume 50 ml) was stirred in the dark, at room temperature overnight.

#### 2.3.3. LDH–ampicillin

To prepare LDH–ampicillin, 0.74 g (2.0 mmol) of sodium ampicillin was dissolved in water containing 10 mM imidazole as a buffer. The pH of the solution was then adjusted to 9.0 and an appropriate amount of LDH suspension containing 0.20 g of LDH-$NO_3$ was added. The solution (final volume 40 ml) was stirred at room temperature for 4 days.

#### 2.3.4. LDH–nalidixic acid

To obtain LDH–nalidixic acid, 0.50 g (1.3 mmol) of the antibiotic was added to water containing 10 mM Tris as a buffer. A concentrated solution of NaOH was then added until the acid was completely dissolved (pH 10.5). Finally the appropriate volume of the LDH suspension equal to 0.30 g of LDH-$NO_3$ was added and the solution (final volume 80 ml) was stirred at room temperature for 4 days.

### 2.4. Characterization

Powder X-ray diffraction (XRD) patterns were recorded on a Rigaku RINT 2000 powder diffractometer, using Cu Kα radiation ($\lambda = 1.54$ Å) at 40 kV and 178 mA. Fourier transform infrared (FT-IR) spectra were recorded using KBr disks in a Perkin-Elmer 1760 X FT-IR spectrometer. Ultraviolet–visible (UV–vis) spectra were recorded by using an SLM-Aminco DW2000 spectrometer.

The amount of the intercalated antibiotics was measured by using UV–vis spectroscopy. An appropriate amount of each nanohybrid was suspended in 80% (v/v) ethanol in water (for amphotericin B, 80% v/v DMSO) containing 0.2 M HCl so that the final nanohybrid content was 50–500 μg/ml. The solution was then stirred until the LDH layers were completely dissolved and the absorption of each sample was measured at the characteristic wavelength of the absorbance maximum of the corresponding antibiotic. Final quantification of the antibiotics was based on a standard curve that was obtained under the same conditions. The selected wavelengths for the measurement of the acidified antibiotics were 270 nm for ampicillin, 282 nm for

Table 1
Antibiotic content and *d*-spacing values of each nanohybrid

| Nanohybrid | *d*-Values (Å) | | | Antibiotic content (%, w/w) |
|---|---|---|---|---|
| | $d_{003}$ | $d_{006}$ | $d_{009}$ | |
| LDH-$NO_3$ | 8.7 | 4.4 | 3.0 | – |
| LDH–cholate–gramicidin | 35.6 | 17.7 | 11.6 | 2.2 |
| LDH–cholate–amphotericin B | 35.9 | 17.6 | 11.6 | 2.7 |
| LDH–amphotericin B | ~34 | 17.0 | – | 9.7 |
| LDH–ampicillin | 20.7 | 10.2 | 6.9 | 51.7 |
| LDH–nalidixic acid | 22.3 | 10.9 | 7.6 | 40.0 |

gramicidin, 320 nm for nalidixic acid and 417 nm for amphotericin B.

### 2.5. Release rate determination

To measure the extent of release of the antibiotics, each nanohybrid was suspended in an appropriate solution buffered at pH 7.0 with 50 mM Tris and was stirred at room temperature. The amount of nanohybrid that was used varied from 40 μg/ml for LDH–nalidixic acid to 1.0 mg/ml for LDH–cholate–gramicidin. At specified time intervals a 1.5 ml sample was removed, centrifuged for 1 min and the resulting supernatant was filtered through a 0.2 μm membrane filter. The absorbance of the filtrate, at the characteristic wavelength for each antibiotic, was measured and plotted against time. The selected wavelengths for the measurement of the antibiotics at neutral pH were 257 nm for ampicillin, 283 nm for gramicidin, 335 nm for nalidixic acid and 415 nm for amphotericin B.

## 3. Results

### 3.1. Characterization

Successful intercalation of each antibiotic into the LDH host is demonstrated by the XRD diagrams of the nanohybrids (Fig. 2). During ion exchange of the nitrate anions, the layers of LDH expand to host the antibiotic anions and this expansion is reflected by the *d*-spacing values that are calculated from the mean value of the first, second and third order peaks of the XRD diagrams (Table 1). These values are 20.6 Å for LDH–ampicillin, 22.3 Å for LDH–nalidixic acid and about 34 Å for LDH–amphotericin B. In the case of gramicidin or amphotericin B co-intercalation with cholate anions, the XRD patterns were almost identical (Fig. 2c) with a *d*-spacing of 35.3 Å.

