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# New Layered Double Hydroxides/Phospholipid Bilayer Hybrid Material with Strong Potential for Sustained Drug Delivery System

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A new supra-organized hybrid material obtained in aqueous medium via anionic exchange of self-assembled unilamellar anionic liposomes with the nitrate ions present in the interlayers of layered double hydroxides (LDH) is fully characterized. This material presents original properties linked to the simultaneous presence of a phospholipid bilayer derived from liposomes, still used as vectors for lipophilic drugs, and LDH, which protects the bilayer and brings about a pH sensitivity. The exchange rate is controlled via the added amount of liposomes. TGA, XRD, and TEM confirm the organization of the entrapped phospholipids as a bilayer. The presence of the latter allows the material to load lipophilic and neutral drugs, which represent the largest fraction of those newly synthesized. Furthermore, in physiological conditions, preliminary tests show a sustained release of phospholipids (1.5% for 7 days and 6% for 14 days), whereas a fluorescent lipophilic drug-mimic reveals the reorganization of the phospholipids into liposomes in the release medium. In the field of biocompatible materials, these new hybrid particles have a strong potential for the storage and sustained release of neutral or lipophilic drugs.

## Introduction

Recently, hybrid structures have attracted considerable interest because of their original physicochemical properties and much attention has been focused on their use as carriers for gene or drug delivery.[1−4] Layered double hydroxides (LDH) are among the most promising inorganic host structures available for the preparation of hybrid materials.[5−7] LDH are made of positively charged mixed metal hydroxides layers exhibiting a brucite-like structure ($Mg(OH)_2$), in which some trivalent cations are isomorphically substituted for divalent ones. These layers are held together by intercalated anions, and water molecules fill the remaining interlayer volume. The ideal formula for an LDH is $\{M^{II}_{1-x}M^{III}_x(OH)_2\}\{(A^{n-})_{x/n}\cdot m_{H_2}O\}$, in which $x$ ranges between 0.2 and 0.33 and $M^{II}$ (Mg, Zn, Ca, etc.) and $M^{III}$ (Al, Fe, Mn, etc.) stand for various divalent and trivalent metal cations, respectively. A variety of inorganic and organic anions are introduced in the interlayer space, either during the synthesis of the LDH or in an anion exchange reaction, or else by reconstruction of the thermally decomposed LDH contacted with a solution of the desired anionic species, using the so-called memory effect.[8]

Properties of LDH such as pH sensitivity, anionic exchange capacity (AEC), and biocompatibility may be tuned following changes in their composition. LDH sensitivity to pH depends of the composition of the metal hydroxide layers. For example, a Mg/Al LDH corresponding to the natural mineral hydrotalcite has been widely used for its stability at physiological pH, whereas Zn/Al LDH was used for applications needing stability at more acidic pH. Anionic exchange of LDH used as a host appeared highly suitable for the preparation of composites because of its versatility. It was successfully used for instance for the intercalation of negatively charged functional biomolecules.[9] Moreover, the interlayer space can be expanded to accommodate anionic species of large size, and an adjustment of the composition, i.e., the $M^{II}/M^{III}$ molar ratio, allows the control of the AEC. LDH organic hybrids are well-adapted for applications in the field of pharmacy.[10] This is not only because of their biocompatibility (e.g., Mg/Al LDH)[11] but also to the

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anionic exchange done in an aqueous medium, avoiding so the use of toxic solvents. For instance, mammalian cells were efficiently transfected thanks to LDH intercalated with anionic biomolecules.[12,13] As the preparation of these LDH based hybrid materials occurred under mild conditions (room temperature, soft stirring), it could be extended to incorporate fragile or sensitive guest entities such as peptides,[14] enzymes,[15] metal-containing complexes,[16] pharmaceutical drugs alone[17−19] or loaded into anionic micelles,[20] natural biopolymers (alginate, carrageenan and pectin),[21] or DNA.[22]

However, in the pharmaceutical domain, many recent drugs of interest are poorly water-soluble and neutral, properties that preclude their easy formulation and hence their biodisponibility. So improvements in their solubilization are needed to develop suitable pharmaceutical formulations. Several approaches were developed to improve the overall drug solubility: introduction of polar or ionizable groups, use of soluble prodrugs or polymorphous and amorphous forms of the drug, complexation methods, or solid dispersions; or formulation into the phospholipid bilayer of liposomes.[23,24] The latter vesicles include a water pool surrounded by one or several phospholipid bilayers. They are biodegradable and biocompatible and act as drug carriers in pharmaceutical preparations.[25] Furthermore, because of their constitution, they may carry hydrophilic drugs dissolved in the aqueous core, as well as hydrophobic drugs solubilized in the lipid bilayer. Liposomes, however, present a major drawback for drug delivery applications as they are unstable in physiological media and cannot be used as vectors in sustained release. Therefore, the intercalation of lipophilic drug-loaded phospholipid bilayers between the inorganic LDH layers was expected to lead to a new class of hybrid material affording protection and stabilization of the bilayer and of the drug over time, as well as presenting a strong potential for sustained release.

The goal of the present work was then to explore the intercalation into the guest $NO_3$−Mg/Al of lipid bilayers originating from liposomes made from 1,2-dimyristoyl-*sn*-glycero-3-phosphate monosodium salt (DMPG). The latter liposomes were loaded with a neutral lipophilic fluorescent probe (1,6-diphenyl-1,3,5-hexatriene), (DPH) mimicking a lipophilic drug. Such a host−guest system was expected to achieve the sustained release of the drug by the in situ degradation of phospholipids or by the slow erosion of the LDH layer at physiological pH after subcutaneous administration, for example. Several such self-assembled hybrid materials were obtained by varying the amount of intercalated phospholipids (from 6.7 to 100% of the AEC of the LDH) and then fully characterized. Finally, release experiments of phospholipids were carried out in physiological conditions, taking advantage of the fluorescence of the model drug DPH previously loaded into the phospholipid bilayer to monitor the process.

