Intercalation of Vitamer into LDH and Their Controlled Release Properties

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Received October 9, 2003

Biofunctional nanohybrids are synthesized from layered double hydroxide (LDH) and the vitamins such as ascorbic acid and topopherol acid succinate. Either ion exchange or copricipitaion leads to successful intercalation of the vitamins into gallery space of LDH that offers a new route to safe preservation of bioactivity as well as controlled release. Intercalations of vitamins are clearly reflected on the increase in the basal spacing of ZnAl-(Nitrate) LDH from 8.5 Å to 10.5 Å for ascorbate, and 49.0 Å for tocopherol acid succinate, respectively. No significant change in UV-Vis and IR absorption characteristics of the intercalated vitamins strongly supports the safe maintenance of their bioactivities without any deterioration of chemical and structural integrity. Furthermore, it is shown that the hybridized vitamins could be discharged in a controlled kinetics.

Key Words : Layered double hydroxide (LDH), Vitamins, Intercalation, Controlled release, Safe delivery

Introduction

Hybridization of two distinct materials in the nanometer scale leads to extraordinarily high synergic and complementary effects on their physico-chemical and functional properties.[1-5] Recently, our attention has been given to the nanohybrids originated from layered double hydroxides (LDHs) because of their unique microstructure and physicochemical property.[6-11] Especially, their intercalation property allows exploration of new potential for bioactive nanohybrid.[12-14]

LDH consists of positively charged hydrotalcite-like layer of metal hydroxide and the interlayer region typically occupied by anionic species and water molecules. Layer structure is stabilized by hydrogen bonding among water molecule, anionic species and hydroxide layer. Various kinds of inorganic or organic anions could be readily introduced and stabilized into the hydroxide interlayer by simple ion-exchange reaction or coprecipitation.[15-17] Thus, LDH could act as a stable host matrix for storage and delivery of these intercalated substances. Especially, when the labile bioactive substances like many vitamins are intercalated into LDH interlayer, they could be effectively protected against rapid degradation by light, temperature, oxygen, alkali metal, etc. In fact, their fast degradation greatly limits their application as active ingredients to cosmetics, foods, and drugs.

Vitamin C is very sensitive to oxidation in the presence of oxygen. A small amount of metal ions assist this reaction under neutral and alkaline conditions. Many enzymes also easily decompose vitamins within biosystem. On the other hand, human body requires only a very small amount of vitamins for physiological functions. Both insufficient and excessive supplies of vitamins cause harmful effects on human body. For reliable supply, it is necessary to develop a controlled delivery system for vitamins. In this regard, LDH could be an excellent candidate matrix due to high anion exchange capacity and biocompatibility.[18,19] Not only LDH could protect vitamins against decompositions, but also intercalated vitamins could be intentionally taken out from the LDH lattice in an ambient condition. The schematic illustration is shown in Figure 1. Their releases in carbonated aqueous solution seem to take place mainly by ion exchange and diffusion reactions that could be readily controlled by the manipulation of reaction conditions and structural properties of LDH.

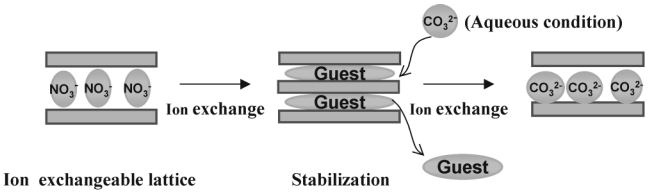

Figure 1. Schematic illustration for slow release of the guest from LDH hybrid.

Recently we succeeded in intercalation of the anionic vitamin derivatives in LDH and metal oxide.[20,21] However, detailed properties of intercalated vitamins are not well understood yet. In the present study, we focus on the characterization of vitamin-LDH hybrids. Furthermore, their release behaviors are also examined by spontaneous deintercalation with carbonate anion.