The presence of the antibiotics in the nanohybrids can be verified by infrared spectroscopy. Fig. 3 presents the infrared spectra of LDH–ampicillin and LDH–nalidixic acid. The characteristic β-lactam $\nu_{C=O}$ of ampicillin is evident in the LDH–ampicillin spectrum at 1763 $cm^{-1}$ along with the amide $\nu_{C=O}$ at 1655 $cm^{-1}$ (Di Stefano et al., 2002). Additionally, the antisymmetric and symmetric stretching vibrations of the carboxylate group appear at 1584 and 1397 $cm^{-1}$, respectively. LDH–nalidixic acid shows a strong absorption at 1627 $cm^{-1}$ attributed to the stretching vibration $\nu_{C=C}$ of the aromatic rings (Neugebauer et al., 2005). The stretching vibration of the ketone at C4 appears at

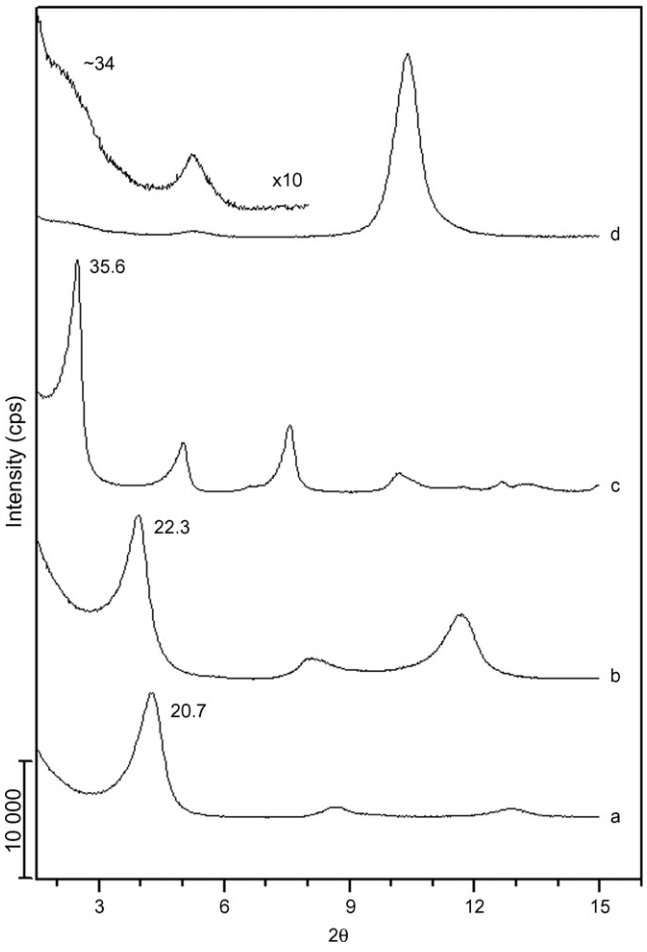


Fig. 2. X-ray diffraction patterns of the nanohybrids: (a) LDH–ampicillin, (b) LDH–nalidixic acid, (c) LDH–cholate–gramicidin, and (d) LDH–amphotericin B.

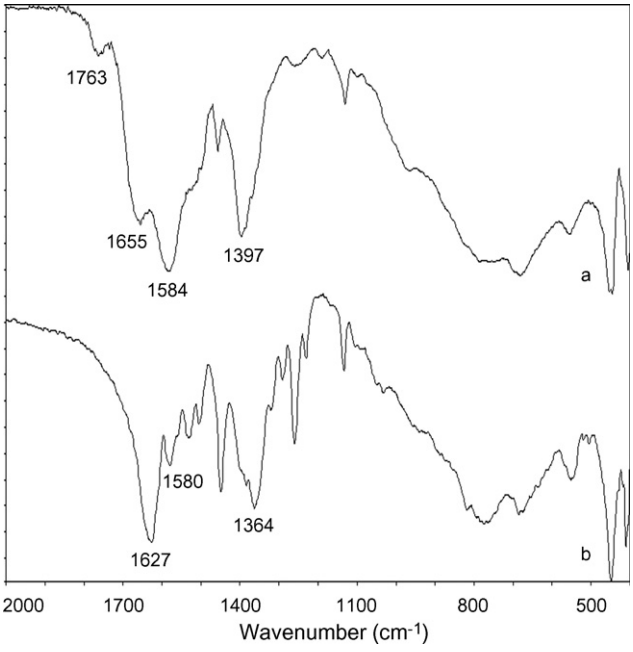


Fig. 3. Infrared spectra of (a) LDH–ampicillin and (b) LDH–nalidixic acid.