## Experimental Section

**Liposome Preparation.** For the synthesis of liposomes, a suitable amount of 1,2-dimyristoyl-*sn*-glycero-3-phosphate monosodium salt (DMPG), a negatively charged phospholipid, (Figure 1) (Lipoïd, Germany) with a sol−gel transition phase temperature of 23.5 °C was dissolved in a chloroform:methanol mixture (3:1). After complete removal of the solvents at 40 °C under reduced pressure, a NaCl solution (5 mM, pH 7.4) was added in order to obtain a lipid suspension (10 mg $mL^{-1}$).[26] After a 12 h hydration step at room temperature, multilamellar liposomes were formed. To obtain unilamellar liposomes, this suspension was extruded above 25 °C (extruder Lipex Biomembranes Inc., Canada) using polycarbonate membranes (GE water and process technologies, US) with mean pore size diameters of 400, 200, and 100 nm, successively.

**LDH Precursor and Hybrid Preparation.** The host $NO_3^-$Mg/Al (hereafter named LDH) was synthesized by a conventional coprecipitation method under ambient atmosphere:[27] 38.3 g of $Mg(NO_3)_2 \cdot 6H_2O$ (99%, Aldrich) and 28.0 g of $Al(NO_3)_3 \cdot 9H_2O$ (Fluka) ($Mg^{2+}/Al^{3+}$ molar ratio of 2) were dissolved in 250 mL of deionized water. This solution was added dropwise at a rate of 2 mL $min^{-1}$ into a beaker. Simultaneously, a NaOH solution (2 M) was added to the same beaker at a controlled rate to maintain the pH close to 10 using a pH-STAT Titrino (Metrohm, France) apparatus. After complete precipitation, the gel obtained was refluxed at 80 °C for 12 h. It was then repeatedly washed at 25 °C (cycles of suspension in distilled water followed by centrifugation, Sigma 2K15 centrifuge, Fisher Bioblock Scientific, France) and finally dried overnight at 80 °C to give a homogeneous dry powder.

Several attempts were performed to optimize the phospholipid intercalation by (i) anionic exchange of the LDH with a solution of phospholipids in chloroform or (ii) anionic exchange of the LDH with the liposome suspension or (iii) reconstruction of the calcined LDH in contact with the liposome suspension. These experiments were performed using unilamellar liposomes with a mean diameter of about 100 nm in a NaCl aqueous

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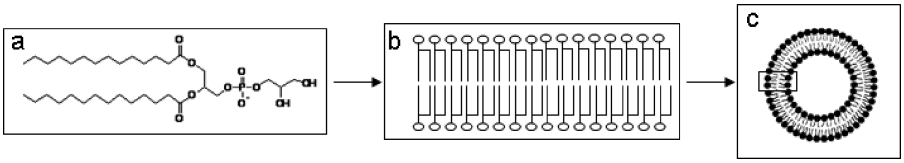


**Figure 1.** Schematic representation of (a) DMPG phospholipid, (b) phospholipids under bilayer organization, and (c) unilamellar liposome.

solution (5 mM) at pH 7.4. The amounts of phospholipids or liposomes corresponded to 6.7, 13.3, 20, 40, 60, and 100% of the AEC for each method. For the anionic exchange in chloroform, 200 mg of LDH were contacted during 1 h with the required amount of phospholipids under stirring. For anionic exchange with an aqueous suspension of liposomes, 200 mg of LDH were added to the required amount of the liposome suspension (i.e., 32 mL of liposome suspension at 10 mg $mL^{-1}$, for 60% of the AEC). The reconstruction process was performed by contacting 200 mg of LDH previously calcined at 450 °C for 5 h with an aqueous suspension of 100 nm liposomes at 40 °C. In any case, the solids were then separated by centrifugation (10 min at 10 000 g), washed three times with the NaCl solution and dried for 24 h at 40 °C. The resulting samples were hereafter named DMPG-*x*-Mg/Al where x represents the expected DMPG content in percent of the AEC (6.7, 13.3, 20, 40, 60, and 100%).

**Liposome Characterization.** The liposome mean size was measured by quasi-elastic light scattering (QLS) at a 90° angle with an excitation wavelength at 633 nm (SEMATECH, SM 633/RTG, France). The determination of the phospholipid content requires the destruction of the phospholipids and the mineralization of phosphorus by a strongly oxidant acid (the most suitable being $HClO_4$).[28] Inorganic phosphor is then converted to phosphomolybdate by addition of ammonium molybdate, which is then quantitatively reduced by 1-amino-2-naphtol-4-sulfonate (Fiske and Subbarow reagent). The intensity of the blue color is determined by spectrophotometry either at 830 nm (high sensitivity) or 660 nm (low sensitivity). The thickness of the phospholipid bilayer was determined by X-ray diffraction using a D8 Advance X-ray diffractometer (Bruker, Germany) on a powder of dried liposome suspension (see experimental conditions for LDH).

The characterization of the phospholipid bilayer involved steady state fluorescence measurements as well as the monitoring of the fluorescence anisotropy of the lipophilic DPH probe.[29] In order to control the phospholipid organization of the liposome, especially during the release, a small volume of a solution of DPH dissolved in tetrahydrofuran (SDS, Peypin, France) (0.25 mg $mL^{-1}$) was added to the DMPG liposome suspension to reach a DPH:DMPG ratio of 1:200. The suspension was then stirred for 1 h at 45 °C (above the high-order transition temperature of the phospholipids) in order to fully load the DPH probe into the DMPG bilayer.