Experimental Section

Preparation ascrobate-LDH (*VC*-LDH). The pristine-$Zn_2Al(NO_3)$-LDH was prepared under N_2 atmosphere. A mixed aqueous solution containing Zn^{2+} (0.02 mol, from $Zn(NO_3)_2 \cdot 6H_2O$) and Al^{3+} (0.01 mol, from $Al(NO_3)_3 \cdot 9H_2O$) was titrated dropwisely with NaOH (0.1 M) solution. The

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resulting white precipitate was isolated by centrifugation, washed with decarbonated water to remove the excess nitrate ion, and then freeze-dried. The ***VC***-LDH hybrid was synthesized by anion exchange reaction of the pristine LDH with ascorbic acid (Sigma Chemical Co.) at pH = 7. The pristine LDH (0.001 mole) was dispersed in decabonated aqueous solution containing excess amount of ascorbic acid (0.003 mole), and reacted for 48 hrs in the ice-bath (at 0 °C) with constant stirring. The reaction product was isolated, washed, and freeze-dried.

Preparation α-tocopherol acid succinate-LDH (*VE*-LDH). ***VE***-LDH hybrid was directly synthesized by the coprecipitation reaction under N_2 atmosphere. A mixed aqueous solution containing Zn^{2+} (6.657×10^{-4} mol) and Al^{3+} (2.219×10^{-4} mol) was dropwisly titrated with NaOH (0.1 M) solution in the presence of vitamin ((+)-α-tocopherol acid succinate; 3.768×10^{-4} mol, Sigma Chemical Co.) with vigorous stirring. The solution pH was adjusted to 7.5 ± 0.2 at 25 °C. The resulting precipitates were washed with deionized water and ethanol thoroughly.

Characterization. X-ray diffraction (XRD) patterns were obtained at 20 mA, 40 kV with Philips PW 3710 powder diffractometer equipped with Ni-filtered Cu K_α radiation (λ = 1. 5405 Å). Infrared spectra were obtained with a Bruker IFS-88 FT-IR spectrometer by a standard KBr disk method. UV-VIS spectra were recorded on a Perkin-Elmer Lambda 12 spectrometer. The stoichiometry of each vitamin-nanohybrid was determined by elemental analysis (CHN), thermogravimetry (TG-DTA), and inductively coupled plasma spectrometry (ICP).

Deintercalation kinetics. Deintercalation of vitamin C from the hybrid was carried out in CO_3^{2-} saturated aqueous solution, whereas that of vitamin E was performed in 50% ethanol solution saturated with CO_3^{2-}, respectively. Each sample was stirred in incubating shaker for 0, 2, 4, 6, 8, 10, 12, and 24 hr, respectively. The concentrations (wt %) of deintercalated vitamin were estimated by measuring the UV absorbance of vitamins C and E at 265 and 277 respectively.

Results and Discussion

The XRD patterns for the pristine and LDH hybrids exhibit the well developed (00*l*) reflections such as (003) and (006), as shown in Figure 2. The intercalation of vitamins into the lamellar host structure is clearly observed by the increase in the basal spacing upon replacing NO_3^- ions with vitamin molecules.

The diffraction peak at 8.7 Å for the pristine LDH corresponds to the basal spacing of LDH with NO_3^- in the interlayer. Although expansion of basal spacing by vitamin intercalation depends on the kind of vitamin, the (*00l*) reflection peaks shift to lower angles upon the intercalation of the vitamins. Configuration of intercalated vitamin within interlayer space could be depicted from the interlayer spacings of vitamin-LDH hybrids. The interlayer spacing (5.7 Å) of the ***VC***-LDH hybrid is closely related to the calculated thickness of the ascorbate molecules when it adopts a mono-layer configuration parallel to the basal plane of LDH. On the other hand, the gallery heights, 49 Å for ***VE***-LDH hybrids, suggest that α-(+)-tocopherol acid succinate have a double-layer configuration. In addition, the well-ordered (*00l*) peaks imply that the anion-exchange reaction occurs without any deterioration of the layer structure of LDH.