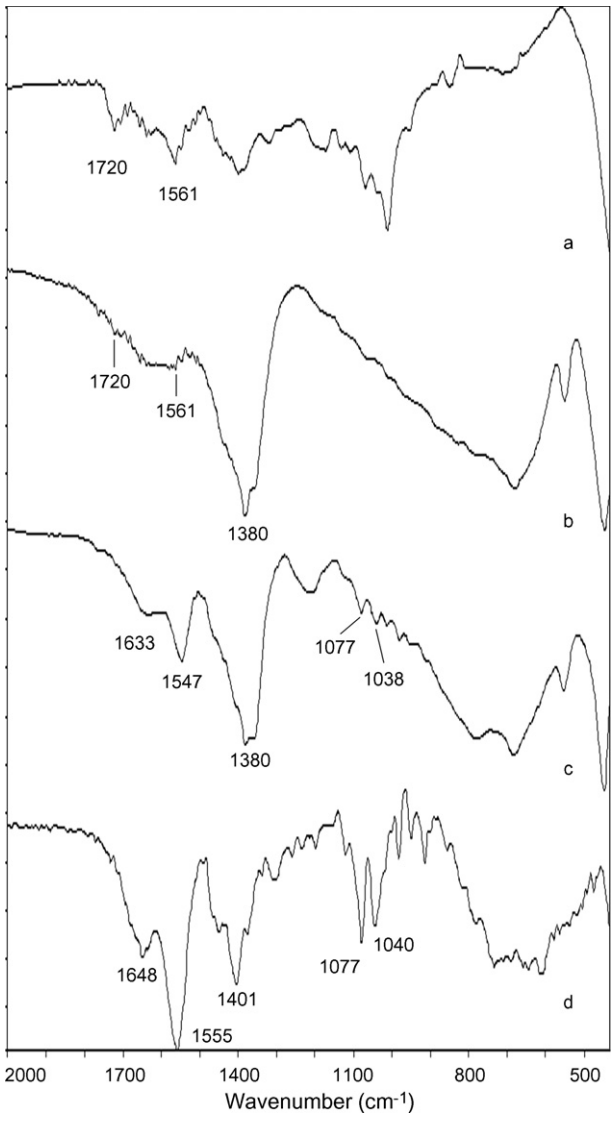


Fig. 4. Infrared spectra of (a) amphotericin B, (b) LDH–amphotericin B, (c) LDH–cholate–amphotericin B, and (d) sodium cholate.

1580 $cm^{-1}$, while the symmetric stretch of the carboxylate group appears at 1364 $cm^{-1}$.

The infrared spectra of amphotericin B, LDH–amphotericin B and LDH–cholate–amphotericin B are shown in Fig. 4. The characteristic peaks at 1720 and 1561 $cm^{-1}$ attributed to the stretching vibrations of the C=O and C=C bonds, respectively (Gagoś et al., 2005), are seen in both spectra. In addition, there is a peak at 1380 $cm^{-1}$ in the spectrum of LDH–amphotericin B due to nitrate anions that were not exchanged. When amphotericin B and sodium cholate are co-intercalated the characteristics of the IR spectrum of cholate anion are dominant. Thus, the two peaks at 1547 and 1633 $cm^{-1}$ of the anionic carboxylate group and the peaks at 1077 and 1038 $cm^{-1}$ that are evident in the LDH–cholate–amphotericin B spectrum (Fig. 4c), are characteristic of the anionic form of cholic acid as shown in Fig. 4d.