Steady-state fluorescence depolarization experiments were performed in a spectrofluorimeter (Shimadzu RF 5310, France) equipped with polarizers (UV−vis) in the excitation and emission beams. The excitation and emission wavelengths were set to 360 and 430 nm, respectively. The relative intensities for the two combinations of vertically and horizontally polarized excitation and emission beams were recorded in the ratio mode in order to eliminate source intensity fluctuations. The steady-state emission anisotropy r was calculated as

$$r = 2p/(3-p)$$

where

$$p = (I_{||} - I_{\perp}G)/(I_{||} + I_{\perp}G)$$

The *G* factor corrects for the unwanted polarization due to the spectrometer optics.[30,31] There was no contribution due to light scattering because dilution of the DPH-labeled vesicles had no effect on the measured fluorescence anisotropy. The temperature in the cell was monitored with an immersed thermistor during the experiments. After cooling to 17 °C, $I_{||}$ and $I_{\perp}$ data were collected in triplicate as the sample temperature was increased from 17 to 45 °C at a rate of 1 °C $min^{-1}$.

**LDH Precursor and Hybrid Characterization.** Chemical analyses were carried out by inductively coupled plasma atomic emission spectroscopy (ICP-AES) in the Central Analysis Service of the CNRS (Solaize, France). X-ray diffraction (XRD) patterns were recorded on a D8 Advance X-ray diffractometer (Bruker, Germany) with a $\theta-\theta$ Bragg−Brentano geometry using the Cu K$\alpha$1 radiation ($\lambda$ = 1.542 Å, 40 kV and 50 mA) and equipped with a scintillation counter detector and an air-scatter screen serves as a device to reduce the background caused by air scattering. The duration of a step was 1 s (step: 0.020°). TGA-DTG experiments were carried out using a TG 209 C (Netzsch, Germany) thermogravimetric analyzer at a typical heating rate of 5 °C $min^{-1}$ from room temperature up to 800 °C in a stream of synthetic air (5/95) (flow rate: 20 mL $min^{-1}$). The IR spectra (Bruker, Equinox 55 with SPECAC diffuse reflectance cell) were collected at room temperature. The size of the dried hybrid powder was evaluated by laser granulometry using a Mastersizer 2000 (Malvern, UK). Transmission electron microscope (TEM) analysis was performed with a H-800 apparatus (Hitachi, Japan), using an accelerating voltage of 200 kV. The powder samples were scraped from the spin coated LDH film under a microscope, diluted and dispersed in water, and finally pipetted onto uncoated copper TEM grids.

**In vitro Release Study.** To monitor the phospholipid release from the LDH hybrid material, we prepared a batch of liposomes loaded with the fluorescent lipophilic probe (DPH) mimicking the drug. A small amount of DMPG-60-Mg/Al (20 mg, loaded with DPH) was added to a flask containing 10 mL of phosphate buffer solution (150 mM, pH 7.4, 37 °C) and set under soft stirring. At selected contact times, 1, 4, 24, and 36 h and 7−14 days, the suspension was centrifuged (3 min at 1600 g) and the supernatant was analyzed first by steady-state DPH fluorescence depolarization in order to control the release of phospholipid organized or not as a bilayer, and second by quasi light scattering in order to measure the size of the released

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objects. The solid residue was controlled after a drying step (50 °C overnight) using XRD and TGA-DTG analysis.

## Results and Discussion

**Liposome Characterization.** The average size of the liposomes is 105 nm and their polydispersity index (p.i.) is valued at 0.151 using a monomodal analysis. It corresponds to an unilamellar form (Figure 1) with a phase transition temperature of 23.5 °C determined using their temperature-dependent fluorescence anisotropy as described in the literature. The phospholipid concentration in the final liposome preparation is 9.5 mg $mL^{-1}$.

**X-ray Diffraction.** The XRD pattern of dried DMPG liposomes is characteristic of a layered structure (Figure 2A). Up to 14 Bragg orders can be detected for the reflections on the lamellar bilayer accounting for the regular stacking of the dried DMPG structure. The position of the peaks corresponds to a lamellar repeat distance of 4.47 nm, slightly lower than the 4.79 nm reported by Tajima et al. for bilayer structures of NaDMPG.[32] However, the dissymmetric shape of the peaks toward low 2Θ angles suggests that domains with interlayer distances larger than 4.47 nm could also exist for DMPG in the present study.

The $NO_3$−Mg/Al host sample displays an XRD pattern typical of the LDH structure (Figure 2B). The diffraction peaks are indexed based on a hexagonal unit cell with a $R\bar{3}m$ rhomboedral symmetry. The two sharp and symmetric diffraction peaks appearing below 25° 2Θ can be ascribed to the (003) and (006) reflections. Their position allows calculating an interlayer distance of 0.88 nm, which is in agreement with the presence of nitrate anions.[33]

The powder XRD patterns of the hybrid materials (DMPG-20-Mg/Al) obtained following the three synthesis methods referred to above are displayed in Figure 3. The sample prepared following the reconstruction process from the Mg(Al)O mixed oxide contacted with an aqueous solution of DMPG liposome exhibits the XRD pattern of the lamellar structure (Figure 3(c)). The position of the (003) reflection leads to an interlayer distance of 0.75 nm accounting for intercalation by $Cl^-$ anions instead of DMPG as confirmed by the absence of (00*l*) reflections in the low 2Θ range (data not shown). On the other hand, XRD patterns of the hybrid material obtained by anionic exchange with a DMPG liposome suspension (Figure 3a) or with a DMPG chloroform solution (Figure 3b), both displayed new reflection peaks in the low 2Θ domain (see inset in Figure 3) characteristic of the presence of DMPG bilayers.

The anionic exchange of unilamellar liposome procedure was however preferred to restrict the use of an organic solvent during the hybrid material preparation. Thus, this procedure was used for the rest of the study.