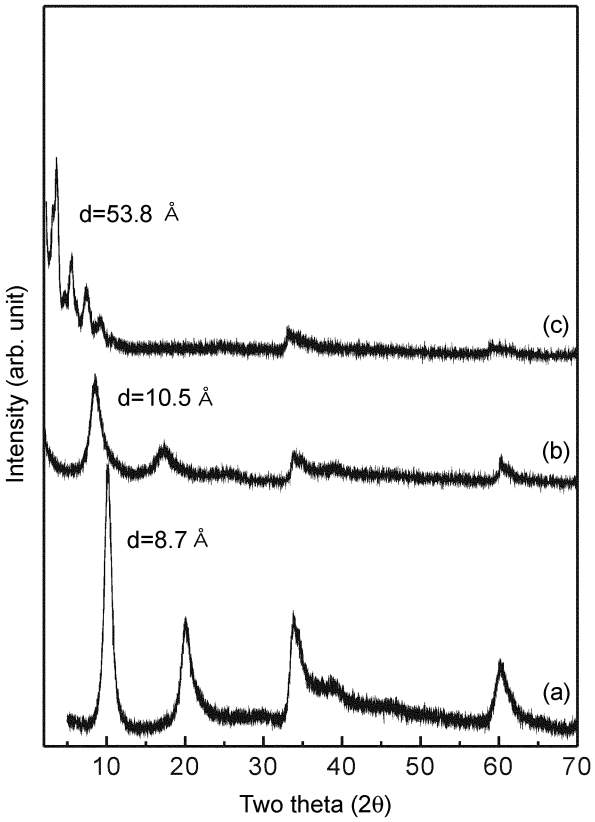

Figure 2. Powder X-ray diffraction patterns for (a) $Zn_3Al(NO_3)$-LDH, (b) Zn_2-Al(vitamin C)-LDH and (c) Zn_3Al(vitamin E)-LDH.

Calculated chemical compositions of synthesized vitamin-LDHs are shown in Table 1. The intercalated vitamin content is estimated to be 23.1% for ***VC***-LDH, and 60.8% for ***VE***-LDH, respectively. In addition, the cell parameters of hybrids are calculated from the relations of $c = 3d_{003}$ and $a = 2d_{110}$ along with the charge density (Table 2). A close relationship of the intercalate vitamin to layer charge indicates that vitamin molecules are stabilized by electrostatic interaction in interlayer space of LDH. The calculated cell parameters also confirm that intercalation leads to a simple layer expansion without any effects on the hydroxide lattice.

Figure 3 shows the infrared spectra for the ***VC***-LDH and ***VE***-LDH hybrids and pristine LDH. The absorption band at

Table 1. Chemical compositions of vitamin-LDH hybrids

Sample	Chemical composition
Pristine LDH	$Zn_{2.04}Al_{1.00}(OH)_{6.08}(NO_3)_{1.00}\cdot 1.2H_2O$
Zn_2Al(vitamin C)-LDH	$Zn_{2.04}Al_{1.00}(OH)_{6.08}(C_6H_7O_2)_{0.56}(NO_3)_{0.06}\cdot 1.25H_2O$
Zn_3Al(vitamin E)-LDH	$Zn_{3.02}Al_{1.00}(OH)_{8.04}(C_{33}H_{53}O_5)_{1.09}\cdot 0.57H_2O$

Table 2. Lattice parameter, charge density and mean volume of crystallite

Sample	c (Å)	a (Å)	$d_{003(cal)}$ (Å)	$d_{003(obs)}$ (Å)	d_c (e/nm^2)	V (Å^3)
Zn_2Al(Vitamin C)-LDH	31.57	3.06	10.18	10.50	3.94	19.30
Zn_3Al(Vitamin E)-LDH	161.34	3.09	54.00	53.78	3.02	99.68

While the c value depends on the thickness of brucite-like layer and the size of gallery species, the a value is only influenced by the ionic radii of interlayer cations. Mean volume (V) could be calculated on basis of the dislike morphology (hexagonal area • height); $V = \pi r^2 h = \pi(^1/_2\ D_{110})^2\ D_{003}$. Positive charge density in the LDH could thus be calculated; $d_c = 1/S_{unit\ charge} = ex/a^2 \sin 60^o$ (x = $Al^{3+}/(Zn^{2+} + Al^{3+})$Å).[15]