The characteristic electronic absorption spectrum of each antibiotic is also observed in the nanohybrids (Fig. 5). The

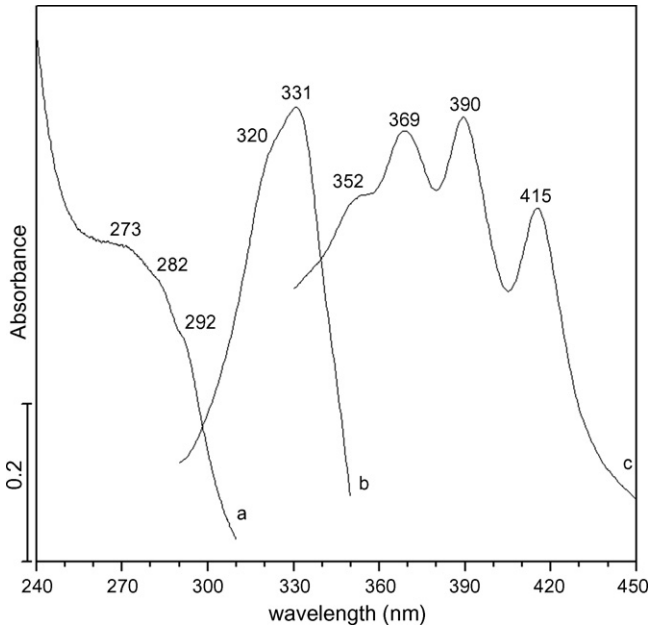


Fig. 5. UV–vis spectra of (a) LDH–cholate–gramicidin, (b) LDH–nalidixic acid, and (c) LDH–amphotericin B.

three overlapping peaks of gramicidin around 280 nm are visible in the spectrum of LDH–cholate–gamicidin (Fig. 5a), while LDH–nalidixic acid (Fig. 5b) shows an absorption maximum at 331 nm with a shoulder at about 320 nm. Amphotericin B absorbs visible light in the region of 400 nm. The yellow colored nanohybrid LDH–cholate–amphotericin B (Fig. 5c) shows the characteristic absorption pattern of the antibiotic with maxima at 352, 369, 390 and 415 nm. The same spectrum was obtained for LDH–amphotericin B without intercalated cholate.

UV–vis spectroscopy was also applied to determine the quantity of the antibiotics in each nanohybrid. As shown in Table 1 the two hydrophobic antibiotics are present in the nanohybrids in small amounts, 2.2% (w/w) for gramicidin, 2.7 and 9.7% (w/w) for amphotericin B. In the case of ampicillin and nalidixic acid the antibiotic content is much higher, 51.7 and 40.0% (w/w), respectively. It should be mentioned that during nalidixic acid determination, the species detected at 320 nm is the complex of nalidixic acid with $Mg^{2+}$ that is produced by the dissolution of the LDH layers (Park et al., 2000; Turel, 2002). To compensate the wavelength shift, 5 mM $MgCl_2$ was added to each sample measured for preparation of the standard curve.

### 3.2. Release rate determination

The intercalation of the antibiotics into the layers of LDHs is reversible and thus, when the nanohybrids are suspended in the proper medium the antibiotics are released. The release occurs either in acidic medium, where the metal hydroxide layers of the LDH are dissolved, or in the presence of anions that exchange with the hosted drugs. The hydrophobic antibiotics were released in a solution of 0.5% (w/v) dodecyl mal-