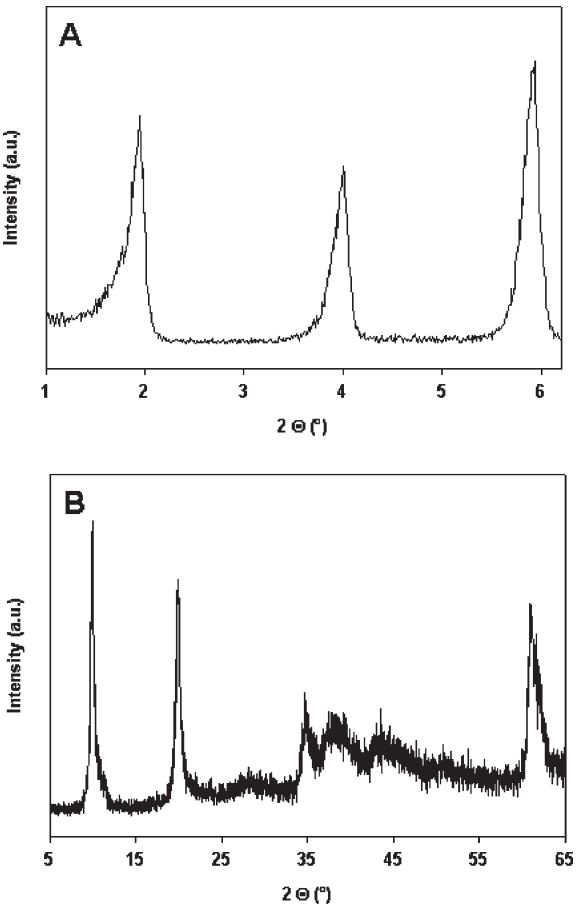


**Figure 2.** Powder XRD patterns (A) in the low 2Θ domain for DMPG and (B) in the large 2Θ domain for LDH.

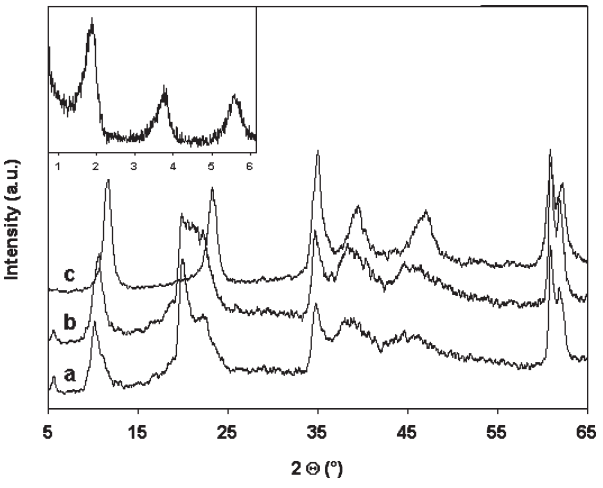


**Figure 3.** Powder XRD patterns in the large 2Θ domain for hybrid materials resulting from 3 different synthesis methods with an AEC of 20%: (a) LDH exchanged with a suspension of DMPG liposomes in NaCl, (b) LDH exchanged with a solution of DMPG in chloroform, (c) calcined LDH reconstructed with an aqueous DMPG suspension. In insert, powder XRD pattern in the low 2Θ domain for LDH exchanged with a suspension of DMPG liposomes in NaCl (a) or a solution of DMPG in chloroform (b).

Hybrid materials with different DMPG exchange rates have been characterized and as expected, several important changes occur in the XRD patterns of the DMPG-Mg/Al hybrids compared to that of the LDH host, as reflections appear in the 2Θ range from 1 to 7°

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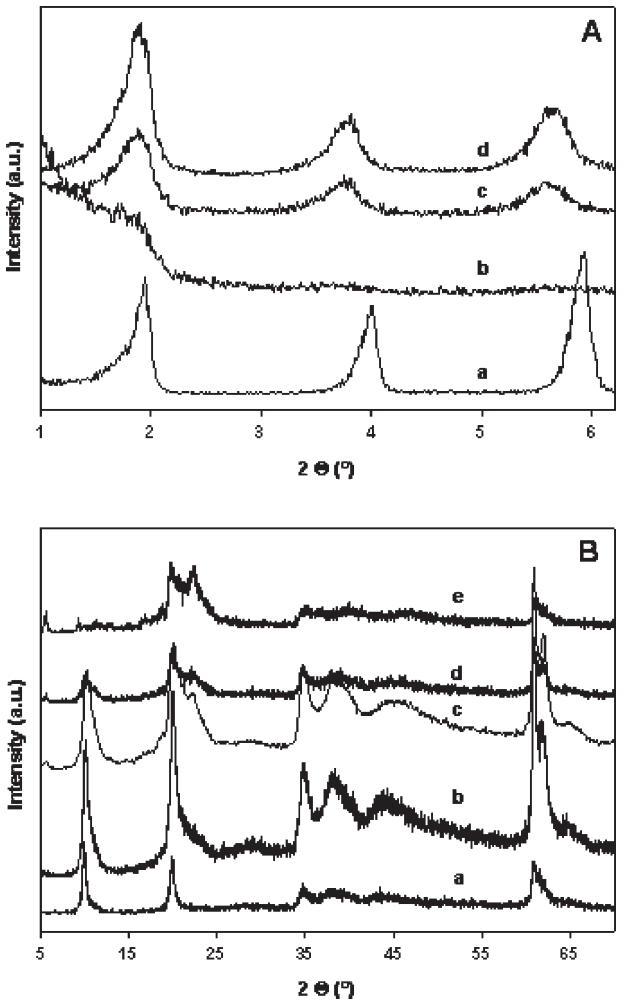


**Figure 4.** Powder XRD pattern of hybrids obtained by exchange with DMPG liposome suspension for different DMPG percentages. (A) In the low 2Θ domain: (a) DMPG, (b) DMPG-6.7-Mg/Al, (c) DMPG-20-Mg/Al (d) DMPG-100-Mg/Al. (B) In the large 2Θ domain: (a) LDH, (b) DMPG-6.7-Mg/Al, (c) DMPG-13.3-Mg/Al, (d) DMPG-20-Mg/Al, (e) DMPG-100-Mg/Al.