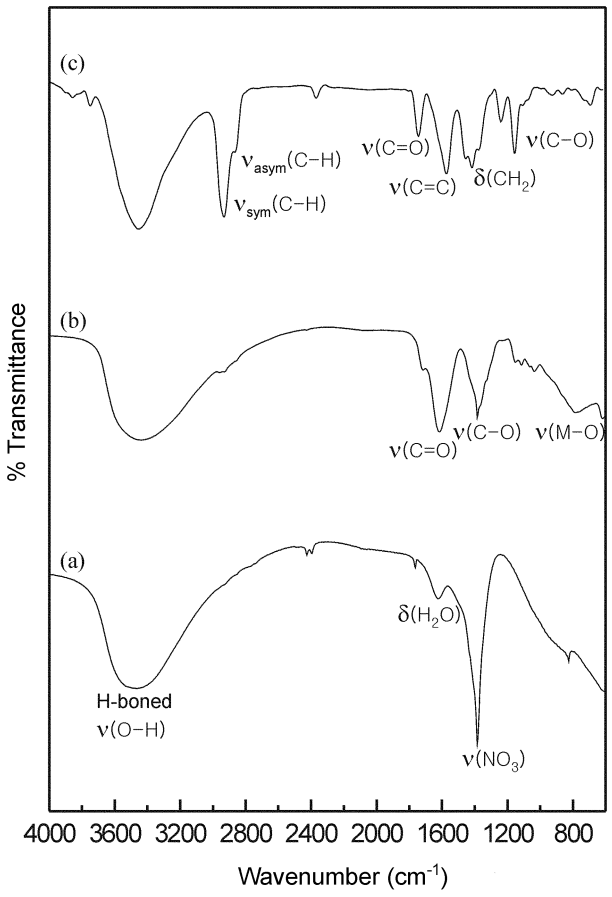

Figure 3. Infrared spectra for (a) $Zn_3Al(NO_3)$-LDH, (b) Zn_2Al(vitamin C)-LDH, and (c) Zn_3Al(vitamin E)-LDH.

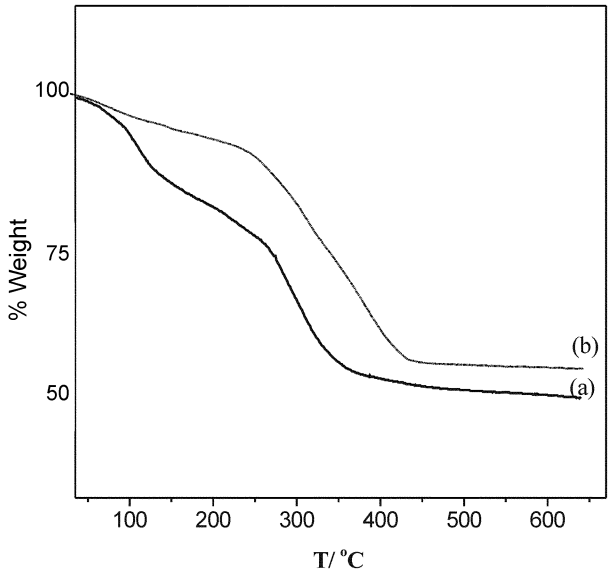

Figure 4. TG profiles for (a) Zn_2Al(vitamin C)-LDH and (b) Zn_2Al(vitamin E)-LDH.

1387 cm^{-1}, assigned to the stretching vibration of NO_3^- in the pristine LDH, completely disappears after the ion-exchange reaction because the interlayer NO_3^- is completely replaced by vitamin molecules. The absorption bands at 2930-3100 cm^{-1} correspond to the ν_{asym} and ν_{sym} (C-H) modes of CH_2 group in the vitamin molecules. The other vitamin bands originated from various functional groups are also found at 1740-1700 (C=O), 1300-1000 cm^{-1} (C-O), and 1600-1475 cm^{-1} (conjugate C=C), respectively. The band broadening by intercalation results from the electrostatic interaction between vitamin molecules and hydroxide sheets to suggest their safe stabilization in the interlayer space of LDH.[22]

The pristine LDH is well known to exhibit a distinctive reduction in mass between 80 and 200 °C owing to the loss of surface-adsorbed and interlayer water. And the other major mass loss occurs in the temperature range from 280 °C to 500 °C due to the concomitant dehydroxylation of inorganic layer and the decomposition of intercalated nitrate anion.[23] As shown in Figure 4, ***VC***-LDH shows a mass loss (*ca*. 15% mass) between 80 and 150 °C because of evaporation of surface-adsorbed and interlayer waters. On the other hand, the gradual mass reduction could be seen from 25 °C to 250 °C for ***VE***-LDH. Such results could be explained by the fact that ***VE***-LDH do not include a lot of interlayer water molecules due to hydrophobic property of α-(+)-tocopherol acid succinate. Decomposition of intercalated vitamins occurs around 350 °C along with dehydroxylation of the hydroxide layer.