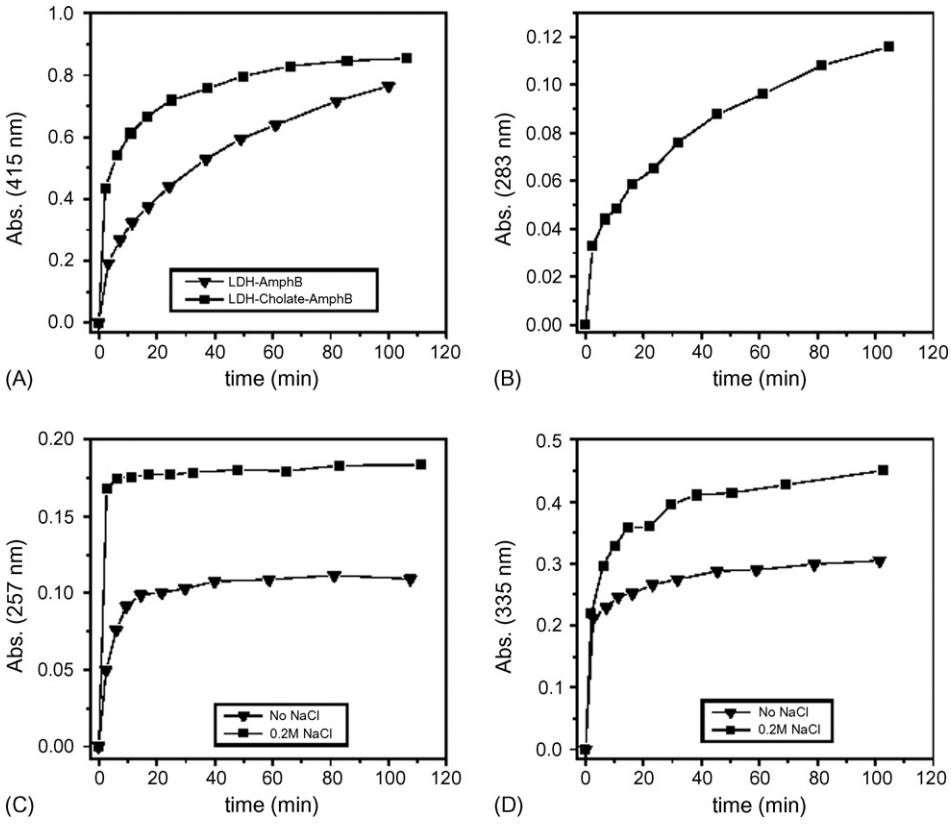


Fig. 6. Release profiles of the antibiotics: (A) amphotericin B, (B) gramicidin, (C) ampicillin, and (D) nalidixic acid, from the corresponding nanohybrids.

toside with the addition of 0.2 M NaCl in the case of the two LDH–amphotericin B nanohybrids and without NaCl in the case of LDH–cholate–gramicidin. In order to compare the release rate of amphotericin B from the two nanohybrids, the proper amounts of LDH–amphotericin B and LDH–cholate–amphotericin B were used to equalize the final antibiotic concentration. The release profiles of these antibiotics are seen in Fig. 6A and B. The release of ampicillin and nalidixic acid was studied in the presence of 0.2 M NaCl or without NaCl. The results are presented in Fig. 6C and D, respectively.

## 4. Discussion

### *4.1. LDH–cholate–gramicidin*

Gramicidin is hydrophobic, has no charged amino acids, and even the terminal groups of the backbone are modified and bear no charge. Therefore gramicidin cannot be intercalated directly into the LDH via anion-exchange. For this reason a two-step strategy was used to incorporate gramicidin in LDH; first gramicidin was solubilized in a negatively charged micelle and second, the micelles were intercalated into the LDH via anion-exchange. Gramicidin was first dissolved in 2,2,2-trifluoroethanol in order to be single stranded and then form helical dimers in the micelles (Wallace, 1998). Besides sodium cholate, gramicidin was solubilized in SDS micelles and mixed micelles of non-ionic surfactants with palmitic acid resulting in LDH–SDS–gramicidin and LDH–palmitate–gramicidin nanohybrids, respectively (results not shown). In all cases the characterization gave similar results with the ones presented for LDH–cholate–gramicidin in this study.

The *d*-spacing of 35.3 Å obtained from the XRD diagram of the nanohybrid LDH–cholate–gramicidin shows the formation of a bilayer of cholate molecules in the interlayer between the inorganic layers. A similar *d*-spacing value is observed for LDH–cholate (33.9 Å, not shown) and for LDH–deoxycholate (Ogawa and Asai, 2000) (32.9 Å). Gramicidin is probably hosted in this bilayer of cholate molecules that form an artificial membrane in the LDH. The XRD pattern shows the formation of a cholate bilayer but does not give evidence for the presence of gramicidin in it. The characterization with UV–vis spectroscopy proves the incorporation of gramicidin in the bilayer as it reveals the characteristic spectrum of the tryptophan residues of the polypeptide chain in the region of 280 nm.

The release of gramicidin depends on the medium where the nanohybrid is suspended. When deionized water is used there is no release at all, while in ethanol, which is a good solvent for gramicidin, the release is almost complete in the first 2 min. A slow release profile is obtained only in the solution containing 0.5% (w/v) dodecyl maltoside (∼15 mM). Since the CMC of dodecyl maltoside is 0.15 mM the release medium consists of detergent micelles. These results show that using this nanohybrid in a controlled release system would lead to the specific release of gramicidin in biological membranes, which is the targeted domain for its action, and not in the bulk solution.