(Figure 4A) and variations in 2Θ from 5 to 65° are observed (Figure 4B) except for DMPG-6.7-Mg/Al, which displays a pattern rather similar to that of $NO_3$-LDH reflecting thus the low amount of DMPG introduced. For the other hybrids, as the exchange rate of DMPG for $NO_3^-$ proceeds, the intensities of the peaks located at 1.8° 2Θ and at 7.8° 2Θ, characteristic of the phospholipids and $NO_3^-$ ions, respectively, behave in opposite directions (Figure 4A and B). Three harmonic reflections corresponding to planes (003), (006) and (009) with respective interlayer repeat distance of 4.64, 2.32, and 1.55 nm are clearly identified in the low 2Θ range, they are assigned to intercalated DMPG species. Their intensity increases with the DMPG hybrid content (c and d in Figure 4A). Concurrently, the intensity of the peaks situated at 10 and 20° 2Θ assigned to reflections from the (003) and (006) planes intercalated with $NO_3^-$ decreases (d and e in Figure 4B). This evolution confirms the exchange of DMPG for $NO_3^-$ anions. From the observed interlayer spacing of 4.64 nm and considering that the width of the brucite-like layer is 0.48 nm, the gallery height is 4.16 nm. It is lower than the repeat distances of 4.79 or 4.75 nm reported for the two different conformations of the DMPG bilayers below and above their transition temperature (23.5 °C), respectively.[34]

We can suggest that either interdigitation of the C14-chains or tilting of these chains relatively to the plane of the brucite-like layers must occur in the constrained interlayer space of the LDH host. From a gallery height of 4.16 nm and DMPG repeat distances of 4.79 or 4.75 nm in the two different conformations, the angles of tilt would be close to 29 and 31°, respectively. These values are lower than the tilt angle of 49.4° reported for the dodecyl sulfate (SDS) anion in LDH or that found in the range 49.3−59.1° for $C_{14}$ alkylammonium intercalated into vermiculites.[35] However, the exchange of the nitrate compensating anions of the host structure by DMPG entities is performed at 40 °C, which is above the temperature corresponding to a high-order transition known to occur at 31.7 °C.[32] In the latter conditions, a conformational change occurs and is assigned to a rotation of the terminal glycerol groups of DMPG, leading to a stretched conformation of the lipids. This results in a bilayer thickness of 5.55 nm.[32] Furthermore, as a result of this transition, the DMPG molecules remain frozen in that stretched conformation when the temperature is brought to a lower temperature, a fact that indicates the presence of a sizable barrier for the return to the original conformation. Going back to our data, that organization of the lipids would correspond to a tilt angle of 41.5° in the hybrid materials, which is closer to the values of the literature given above for analogous systems. Also as a consequence of the existence of the conformational energy barrier, it is clear that the change in geometry occurring in DMPG molecules could also occur during the drying step of the hybrids because it is performed at a temperature close to 40 °C.

The formation of these hybrids raises the question of the mechanism of intercalation of a bilayer. Obviously, this must be a complex multistep process that may begin with the adsorption of the liposome by its external lipid layer to the rim of the mineral LDH. In a following step, as the liposome cannot squeeze itself in-between the layers as this would lead to intercalation of a stack of two bilayers, the external layer of the liposomes could so to speak flow from each side of the contact area to form the intercalated bilayer, while being continuously fed from the internal layer of the liposome by a flip-flop mechanism.[36] One could also imagine an adsorption of the liposome onto the external brucite-like layer of the LDH particles, with the formation of a bilayer followed by a diffusion of the latter over the edge and into the intra lamellar space. We are presently starting to work along these speculative lines to obtain some experimental lead.

The resolution of the in-plane (110) and (113) reflections typical of the LDH structure between 60 and 65° 2Θ

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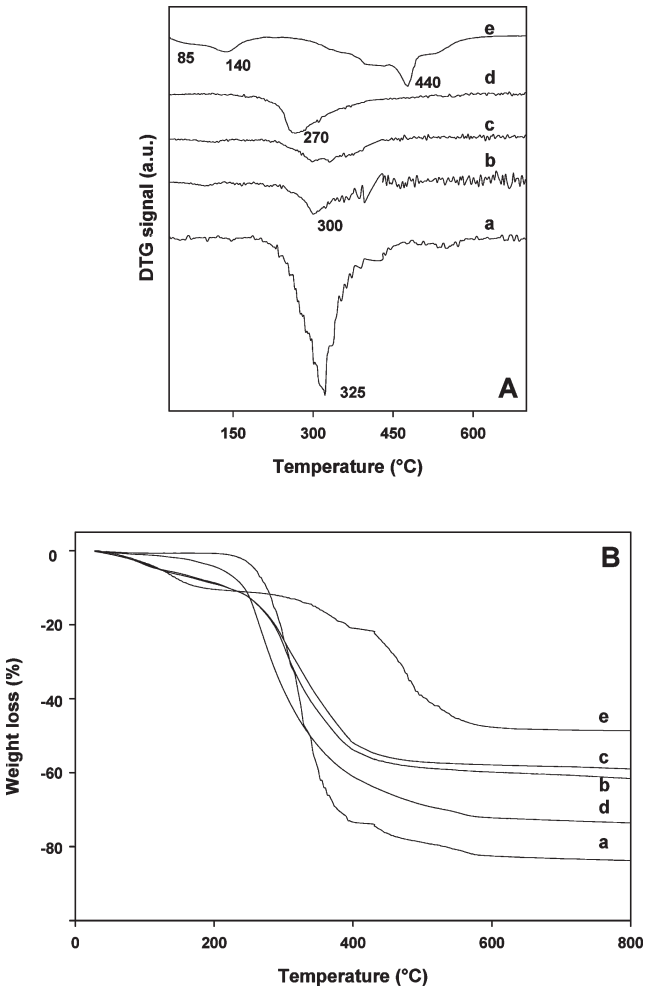


**Figure 5.** (A) DTG and (B) TGA profiles in the temperature range from 30 to 800 °C of (a) DMPG, (b) DMPG-13.3-Mg/Al, (c) DMPG-20-Mg/Al, (d) DMPG-100-Mg/Al, and (e) LDH.

increases with the DMPG content (Figure 4B), a fact that could reveal an improvement in the intralamellar organization or, likely, a well co-organized hybrid with well-stacked layers.