According to the UV-vis absorption spectra, intercalated vitamins exhibit the characteristic absorption bands in the range of 250-300 nm. These bands are attributed to $\pi \rightarrow \pi^*$ (enone) transition for vitamin C, and n $\rightarrow \pi^*$ (carboxylate) and $\pi \rightarrow \pi^*$ (benzene) transitions for vitamin E. The absorption maximum of vitamin C at 265 nm in water arises from deprotonation of ascorbic acid to ascrobate at pH 7 (Fig. 5(a)). Any significant differences are not found between vitamin C and ***VC***-LDH hybrids. The UV-Vis spectrum of pure α-(+)-tocopherol acid succinate occur at 277nm in 50% ethanol solution. The spectra of ***VE***-LDH show a red shift by about $\Delta\lambda_{max}$ = 5 nm (Fig. 5(b)). According to the Kasha's theory for the bilayers,[24,25] a blue shift should be observed by the neighboring transition dipole of the isolated chromophor (the H-aggregate) in parallel-alignment. Conversely, a

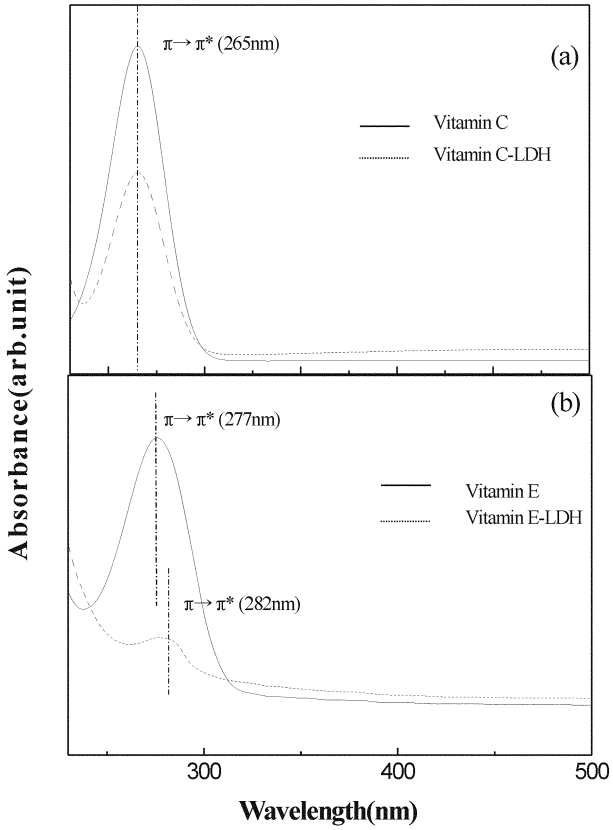

Figure 5. Comparison of UV-Vis spectra for (a) Zn_3Al(vitamin C)-LDH and (b) Zn_2Al(vitamin E)-LDH to those of free vitamin.

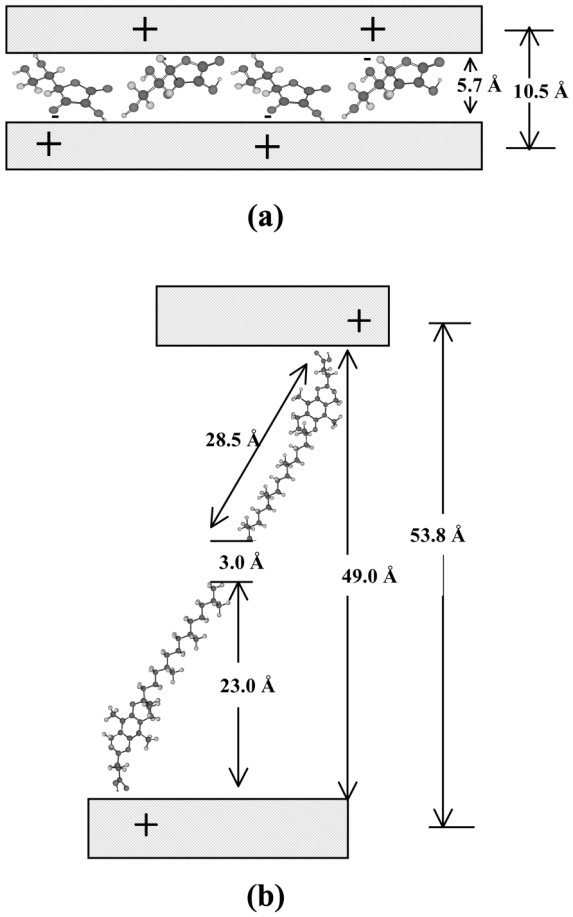

Figure 6. Schematic illustrations of (a) Zn_2Al(vitamin C)-LDH, and (b) Zn_3Al(vitamin E)-LDH.