### 4.2. LDH–cholate–amphotericin B and LDH–amphotericin B

The two-step strategy that was used for gramicidin, as described in the previous section, was also applied in order to synthesize LDH–cholate–amphotericin B. The difference in the case of amphotericin B is that it has a carboxylic group and thus can also be intercalated directly into LDHs. For that purpose the antibiotic was solubilized in the non-ionic surfactant Hecameg and the subsequent anion-exchange reaction resulted in the formation of LDH–amphotericin B. The surfactant was not intercalated in the final product as shown by the absence of any characteristic peaks in the IR spectrum.

As in the case of gramicidin, when cholate is co-intercalated, the antibiotic cannot be detected by XRD and IR because the characteristics of cholate dominate. Thus, the *d*-spacing of 35.3 Å corresponds to the bilayer of cholate molecules while the peaks at 1633, 1547, 1077 and 1038 $cm^{-1}$ observed in the IR spectrum are typical for anionic cholate and they are also seen in the spectra of sodium cholate and LDH–cholate. However, the presence of the antibiotic is obvious from the yellow color of the nanohybrid and the characteristic visible absorption spectrum. The absorption spectrum gives additional information about the aggregation state of amphotericin B. When amphotericin B is aggregated the peak height ratio is altered and the maximum is observed around 340 nm (Hargreaves et al., 2006). These changes are not observed in the nanohybrid that exhibits a spectrum similar to that of monomeric amphotericin B dissolved in organic solvent.

Cholate is absent in LDH–amphotericin and it does not interfere with the XRD and IR characterization. The large diffraction peak around 11° is due to nitrate that has not been exchanged but, as shown in the enlarged region in Fig. 2d, there is a clear peak at 17.0 Å which is apparently a second order peak and the first is the shoulder around 34 Å. This expansion of the LDH layers is convincing evidence for the intercalation of the antibiotic. The infrared spectrum of LDH–amphotericin B, compared with the spectrum of amphotericin B in Fig. 4, gives further evidence of the presence of the antibiotic because there are peaks in the region of 1550–1750 $cm^{-1}$ that are attributed to amphotericin B vibrations. In addition, the characteristic visible absorption spectrum of monomeric amphotericin B is also observed for this nanohybrid.

A solution containing both micelles and salt is required for the release of amphotericin B because it is electrostatically associated with the inorganic layers of the LDH. Suspension of the nanohybrids in water or organic solvents such as ethanol or DMSO does not result in release of the antibiotic. Thus, as in the case of the gramicidin containing nanohybrid, the antibiotic is released specifically in biological membranes and not in the bulk solution. As shown in Fig. 6A, the release is faster from the cholate containing nanohybrid despite the fact that it contains a smaller amount of amphotericin B. It is likely that the deintercalation of cholate molecules, due to exchange by $Cl^-$, facilitates the release of the antibiotic.

### 4.3. LDH–ampicillin and LDH–nalidixic acid

Ampicillin and nalidixic acid were intercalated with simple anion-exchange reactions simply by mixing the antibiotic and LDH solutions. The antibiotic content of both nanohybrid products was high, 51.7 and 40.0% (w/w) for ampicillin and nalidixic acid, respectively, which corresponds to almost complete exchange of the nitrate ions of the starting LDH material. Surprisingly, although ampicillin is a larger molecule than nalidixic acid (Fig. 1), the *d*-spacing value is greater for LDH–nalidixic acid (22.3 Å) than for LDH–ampicillin (20.6 Å). This discrepancy can only be interpreted by assuming a bilayer formation for nalidixic acid and a monolayer for ampicillin, as shown in Fig. 7.

The IR spectrum of ampicillin shows many characteristic peaks of the antibiotic. These are the carbonyl group of the β-lactam, the amide carbonyl and the ionized carboxylic group. According to the spectrum the ampicillin molecule is intercalated intact and in the ionized, negatively charged form. The case of LDH–nalidixic acid is more complicated because a large number of peaks appear in the region from 1300 to 1700 $cm^{-1}$ of the IR spectrum. In addition to the peaks labeled on the spectrum in Fig. 3, many unassigned bands still remain. The multiplicity of bands in this region of the spectrum indicates a diversity of carboxylic groups in the interlayers. A similar situation was observed in fatty acid intercalated LDHs (Borja and Dutta, 1992) where both protonated and ionized fatty acids were present.