**Hybrid Material Thermal Behavior.** The TGA-DTG profiles of the hybrids (13.3, 20 and 100% of the AEC) are compared to those of the LDH host and of DMPG (Figure 5). LDH presents the characteristic two weight-loss domains generally observed in LDH. The first one between 25 and 200 °C corresponds to the loss of weakly bound surface and interlayer water molecules giving the DTG peaks at 85 and 140 °C, respectively (Figure 5A). The second weight loss above 200 °C is due to the dehydroxylation of the layers and the decomposition of the nitrate anions, the latter giving rise to the DTG peak at 440 °C. In contrast with this behavior, pure DMPG is decomposed in one step between 200 and 500 °C, with a maximum in the DTG profile peaking at 325 °C. A slight weight loss corresponding to less than 6% is measured only above 450 °C.

Similarly to the host structure, two weight-loss domains are observed for the DMPG-Mg/Al hybrid materials. The total weight losses amount to ca. 62 and 74% of the initial samples weight up to 800 °C for DMPG-13.3-Mg/Al and DMPG-100-Mg/Al LDH, respectively (Figure 5B). They fit well with the expected values corresponding to the complete decomposition of the hybrids into their mixed oxide forms. The asymmetric shape of the intense DTG peak observed above 200 °C corresponding to the maximum rate of the second weight loss accounts for the decomposition of several types of compensating anions in DMPG-13.3-Mg/Al and of DMPG in several steps in DMPG-100-Mg/Al. It is worth noting that this peak is shifted from 325 °C in free DMPG, to 300 and 270 °C in DMPG-13.3-Mg/Al and DMPG-100-Mg/Al, respectively. Intercalated DMPG appear therefore less thermally stable than free DMPG and their thermal stability decreases with the loading. This behavior is opposite to that generally observed in the case of biopolymers,[21] drugs,[37,38] or dyes,[39] where intercalation between the LDH layers enhances the thermal stability. However a decrease of the thermal stability has also been reported in the case of pectin intercalated in Zn/Al LDH because of the different conformations of the native and intercalated biopolymer.[21]

**Hybrid Material Structural Formulas.** The elemental analyses of the host structure and of some hybrids reported in Table 1 allow determining the structural formulas. The Mg/Al molar ratio of 2 in the LDH host sample is the same as that of the initial aqueous solution of Mg and Al nitrates. This ratio is consistently maintained at 2 in the different hybrids prepared by exchanges performed at pH = 7.4, conditions in which the Mg−Al brucite-like layers are known to be chemically stable. The amount of nitrate exchanged increases in the hybrid materials with the content of DMPG in the suspension. However the C/P molar ratios in the hybrids are always higher than the nominal value of 34 corresponding to pure DMPG expected since the phospholipids are chemically stable in these conditions.[32] The higher than expected C/P values show that carbonation must occur during the exchange because of contamination by $CO_2$ present in air. The DMPG contents were therefore deduced from the phosphorus amount, and the carbonate content was estimated from the carbon not belonging to DMPG. On this assumption, the balance expected between the positive charge of the layers and the negative charge contributed by the nitrates carbonate and monovalent DMPG anions is a strong evidence for the presence of DMPG intercalated in the hybrids. Unexpectedly, chloride ions present in the DMPG suspensions are not present in the hybrids. As will be shown hereunder, they appear as solid NaCl crystallites. Carbonate contents in the 15−20% range of the anionic content are reached in all these hybrids in agreement with the well-known high affinity of these anions toward LDH.[33] However, the limited amount of intercalated DMPG (valued at ~80% of the AEC due to residual $NO_3^-$ (Table 1)) can also

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**Table 1. Composition Obtained from Chemical Analysis and Proposed Formula of the DMPG-*x*-Mg/Al Samples**

| | chemical composition (weight %) | | | | | | |
|---|---|---|---|---|---|---|---|
| sample | Mg | Al | N | P | C | C/P mol/mol | formula |
| $NO_3$−Mg/Al | 18.20 | 10.30 | 5.26 | | 0.41 | | $Mg_{0.66}Al_{0.33}(OH)_2\ (NO_3)_{0.27}$ $(CO_3)_{0.03}$, 0.54 $H_2O$ |
| DMPG-6.7-Mg/Al | 17.17 | 9.40 | 3.03 | 0.41 | 6.10 | 38.40 | $Mg_{0.67}Al_{0.33}(OH)_2\ (NO_3)_{0.203}\ (C_{34}O_{10}H_{36}P)_{0.012}(CO_3)_{0.057}$, 0.60 $H_2O$ |
| DMPG-13.3-Mg/Al | 14.42 | 8.13 | 2.13 | 1.14 | 16.44 | 37.25 | $Mg_{0.67}Al_{0.33}(OH)_2\ (NO_3)_{0.169}\ (C_{34}O_{10}H_{36}P)_{0.041}(CO_3)_{0.067}$, 0.66 $H_2O$ |
| DMPG-20-Mg/Al | 14.04 | 7.79 | 2.11 | 1.26 | 19.50 | 40.00 | $Mg_{0.67}Al_{0.33}(OH)_2\ (NO_3)_{0.172}\ (C_{34}O_{10}H_{36}P)_{0.046}(CO_3)_{0.06}$, 0.67 $H_2O$ |
| DMPG-60-Mg/Al | 9.11 | 5.05 | 0.70 | 2.70 | 35.98 | 34.42 | $Mg_{0.67}Al_{0.33}(OH)_2\ (NO_3)_{0.09}\ (C_{34}O_{10}H_{36}P)_{0.154}(CO_3)_{0.055}$, 0.60 $H_2O$ |
| DMPG-100-Mg/Al | 6.59 | 3.62 | 0.05 | 3.32 | 43.86 | 34.12 | $Mg_{0.67}Al_{0.33}(OH)_2\ (NO_3)_{0.009}\ (C_{34}O_{10}H_{36}P)_{0.262}(CO_3)_{0.05}$, 0.56 $H_2O$ |

originate from steric hindrance. Indeed, for an area of 0.53 $nm^2$ per headgroup of DMPG bearing one negative charge,[34] the surface area corresponding to one positive charge[35] in LDH is valued at 0.50 $nm^2$. Therefore, less than 1 mol of DMPG can theoretically be accommodated per mole of LDH. The maximum amount of DMPG retained represents around 115 mequiv/100 g. It is similar to the 128 mequiv/100 g of the anionic biopolymer $\kappa$-carrageenan intercalated in Cl−Zn/Al LDH[21] or to the 140 mequiv/100 g of the drug indomethacin intercalated in Cl−Mg/Al LDH[17] previously reported, but far higher than the 23 mequiv/100 g of ATP intercalated in $NO_3$−Mg/Al LDH.[40]