red shift is attributed to the tilted chromophore orientation (J-like aggregate). Therefore, the red shift observed for ***VE***-LDH suggests that α-(+)-tocopherol acid succinate stacks diagonally with respect to the basal plane. This configuration is also clearly reflected on the XRD pattern (Fig. 1). Assuming the carbon backbone of vitamins is aligned with all C-C bonds in trans form, the tilting angle of vitamin-E is about 62^o (Fig. 6). However, no absorption shift could be seen for the intercalated vitamin C because shorter and less conjugated molecule is not complied with Kasha's theory.

Deintercalation of the ***VC***-LDH hybrid was carried out in CO_3^{2-} saturated aqueous solution while that of ***VE***-LDH hybrids were performed in 50% ethanol solution. The concentrations (wt%) of deintercalated vitamins were estimated by the characteristic absorption peaks at 265 and 277 in the UV/VIS spectra of vitamins C and E, respectively. The amount of deintercalated vitamins is plotted with reaction times. Figure 7 clearly shows that the releases of vitamins occur relatively slowly. All two hybrids exhibit the L type of release pattern that results typically from ion exchange reaction. ***VC***-LDH is found to show a considerable deviation from the other. This deviation could be explained by its rapid degradation into oxalic aicd, L-threonic acid, CO_2, L-xylonic acid in the presence of O_2.[26] Because water-dissolved O_2, even at extremely low concentration, results in quick oxidation of deintercalation vitamin C, it is nearly impossible to obtain the exact cumulative amount for the

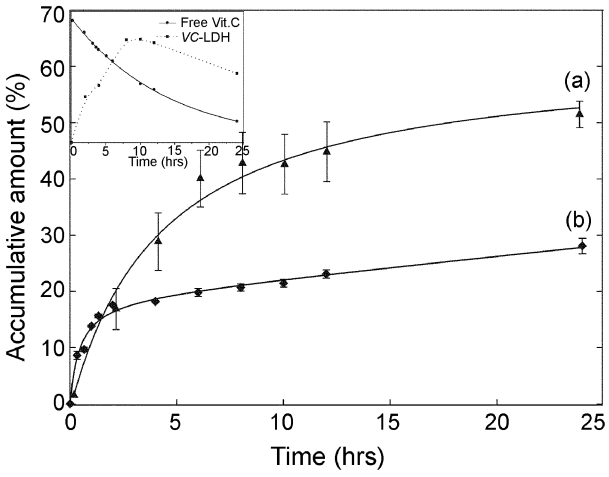

Figure 7. Release patterns of intercalated (a) vitamin C and (b) vitamin E. Inlet shows that free vitamin C (solid) and deintercalated vitamin C (dot).

long time in CO_3^{2-} saturated aqueous condition. In fact, Figure 7 (Inlet) shows that free vitamin C and deintercalated vitamin C content decreases exponentially (solid line) and after 8 hr (dot line), respectively. To revise accumulative amount of vitamin C, it adds deintercalated amount to

decomposed amount of free vitamin C. However, the overall release patterns of vitamins clearly indicate that intercalated vitamins are mainly anion-exchanged by dissolved carbonates. The releasing rates of vitamins from vitamin-LDH hybrids are likely to be affected by the factors such as solvent, kind of anion and charge density that could be easily controlled. Therefore, it is highly feasible that vitamins can be dispensed in a controlled manner even in an ambient condition.

Conclusions

This study clearly shows that the vitamin is stabilized in the interlayer space of LDH without any changes in its chemical and functional integrity through hybridization, and that the stabilized vitamins can be intentionally discharged from the hybrids either through ion-exchange reaction or dissolution of LDH framework in an ambient condition.

Therefore, it is expected that the inorganic LDH can be a excellent host lattice for safe storage and effective delivery of the biological compounds that are rather unstable in the ambient environment or during their delivery within the biosystem.

Acknowledgments. This research is financially supported by a grant of the Korea Health 21 R&D Project (02-PJ1-PG1-CH11-0001), the Korean Ministry of Health and Welfare. Authors are grateful for the BK21 Program.

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