The UV–vis spectrum of nalidixic acid is significantly affected by the state of the carboxylic group (Song et al., 1999). The absorption spectrum of LDH–nalidixic acid suspended in

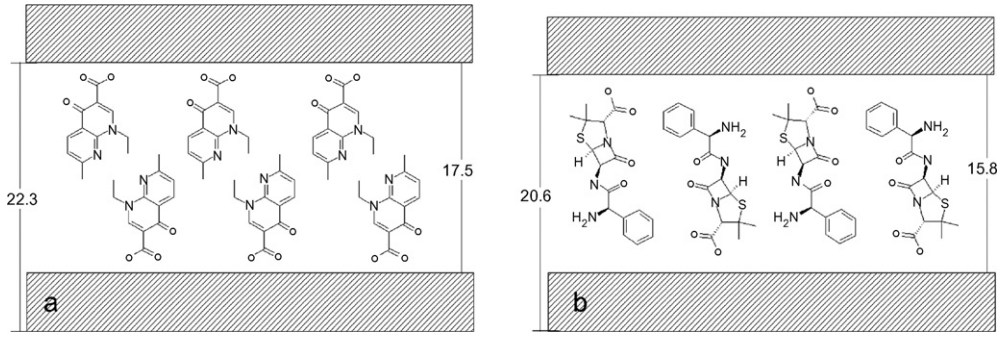


Fig. 7. Schematic representation of the antibiotic conformation in the interlayer space of the nanohybrids based on the XRD data: (a) LDH–nalidixic acid and (b) LDH–ampicillin.

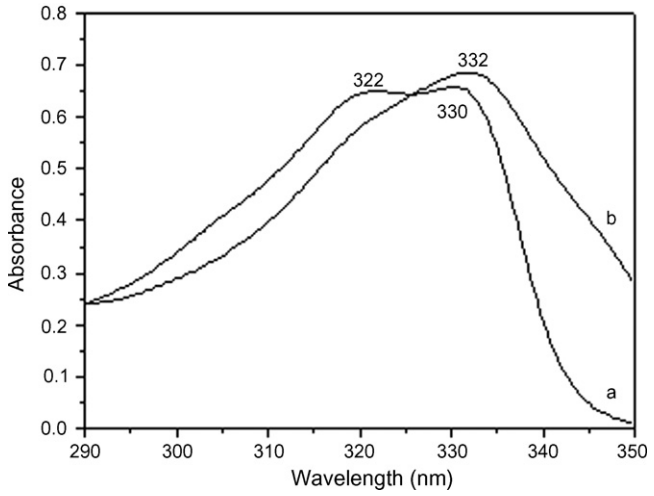


Fig. 8. Comparison of the UV–vis absorption spectra of: (a) nalidixic acid in ethanol and (b) LDH–nalidixic acid in ethanol.

ethanol does not resemble to the typical nalidixic acid spectrum (Fig. 8). On the contrary, it resembles to the spectrum of nalidixic acid in aqueous solution at neutral pH, which is a mixture of both ionized and protonated molecules. Thus, the UV–vis and IR spectra are in agreement and suggest that the neutral and anionic forms of nalidixic acid coexist in the interlayer of the nanohybrid.

The release of the hydrophilic antibiotics occurs by simply suspending the nanohybrids in an aqueous solution containing NaCl. The release of ampicillin is faster than that of nalidixic acid as shown in Fig. 6C and D. This is probably due to higher solubility and greater antibiotic content of LDH–ampicillin compared to LDH–nalidixic acid. Even when the nanohybrids are suspended in plain deionized water buffered at pH 7.0 a significant release is observed. Presumably, even the small amount of $Cl^-$ added to adjust the pH, along with $HCO_3^-$ coming from the atmospheric $CO_2$ are enough to exchange some of the antibiotic. The release in plain water is more extensive in the case of nalidixic acid as there are protonated antibiotic molecules in the interlayer, which are easily released by diffusion and not ion exchange.

## 5. Conclusion

The intercalation and release of the antibiotics gramicidin, amphotericin B, ampicillin and nalidixic acid was studied. The different approaches used for the synthesis of each nanohybrid resulted in successful intercalation in all four cases. Layered double hydroxides have been used as inorganic drug carriers for many different pharmaceutically active compounds and, as shown in this study, they can also serve as carriers for several types of antibiotics. The drug delivery properties of LDHs along with the possibility of targeting through surface modification make these nanohybrids very promising antimicrobial agents. Additionally, the methods presented here can be applied for the formation of nanohybrid materials using almost any biomolecule, ionic or non-ionic, water soluble or hydrophobic.

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