**Size Distribution.** The host structure LDH and the hybrid DMPG-60-Mg/Al show an average size of 53 and 29 $\mu$m, with a uniformity of 1.25 and 1.58, respectively.

**TEM Micrographs.** The samples were also observed by TEM, and as shown in Figure 6, hybrid materials are well-organized compared to LDH. Moreover, the interlayer distances estimated from the TEM results is in agreement with the thickness of 4.2 nm based on the XRD results for the phospholipid bilayer. This gives a positive evidence for the organization of the phospholipids as single bilayers intercalated between the LDH layers.

**Fluorescence Experiments.** Fluorescence excitation and emission spectra of DPH in these hybrid materials in an aqueous suspension were also recorded (Figure 7). Because of its strongly hydrophobic character, DPH remains in the lipid bilayer. Even so, the excitation spectra of DPH in the hybrid materials are markedly different from those in free labeled liposomes (Figure 7). The absorption band is broader, slightly shifted to the red (by 3 nm for all vibronic components) and the relative intensity of the two first vibronic components is totally reversed, with the 1:2 ratio changing from 0.76 to 1.03 going to the intercalated material. If the emission spectrum is similarly hypsochromically shifted by 2 nm, the relative intensity of its vibronic components is less affected. Another difference between both environments is seen in the emission anisotropy. Fluorescence polarization data acquired at 25 °C give an *r* value of 0.25 for the emission anisotropy of DPH in the intercalated material (Figure 7), a value lower than the value 0.32 found in free liposomes.

The latter observation reveals a less compact arrangement of the lipids that could allow larger amplitude for a

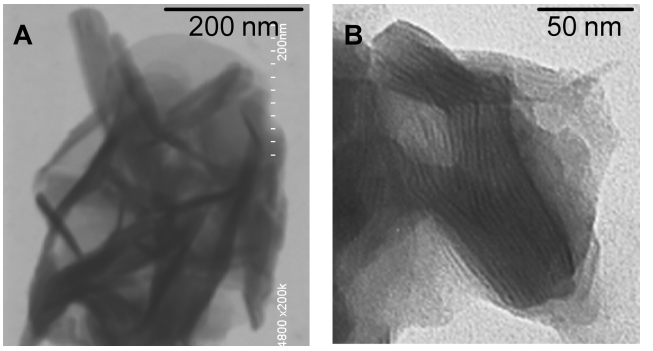


**Figure 6.** TEM photograph of (A) the LDH host and (B) the hybrid DMPG-60-Mg/Al.

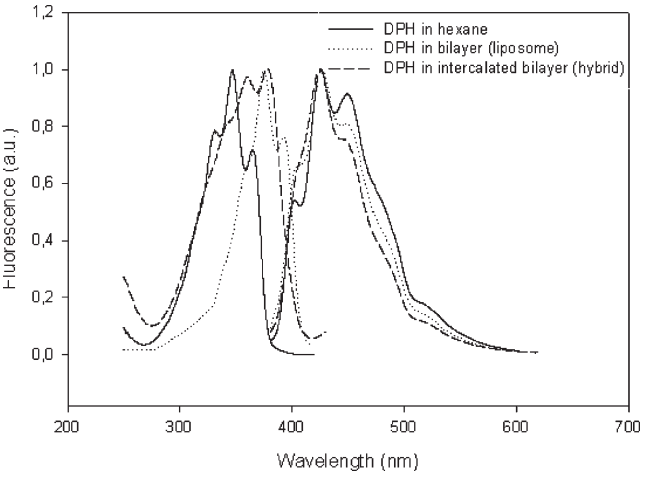


**Figure 7.** Fluorescence excitation and emission spectra of DPH in solution or loaded into phospholipid bilayer (liposome or intercalated bilayer).

wobbling or rotation of DPH around an axis normal to its long molecular axis. This is somehow expected for two reasons. First, the very hydrophobic DPH spends most of its time in the region of the alkyl chains for which more space is available when the molecules lie roughly parallel than when they tend to be in stronger interaction because of the curvature of the liposome bilayer. The second reason is that there is probably more disorder at the level of the alkyl chains in the high energy conformation of the lipids, with the same consequence. The molecule could also probe several microenvironments associated to different local organizations of the trapped bilayer as hinted by the spectral broadening.

Anyway, all experimental data obtained concurred to the same result, namely that a single phospholipid bilayer was intercalated between the LDH layers

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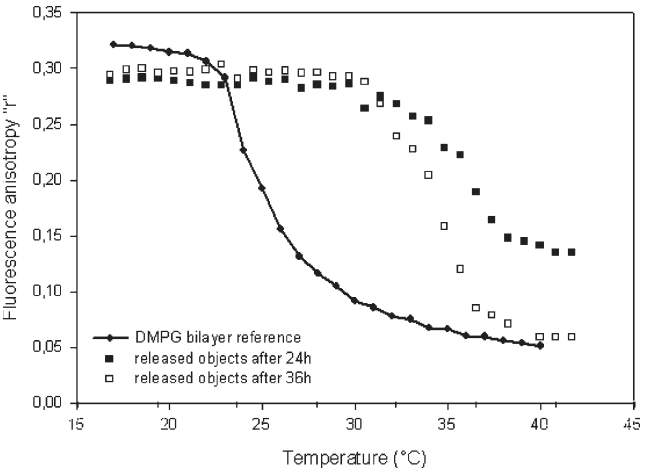


**Figure 8.** Depolarization curves of the reference (liposome DMPG phospholipid bilayer) and released objects (recovered after centrifugation) after 24 or 36 h release study in a release medium (pH 7.4, 150 mM, 37 °C).

**Release Studies.** DPH-loaded hybrid material was used for the release studies. At different times of the release study in a physiological medium (pH 7.4, NaCl 150 mM), the suspension is centrifuged. The supernatant is analyzed by spectrofluorimetry in order to follow the presence of released DPH. Up to 24 h, a depolarization curve is not observed and the weak fluorescence detected results from the release of small amounts of DPH and phospholipids without any detectable organization. From $t = 24$ h on, a low amplitude typical sigmoidal depolarization curve (with r going from 0.3 in solid gel state to 0.18 in fluid gel state) is observed with a phase transition temperature at 36 °C (Figure 8). It is repeatedly seen up to 36 h, with increasing amplitude for the change in $r$ from 0.3 to 0.06 while the phase transition temperature remains the same. That depolarization curve confirms the organization of the phospholipids into bilayers.

The size and shape of these bilayers are ascertained by a QLS analysis that reveals the presence of spherical particles with a mean diameter of 154 nm (p.i: 0.356) after 24 h and of 90 nm (p.i.: 0.309) after 36 h. These results are in agreement with the known fact that lipid bilayers in suspension in an aqueous medium spontaneously form liposomes. The shift in the transition temperature to 36 °C is due to the fact that the lipids from the liposomes formed after the release, are in the stretched conformation referred to above which is different from that present in the initial liposomes used in the synthesis.[32] As the presence of free Mg or Al ions is highly improbable in the present conditions because of the stability of the LDH layers, they cannot affect the compacity of the charged lipid heads. On the other hand, the stretched lipid conformation must allow the formation of a stronger set of H-bonds between the lipid heads, which could explain the presence of the barrier that prevents the return to the initial conformation as well as the higher transition temperature observed.[32] These elements taken together show that the intercalated phospholipids are reorganized as liposomes after the release step (from 24 h) and that these liposomes carry with them the originally encapsulated lipophilic drug.

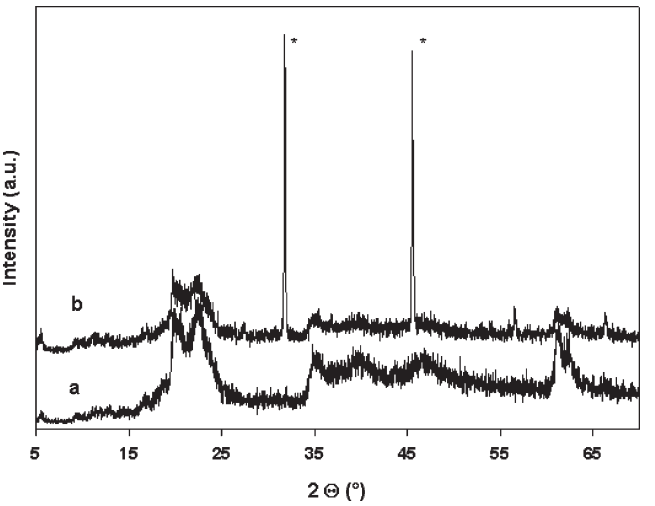


**Figure 9.** Powder XRD patterns of hybrids in the large 2Θ domain (a) before and (b) after the release study, consisting of a 14 day incubation in a release medium (pH 7.4, 150 mM, 37 °C).

The XRD pattern of the recovered dried solid is unchanged with respect to the starting product even after running the kinetics for 14 days (Figure 9). This is not surprising because only 1.5% of the DMPG phospholipids initially present were extracted after 7 days and 6% after 14 days (TGA, data not shown). The presence of sharp diffraction peaks of NaCl crystallites originating from the mother suspension is also seen in the pattern. As the LDH are well-known to be stable at physiological pH, the phospholipid release may occur by anionic exchange with the phosphate buffer anions.

## Conclusions

A new family of hybrid materials involving the self-assembly of two organized subsystems, an inorganic LDH and a lipid bilayer, is shown to afford an interesting storage and release medium for non ionic water insoluble drugs, all the more because cytotoxicity studies have shown the biocompatibility of LDHs. The synthesis consists in mixing together a suspension of liposomes loaded with a lipophilic drug with the host LDH. So the synthesis proceeds in an aqueous medium, under soft conditions of pH, temperature, and stirring. The intercalation of the loaded bilayer results in an important increase of the interlamellar space of the LDH structure, potentially allowing the loading of biomolecules (pharmaceutical drugs) of high molecular weight that could be neutral or lipophilic. There is a special interest in the latter, as they represent the largest fraction of the most recent pharmaceutical molecules. In these hybrids, a sustained drug delivery monitored by means of a fluorescent mimic drug is observed to occur in physiological conditions. The analysis of the release shows that the lipids and the drug are slowly and simultaneously released over weeks to form reconstructed liposomes. Furthermore, an irreversible change in the conformation of the lipids induced by their heating at 40 °C during the synthesis of the hybrids results in an increase in the transition temperature of the reconstructed liposomes which occurs now at 36 °C.

Continuing work is focusing on the improvement of the design of these hybrid materials in order to increase their application scope, the main targets being the optimization of the loading, the downsizing of the particles to nanometric scale to design a subcutaneous implant, and the understanding of the intercalation mechanism.

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**Supporting Information Available:** FITR spectra of LDH and hybrid DMPG-60-Mg/Al. The IR spectra of the LDH, phospholipid bilayer, and hybrid material have been recorded. Some bands could be attributed to one of the components but there was not much to be learnt from them. We have worked on several similar intercalations of large anionic organic molecules into LDH. Few valuable data could be gained, such as variation in band intensity ratios that could confirm the other analytical determination or small shifts that could not be actually interpreted (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.